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Accumulation of T lymphocytes in the Liver of Mice Infected with *Plasmodium berghei* (NK65)

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Key words: immunopathology, *Plasmodium berghei*, thymus, T lymphocytes, liver, detrimental reaction

Introduction

There are considerable evidences that immunological reactions may play an important role in the etiology whereby some of the lesions are induced in malaria. To date, immune complex diseases, anemia, cerebral malaria, autoimmune diseases and immunosuppression have been extensively studied (WHO Technical Report Series, No. 579, 1975). However, informations on cellular aspects of immunopathology in malaria are still limited. Waki and Suzuki (1977) reported that in *Plasmodium berghei* infection, athymic nude mice survived much longer time than that shown by normal mice and that reconstitution of thymus cells in nude mice shortened survival time. The findings closed up immunopathological deterioration caused by T lymphocytes in malarial mice.

Immunopathological host responses relevant to T lymphocytes were also studied by several authors. Wright *et al.* (1971) found that the acute death in hamsters infected with *P. berghei* was associated with the development of cerebral hemorrhages and that cerebral hemorrhages were prevented by the administration of antithymocyte serum. Finley *et al.* (1982) also demonstrated that cerebral malaria lesions were observed in the nu/+ mice but not in the nu/nu mice during the course of *P. berghei* infection. Roberts and Weidanz (1978) showed that anemia and splenomegaly in *P. yoelii* infection were caused by thymus-dependent host responses. These observations had pointed adversed reactions by T lymphocytes in malarial host. However, target organ and immunopathological events caused by T lymphocytes have not yet been fully studied.

The present study was undertaken to examine the behavior of T lymphocytes with special attention to immunopathologic reactions in tissues from malarial mice. The authors followed the number of T lymphocytes in the thymus, blood and various tissues as the infection progressed to see the roles and reactions by the cells in mice infected with *P. berghei* (NK65) which caused fatal malaria of the rodent. We especially remarked the behavior of T lymphocytes in the liver tissue at the acute stage of the infection. Interactions between lymphocytes and infected cells in

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the liver were studied by the use of immunofluorescent technique, light and electron microscopy.

Materials and Methods

Animals: Five- to six-week-old female BALB/c mice weighing 20–22 g (Japan Clea, Tokyo) were used. All animals were kept in plastic cages and fed with sterilized diet and water *ad libitum*. The animal house was air-conditioned at approximately 23–26 C, with 60–70% humidity.

Parasite: The NK65 strain of *Plasmodium berghei* was used. The parasite was originally donated by the late Professor Meir Yoeli in 1969 and since then the strain has been maintained by blood transfer in mice or with occasional freezing at −70 C. The mice were inoculated with 10^7 parasitized erythrocytes by intraperitoneal route. Parasitemias were monitored by examination of Giemsa-stained thin smears of tail blood.

Calculations of weight indices of spleens and thymuses from infected mice: Four group of five mice each were consecutively studied on day 0, day 2, day 4 and day 6 of parasite inoculation. A group of uninfected mice examined on day 0 served as control. Mice were exsanguinated by cardiac puncture with heparinized syringes under ether anesthesia. The thymuses, spleens, livers and lungs were separately taken out from mice of the respective group. The wet weight of each organ was recorded. No remarkable differences were noted in the weight of other organs except spleen and thymus. Hence only weight indices shown by spleen and thymus were calculated by the formula:

$$\text{weight index} = \frac{\text{T. W. or S. W.}}{\text{B. W.}} \times 1000,$$

where T. W., S. W, and B. W. indicate thymus weight, spleen weight and total body weight respectively.

Preparation of cell suspensions of the organs: Thy-1 (+) cells (T lymphocytes) in each organ and in the blood of infected mice were counted either by a cytotoxic test (Trypan blue dye exclusion) or by an indirect fluorescent antibody test (IFAT). The thymuses or spleens were minced with scissors and squeezed between two frosted glass slides. The tissue fragments were extruded through 200-mesh stainless-steel screens to obtain single cell suspensions. The single cell suspensions were washed in Eagle minimal essential medium (MEM, pH 7.2, Gibco Laboratories, New York) by centrifugation (400×g for 5 min). The number of cells was adjusted to 5×10^7 cells/ml by hemocytometer counting. Cell suspensions of liver and lungs were prepared by emulsifying the organs with a polytron (Nihon Seimitsu Co., Model NS-500, Tokyo) and extruded through 200-mesh stainless-steel screens. After final washing by centrifugation, the sedimented cells were resuspended in 5 ml of MEM. Erythrocytes in the both cell suspensions were removed by lysing with 0.83% of anmonium chloride adjusted at pH 7.2 with Tris-HCl buffer. The prepared cells were finally washed three times by centrifugation in MEM. The cell number in the blood was counted after lysing the involved erythrocytes and adjusting the volume to 0.5 ml with MEM.

Preparation of anti-Thy-1 serum: Rabbit anti-mouse brain-associated Thy-1 serum was prepared according to the method described by Golub (1971). Briefly, homogenized brains from BALB/c mice were pooled and emulsified with Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan). The prepared materials were injected subcutaneously at four sites of rabbits. Three consecutive injections were administrated at two-week intervals. Six weeks after the primary immunization, brain emulsion without Freund's complete adjuvant was given subcutaneously. The

animals were bled one week after completion of the immunization schedule. Before use, the obtained serum was inactivated at 56 C 30 min and 5 volumes of serum were absorbed with one volume of washed, packed BALB/c mouse erythrocytes at 0 C for 70 min, followed by the absorption with one volume of BALB/c liver homogenate against 3 volumes of the prepared serum. The specificity of anti-Thy-1 serum was examined by the *in vitro* cytotoxic test as described below. The serum diluted 1:32 killed almost 100% of thymus cells, 30–40% of spleen cells but less than 5% of bone marrow cells. The cytotoxic activity of the serum was completely abolished by absorption with thymus cells.

In vitro cytotoxic test for counting Thy-1 (+) cells in thymus and spleen: Thy-1 (+) cells in the thymuses and spleens from infected mice were determined by the cytotoxic test according to the method described by Barker *et al.* (1973). Briefly, 0.1 ml of cell suspension, 0.1 ml of anti-Thy-1 serum or normal rabbit serum (NRS, both sera were diluted 1:32) and 0.1 ml of complement (guinea pig serum, absorbed with thymus cells and diluted 1:5) were mixed in small tubes. The tubes were incubated at 37 C for 30 min. After incubation, 0.03 ml of 0.5% of trypan blue dye solution was added. The number of dead cells was determined by counting 200 cells in a hemocytometer. The percent lysis for each tube was determined by the following equation.

$$\% \text{ lysis} = \frac{\text{dead cells in antiserum} - \text{dead cells in NRS}}{200 - \text{dead cells in NRS}} \times 100$$

The number of Thy-1 (+) cells in the thymuses or spleens from infected mice was counted by percent lysis multiplied by total number of thymus or spleen cells.

Indirect fluorescent antibody test (IFAT): Numbers of Thy-1 (+) cells in the lungs, livers and blood were examined by IFAT. Two hundred microliter cell suspensions from each organ emulsions adjusted at 5 ml and blood were incubated with 10 microliter of anti-Thy-1 serum or NRS at 0 C for 30 min. The reacted cell specimens were washed twice in MEM, then stained with FITC-conjugated goat anti-rabbit IgG serum (Miles-Yeda Ltd., Israel) diluted 1:30 for 30 min at 0 C. The cells were finally washed in MEM and resuspended in 0.5 ml of MEM with 30% glycerol. The stained cells were counted in a hemocytemeter with a fluorescence microscope (incident light illuminating system, Olympus, Type BHA-RFL, Tokyo).

Preparation of tissue specimens for IFAT: The liver specimens were fixed in a fixative consisting of equal volumes of aceton and ethanol for 1 hr in ice. After fixation, the materials were transfered in an ethanol-aceton-benzen mixture (1:1:2), infiltrated in benzen and eventually embedded in paraffin blocks. Three micron thickness sections were cut, placed on slide glasses, deparaffinized in xylene and dried. The sections were soaked in phosphate buffered saline (PBS, pH 7.2) before subjecting to anti-Thy-1 serum or NRS treatment. After three-time washings in cold PBS, the prepared tissue specimens were incubated with FITC-conjugated goat anti-rabbit IgG serum as previously described, washed in cold PBS three times, mounted in PBS with 30% of glycerol at pH 9.2 and processed for examinations by a fluorescence microscope. After counting Thy-1 (+) cells with IFAT, the same section was stained with hematoxylin and eosin, and again observed by a light microscope. Such combination studies made possible to calculate Thy-1 (+) cells per 1,000 nucleated hepatocytes in the liver specimens and to combine fluorescent staining with histological observations.

Light and electron microscopic studies

on lymphocytes in the liver: The livers from control and infected mice were taken out and cut into small pieces, fixed in 2.5% glutaraldehyde with 1/15 M phosphate buffer at pH 7.4 for 2 hr and post-fixed in 1% osmium tetroxide with phosphate buffer containing 7% sucrose. Both fixations were carried out in an ice bath. After dehydration with graded series of ethanol, the specimens were embedded in a mixture of Epon 812 and Araldite. One micron thickness sections were prepared and stained with toluidine blue for light microscopic studies. Thin sections were cut on a Porter-Blum MT2-B ultramicrotome with a glass knife and were stained with uranyl acetate and lead citrate. The specimens were observed with a transmission electron microscope, Hitachi HU-11D (Hitachi Seisakusho Inc., Tokyo), set at 75 KV of accelerating potential.

Results

I. Population changes of Thy-1 (+) cells localized in organs from malarial mice during the course of infection.

(1) *Reduction of Thy-1 (+) cells in the thymuses of infected mice*: In the course of *P. berghei* infections, a substantial reduction in the size and weight of the thymus was observed as the disease progressed. The relative decrease in thymus weight was proportionately greater, although the body weight of these animals was also slightly reduced. The weight index of thymuses from infected mice was reduced to about 30% of index given by normal mice group on day 6 of infection (Fig. 1).

On day 6 after inoculation the number of Thy-1 (+) cells was reduced to around 3.5% of normal value (Fig. 2A). The proportion of Thy-1 (+) cells in the whole thymus, however, was not changed during the course of infections. The findings indicated that the thymus underwent atrophy due to the loss of T lymphocytes.

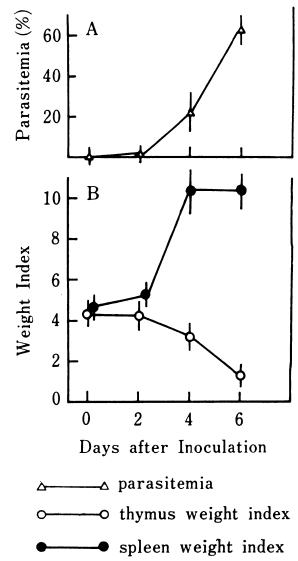

Fig. 1 Development of parasitemia (A) and associated changes of weight indices (B) of the thymus and the spleen during *P. berghei* infection. Each point represents mean ± SD of five mice.

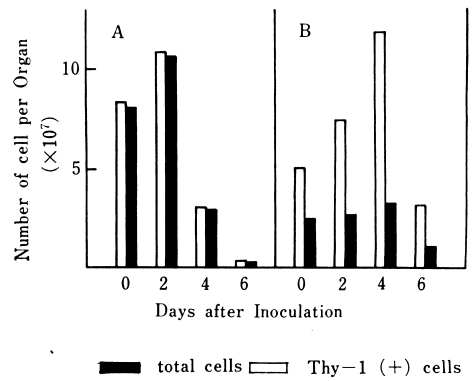

Fig. 2 Total cell number and Thy-1 (+) cell number in the thymus (A) and in the spleen (B) of mice during the course of malaria infection. Mean for each point was obtained from five mice.

(2) *Reduction of Thy-1 (+) cells in the spleens of infected mice*: Spleen weight of infected mice was rapidly increased and manifested an approximate 2-fold increase of the weight index given by normal mice

Table 1 Increase of Thy-1 (+) cell number in the livers and blood of *P. berghei* infected mice

Organs	Thy-1 (+) cell number ($\times 10^5$) Days after inoculation			
	0	2	4	6
Blood	0.7	1.1	4.9	25.0
Lungs	4.2	1.9	4.2	5.4
Liver	4.5	5.0	150.0	230.0

Thy-1 (+) cells in the blood and in the whole organs were counted by the following formula.

$$A = B \times 10^3 \times \frac{0.5 \text{ (volume of final suspension of tested specimen)}}{0.2 \text{ (volume of initial sampling from organ emulsion or blood}} \times C$$

A: No. of Thy-1 (+) cells in the organ or in the blood (1 ml).
B: No. of Thy-1 (+) cells in counted 1 mm^3 specimen.
C: Total volume of organ emulsion (5 ml) or blood (0.5 ml).
Average No. of Thy-1 (+) cells was calculated from 5 mice.

on day 4 and 6 of infection (Fig. 1). The spleen continued to hypertrophy until death of mice. However, the total number of spleen cells dropped precipitously, and the number of Thy-1 (+) cells in the spleen decreased to 44% of the control level on day 6 of infection (Fig. 2B).

(3) *Increase of Thy-1 (+) cells in the blood from infected mice*: Number of Thy-1 (+) cells in the blood remained unaltered on day 2 of infection. On day 4, however, the number of Thy-1 (+) cells in the blood increased remarkably and boosted to 25-fold increase on day 6 of infection as compared with normal level (Table 1). The increase of Thy-1 (+) cells in the blood was accompanied by the atrophy of thymuses of infected mice; hence, it was presumed that T lymphocytes released from thymus and circulated in the blood as the infection progressed.

(4) *Accumulation of Thy-1 (+) cells in the livers of infected mice*: The number of Thy-1 (+) cells obtained from the livers of infected mice was within normal range at the early stage of infection. On day 6, however, infected mice showed more than 50-fold increase of Thy-1 (+) cells of that in the livers from the normal mice (Table 1). Although the number of Thy-1 (+) cells in the lungs was also followed by IFAT, no remarkable changes were noted during the course of infection (Table 1). The increase of Thy-1 (+) cells in the livers of infected mice was paralleled with the decrease of the cell number in the thymuses and spleens. Therefore, it seemed that T lymphocytes were released from lymphoid organs into blood circulation and eventually localized in the liver at the late stage of infection.

II. The behavior of Thy-1 (+) cells in the livers of infected mice.

(1) *Immunofluorescent and histologic observations*: Immunofluorescent staining and histologic studies were undertaken to monitor the number of Thy-1 (+) cells in the liver tissue of malarial mice. Total number of Thy-1 (+) cells in the liver tissue of infected mice was counted per 1,000 nucleated hepatocytes according to the method described in Materials and Methods. Normal mice livers showed very

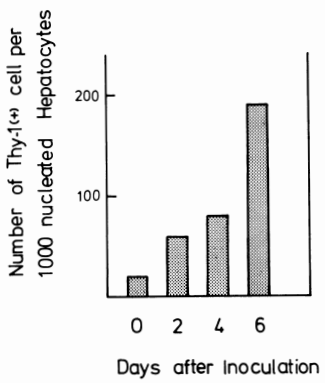

Fig. 3 Increase of Thy-1 (+) cells in the liver as the infection progressed.

few Thy-1 (+) cells if any in the sinusoids. Whereas, infected mice showed marked accumulation of Thy-1 (+) cells in the livers as the disease progressed. The number of Thy-1 (+) cells per 1,000 nucleated hepatocytes on day 6 of infection was about 20-fold greater than that of counted in normal mice (Fig. 3). The Thy-1 (+) cells were scattered evenly in the liver tissue (Fig. 4). Immunofluorescent staining and histologic observation of the same specimens correlated Thy-1 (+) cells and lymphocytes in the liver tissue. By this study, it was revealed that the majority of lymphocyte population localizing in the liver tissue were T lymphocytes. The findings suggested that increased Thy-1 (+) cells in the liver tissue of infected mice were not the passive reflection of the increased Thy-1 (+) cells in the blood circulation of the liver. From the histologic observation it was presumed that Thy-1 (+) cells in the malarial mice actively penetrated into the liver tissue from the blood circulation.

(2) *Studies on one micron thickness sections of livers from uninfected mice*: In order to study the role of Thy-1 (+) cells in the livers, further precise observation was undertaken using one micron thickness sections which allowed correlation between light and electron microscopic studies. Livers from three untreated mice were taken out and subjected to the examination. The light microscopic observations revealed no pathologic changes in the examined livers so far. The plates

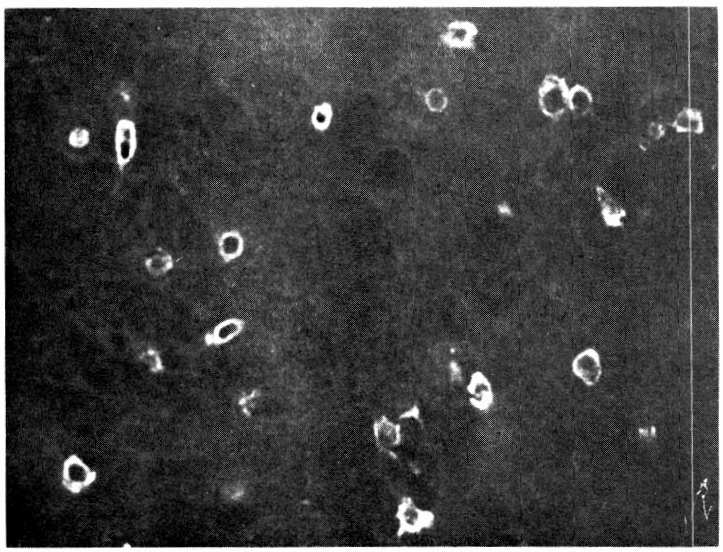

Fig. 4 Immunofluorescent photomicrograph showing even distribution of Thy-1 (+) cells in the liver from a mouse on day 6 of *P. berghei* infection. Magnification, ×750.

of hepatic cells radiated from the central vein toward the periphery of the liver lobules in a normal arrangement. Endothelial cells were lined on sinusoids, occasionally accompanied by Kupffer cells. Both of the cell types showed neither hypertrophy nor unusual multiplication. No remarkable changes were observed both on sinusoids and on bile canaliculi. Blood cells in vessels and sinusoids showed distribution of normal range. Another three mice were used for infection control and were inoculated with normal mouse blood diluted at the same concentration of the blood for infection source. Although some population of the hepatocytes manifested vacuole formations in the cytoplasms, number and size of the vacuoles were limited within normal variation.

(3) *Studies on one micron thickness sections of the livers from infected mice*: Morphologic alterations of the livers in malarial mice were followed on day 0, 2, 3, 4, 5 and 6 since inoculation. Changes in the liver tissue were noted on day 3 after inoculation. Deposits of malarial pigments were observed throughout the section, especially in Kupffer cells and in endothelial cells of the sinusoids. Lymphocytes were occasionally found in sinusoids and some lymphocytes looked to attach to macrophages. Close contacts of lymphocytes with endothelial cells were also observed. On day 4, some sinusoidal spaces were thrombossed with blood aggregants. Depositions of malarial pigments were observed in the cytoplasms of macrophages and sinusoidal lining cells. Lymphocytes occasionally associated with macrophages that engulfed malarial pigments. Most lymphocytes localized in hepatic sinusoids; however, some lymphocytes appeared to infiltrate into liver parenchyma (Fig. 5). On day 6, the features of changes in the livers were similar to those observed in day 4 specimens, but much advanced. Most sinu-

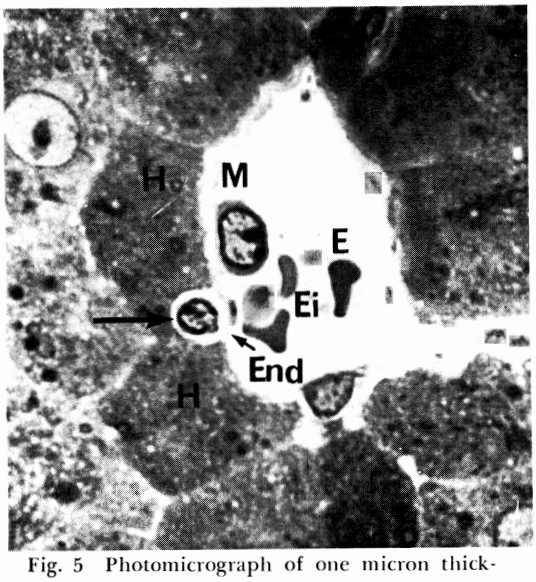

Fig. 5 Photomicrograph of one micron thickness section of the liver from an infected mouse on day 4 after parasite inoculation. A lymphocyte (arrow) penetrating between two hepatocytes (H) is seen. Note that a lymphocyte locates beneath an endothelial cell (End). M; mononuclear cell Ei; infected erythrocyte, E; uninfected erythrocyte, Toluidine blue staining. Magnification, ×1400.

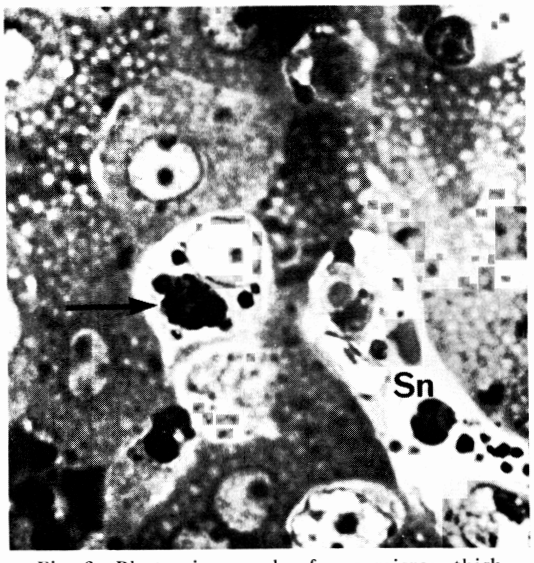

Fig. 6 Photomicrograph of one micron thickness section of the liver from an infected mouse on day 6 after parasite inoculation. A degenerated hepatocyte containing clumped malaria pigment is seen (arrow). Sn; sinusoidal lumen, Magnification, ×1400.

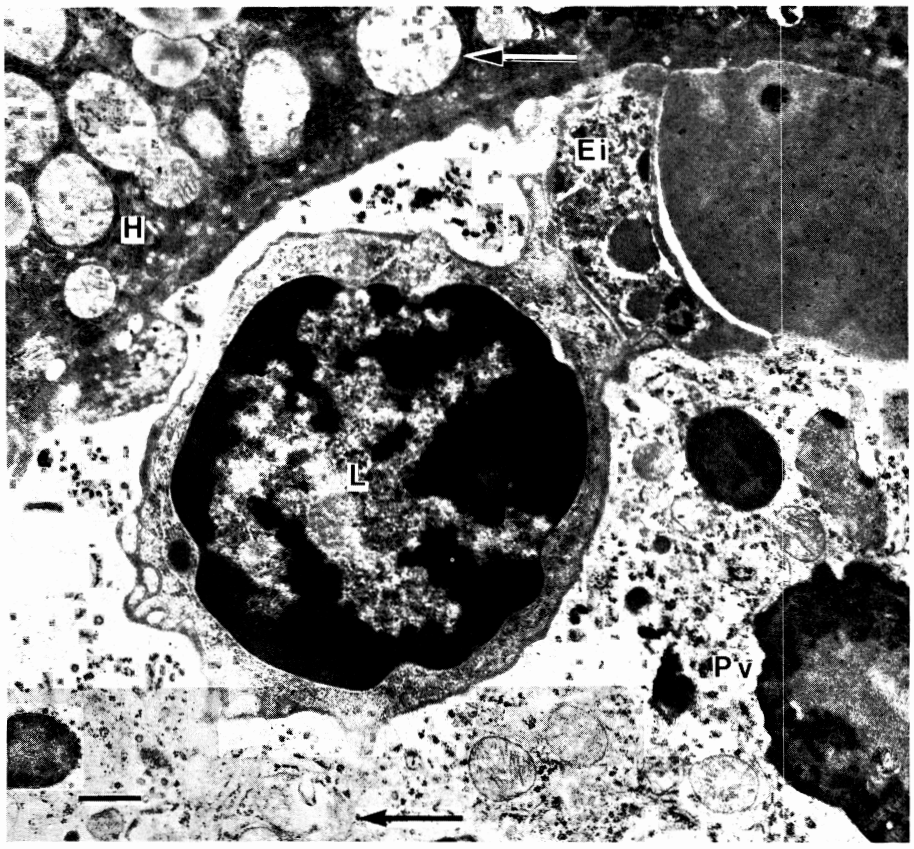

Fig. 7 Electron micrograph of the liver from a *P. berghei* infected mouse. In the lumen of sinusoid, a lymphocyte (L) extrudes a protrusion which covers surface of an infected erythrocyte (Ei). A huge Kupffer cell surrounding the lymphocyte contains several pigment vacuoles (Pv). The cytoplasm of Kupffer cell manifests extremely lowered electron density. Some of the mitochondria in Kupffer cell and in the hepatocyte (H) degenerated (arrow). The bar is equivalent to 1 μm ($\times$7650).

soids were occupied with blood aggregants including infected erythrocytes, monocytes, macrophages and lymphocytes. Vacuoles containing malarial pigments were observed in macrophages, endothelial cells and also in hepatocytes (Fig. 6). Some hepatocytes and phagocytes underwent degeneration manifesting abundant vacuoles. Close contacts between lymphocytes and macrophages harboring malarial pigments were observed. In addition, occasionally, lymphocytes penetrating into hepatocyte line were remarked.

(4) *Electron microscopic observations*: Based on the light microscopic findings, electron microscopic study was undertaken to examine the behavior of lymphocytes in the livers of mice at advanced stage of infection. Specimens from infected mice on day 4 and 6 were studied. In hepatic sinusoids, numerous lymphocytes were found at low magnification. Some of the lymphocytes attached to Kupffer cells with parasites in the phagosomes. A lymphocyte protruding cytoplasmic extension and covering the surface of parasitized erythro-

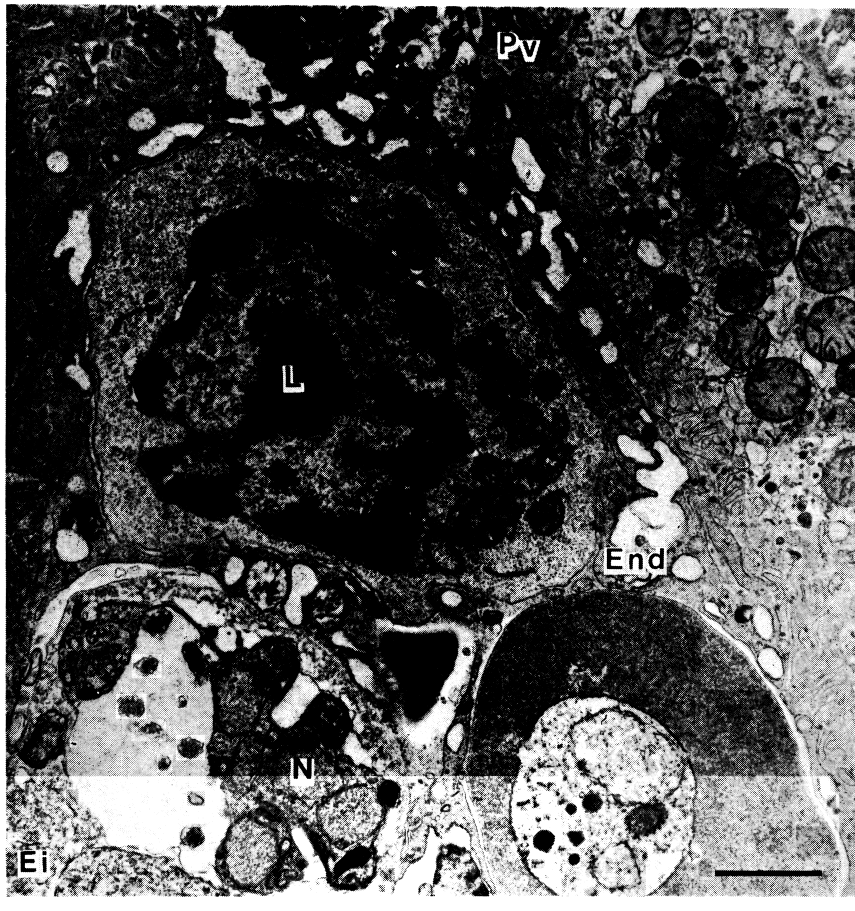

Fig. 8 Electron micrograph of the liver from a *P. berghei* infected mouse. In the lumen of liver sinusoid, a lymphocyte (L) attaches to an endothelial cell (End) with an infected erythrocyte (Ei) in the cytoplasm. The parasite in the erythrocyte has 5 nuclei (N). Structure of erythrocyte hosting the parasite is not preserved. In another part of the endothelial cell, vacuoles containing malarial pigments are seen (Pv). The bar is equivalent to 1 μm ($\times$13700).

cyte was noted (Fig. 7). In this electron microphotograph, a Kupffer cell surrounding the lymphocyte underwent degeneration manifesting lowered electron density of the cytoplasm. Malarial pigments in the degenerated Kupffer cells were prominant. It was also remarked that endothelial cells with parasitized erythrocytes were occasionally found in association with attachment of lymphocytes (Fig. 8). Lymphocytes were detected also in the Disse spaces. As was suggested by light microscopic examination, lymphocytes penetrating into hepatic cell line were confirmed by some electron microphotographs. In such instance, very close apposition of membranes between the attached lymphocyte and hepatocyte was observed in one electron microphotograph.

Discussion

In the present study thymus atrophy (Fig. 1) and reduction of T lymphocytes

in the involved thymus (Fig. 2A) were demonstrated as the malaria infection progressed. Destruction of lymphocytes in the organ by some cytotoxins may cause thymus atrophy. If malaria infection worked as a kind of nonspecific stress on the host, sudden excess secretion of corticosteriod might impair lymphocytes in the thymus, causing atrophy of the tissue. However, the structure of thymuses from *P. chabaudi* infected mice did not present any evidence that supported destruction of thymocytes; rather depletion of thymocytes and loss of distinction between the cortex and medulla were shown (Aikawa *et al.*, 1980). The finding was also true in *P. berghei* infection. The involved thymus consisted mostly of connective tissue and reticular cells, and thymocytes were extremely decreased. These observations suggested that thymocytes were not destroyed due to a kind of stress caused by malaria infection. Atrophy of thymus associated with malaria appeared to be the result of lymphorrhexis. The increased T lymphocytes in the circulating blood (Table 1) supported this view.

The increase of T lymphocytes in the livers of mice during the course of infection was noted (Table 1). The liver has a large network of blood vessels; the fact would allow a speculation that the increase of T lymphocytes was simply a passive reflection of increased T lymphocytes in the circulation. Therefore, the number of T lymphocytes was examined in the lungs where a large volume of blood also circulates. However, no remarkable increase of T lymphocytes in the lungs was observed (Table 1). The results showed that T lymphocytes accumulated selectively in the liver. The finding was comparable with the observation by Dockrell *et al.* (1980). They suggested the protective role of T lymphocytes in the liver and considered that *P. yoelii* parasites were largely destroyed in the liver effected by the accumulated lymphocytes.

We would like to propose another role of T lymphocytes in malaria from the previous and the present findings. One of authors (Waki and Suzuki, 1977) previously documented that survival time of athymic nude mice remarkably shortened in *P. berghei* infection when the nude mice were reconstituted by thymus cell transfer. This finding suggested that some immunopathological reaction in infected mice might be mediated by thymus cells. In another study (Suzuki *et al.*, 1980), it was found that accumulation of Thy-1 (+) cells was remarkable in the liver in case mice were infected with virulent *P. berghei* (NK65). While Thy-1 (+) cells in the livers from mice inoculated with an attenuated *P. berghei* (NK65 XAT), a permanent mutant from virulent NK65 strain (Waki *et al.*, 1982), showed very modest infiltration. The finding would reason the speculation that T lymphocytes might exert adverse reactions in the liver rather than protective reactions. Hence the structure of the involved liver was studied.

Fixation of the liver tissue by acetone-ethanol mixture made feasible to demonstrate the presence of T lymphocytes in the permanent liver specimens by IFAT. The fixation method preserved Thy-1 antigenicity (Fig. 4), although the fixative gave a considerable histological distortion of the liver tissue. By using this fixative, the complete correlation between Thy-1 (+) cells seen by IFAT and lymphocytes observed in hematoxylin and eosin staining was attempted (see Materials and Methods). Immunofluorescent studies followed by histological observation on exactly same specimens revealed that the majority of lymphocytes in the liver tissue of infected mice were T lymphocytes. The coincidence between lymphocytes and Thy-1 (+) cells in the liver thus studied presented an evidence that lymphocytes in the liver observed in one micron thickness sections and electron micrographs were T lympho-

cytes, although the definite conclusion will be given in the future studies using the enzyme-labelled or the ferritin-labelled antibody technique. Studies on one micron thickness sections of the liver tissue strongly suggested that lymphocytes penetrated from sinusoid into parenchymal cells of the liver. Electron microscopic studies provided further details of lymphocyte behavior in the liver tissue in relation to malaria parasite. Except sinusoidal lumina, lymphocytes were found in the Disse spaces and even between two hepatocytes in the tissue. Lymphocytes sometimes kept close proximity with erythrocytes harboring malaria parasites. Indeed, one electron micrograph clearly demonstrated a prominent cytoplasmic protrusion extended from a lymphocyte toward an infected erythrocyte (Fig. 7). The lymphocytes in the liver occasionally located very close to endothelial cells containing infected erythrocytes (Fig. 8). The findings may provide a speculation that some of T lymphocytes infiltrated into parenchymal tissue from the sinusoidal spaces because of their special affinity to liver cells engulfing malaria parasites. Sensitized T lymphocytes of the same haplotype evoked destruction of target cells containing lymphocytic choriomeningitic viruses (Doherty and Zinkernagel, 1974). The finding has been extensively studied in many systems of intracellular microorganisms. If such events are true in the livers of *P. berghei* infected mice, T lymphocytes might cause detrimental reaction to the host cells in which malaria parasites were taken. The parasites in the host cells were then exposed to immune effects of the host. Two aspects of the reactions (Suzuki and Waki, 1978) by T lymphocytes, one detrimental reaction to the host and another killing effect to the parasites, were suggested by the present study.

Summary

Behavior of T lymphocytes with special attention to immunopathologic reactions in *Plasmodium berghei* infected mice was examined. Cytotoxic test and indirect fluorescent antibody test (IFAT) using anti-Thy-1 serum revealed that Thy-1 (+) cells were released from thymus, circulated in the blood and selectively located in the liver as the disease progressed. Correlation between lymphocytes and Thy-1 (+) cells was attempted by combining IFAT followed by hematoxylin and eosin staining on exactly same liver specimens. By the technique, it was revealed that most lymphocytes in the involved liver consisted of T lymphocytes. Histological and electron microscopic observations showed that the lymphocytes in the liver localized not only in hepatic sinusoids but also in the Disse spaces and even between hepatocytes. Specific affinity of the lymphocytes with infected erythrocytes or endothelial cells containing parasitized erythrocytes was suggested. These findings will allow a speculation that T lymphocytes work as effector cells against the host cells harboring malaria parasites and that the lymphocytes exert some detrimental reaction both to the host cells as well as to parasites in the cells.

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マラリア感染マウスにおけるT細胞の肝臓への集積

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マラリア感染に際し，生体に障害的に作用する胸腺細胞ないしはT細胞が存在することがヌードマウスを用いた感染実験により立証されている．そこで，マウスマラリア実験モデルを用いてT細胞の生体内における動態を追求した．

マウス（BALB/C）にマラリア原虫 *Plasmodium berghei*（NK65）を接種し，胸腺，脾，肝，肺，血液中の Thy-1 陽性細胞（T細胞）数の経時的推移を，免疫細胞溶解反応及び間接螢光抗体法により観察した．感染の進行に従って，胸腺，脾臓からの Thy-1 陽性細胞が流血中に放出され，終局的に肝臓内に集積することが観察された．全く同一の肝組織切片を間接螢光抗体法とヘマトキシリン・エオジン染色法を組合せて観察したところ，肝に集積しているリンパ球の大部分は Thy-1 陽性細胞であることが示された．

肝組織内におけるTリンパ球の免疫病理学的動態を追求するため，肝組織におけるリンパ球について光学顕鏡及び電子顕微鏡を用いて観察した．その結果，リンパ球は洞様毛細血管中はばかりでなくディッセ腔，更に肝細胞間にも認められた．またマラリア感染赤血球，感染赤血球を含んだ毛細血管上皮細胞及びKupffer細胞等とリンパ球の密接な接着像が観察された．これらの知見により，肝においてT細胞がマラリア原虫をとりこんだ宿主細胞に対して作動細胞となり，宿主細胞中のマラリア原虫ばかりでなく，マラリア原虫を含む宿主細胞に対しても障害的に作用する可能性が示唆された．