Mesangial proliferative glomerulonephritis in murine malaria parasite, *Plasmodium chabaudi* AS, infected NC mice

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Abstract

Background Malaria is an important tropical disease and has remained a serious health problem in many countries. One of the critical complications of malarial infection is renal injury, such as acute renal failure and chronic glomerulopathy. Few animal models of nephropathy related to malarial infection have been reported. Therefore, we developed and investigated a novel malarial nephropathy model in mice infected by murine malaria parasites.

Methods NC mice and C57BL/6J mice were infected with ~~T~~two different murine malaria parasites, *Plasmodium (P.) chabaudi* AS and *P. yoelii* 17X. After the infection, renal pathology and blood and urinary biochemistry were analyzed.

Results NC mice infected by the murine malaria parasite *P. chabaudi* AS, but not *P. yoelii* 17X, developed mesangial proliferative glomerulonephritis with endothelial damage and, decreased serum albumin concentration and increased proteinuria. These pathological changes were accompanied by deposition of immunoglobulin G and complement component 3, mainly in the mesangium until day 4 and in the mesangium and glomerular capillaries from day 8. On day 21, renal pathology developed to focal segmental sclerosis according to light microscopy. In C57BL/6J mice, renal injuries were not observed from either parasite infection.

Conclusion The clinical and pathological features of *P. chabaudi* AS infection in NC mice might be similar to quartan malarial nephropathy resulting from human malaria parasite *P. malariae* infection. The NC mouse model might therefore be useful in analyzing the underlying mechanisms and developing therapeutic approaches to malaria-related nephropathy.

Key words: animal model・glomerulonephritis・malarial nephropathy・NC mice・nephrotic syndrome

Introduction

Despite efforts to eradicate or control malaria, the disease remains a major public health problem in countries in the tropical and subtropical regions of the world. Approximately 40% of the world's population lives in areas where malaria is transmitted. Approximately 300-500 million cases of infection with malaria parasites occur every year [1]. Four pathogenic species of malaria parasite, *Plasmodium (P.) vivax*, *P. ovale*, *P. malariae* and *P. falciparum*, infect humans and are responsible for approximately 800,000 deaths globally every year due to severe life-threatening complications, such as cerebral malaria, severe anemia, acidosis, respiratory distress, jaundice, acute renal failure, and acute respiratory distress syndrome.

The kidney is one of the critical target organs in malaria, and renal failure contributes to the poor prognosis for malaria [1]. Renal involvement has been reported mainly with *P. falciparum* and *P. malariae* infections [2]. Acute renal failure (ARF) in *P. falciparum* patients and chronic and progressive glomerulopathy in *P. malariae* patients (quartan malarial nephropathy) are the two major renal disorders associated with malaria [1, 2, 3]. Although the pathology of human malarial nephropathy has various sequelae, such as ARF and chronic kidney diseases, most patients with malaria parasite infection in developing countries who die due to lethal complications of multiple-organ failure involving ARF do not undergo pathological examination [4]. Several causal factors, such as immune complexes, cytokines, humoral factors and hemodynamic factors, have been reported to be involved in the pathogenesis of malaria nephropathy [1, 2, 3]. In cases involving the formation of immune complexes, involvement of immunoglobulin (Ig)M, IgG, complement component 3 (C3), and IgA were has been observed in mesangial and subendothelial areas, with IgM complexes predominating and IgA complexes appearing only rarely [1]. Other factors are characterized by endothelial damage due to the effects of cytokines and hemodynamic factors [1]. However, the mechanisms underlying malarial renal failure remain unclear.

Improved treatment of renal damage is important to reduce malaria mortality. Murine

models are useful for analyzing the mechanisms of malaria renal pathology and developing new therapeutic strategies against malaria-associated renal damage. Few studies on renal damage in mice infected with rodent malaria have been reported. The main pathological change in mice infected with *P. berghei* was acute glomerulonephritis (GN) [45]. However, there have been few reports on chronic glomerulopathy with nephrotic syndrome as observed in African children with *P. malariae* infections. The need for another model of malarial nephrosis led us to survey novel animal models that might prove useful for further research into human malaria nephrosis.

The present study describes renal injuries in *P. chabaudi* AS-infected NC mice as a new model of malaria-related nephropathy.

Materials and Methods

Animals

NC and C57BL/6J (B6) mice were purchased from CLEA Japan (Tokyo, Japan). The mice were provided *ad libitum* access to commercial diet and water, and bred in a pathogen-free facility at the Institute for Laboratory Animal Research, Graduate School of Medicine, Nagoya University. Animal care and all experimental procedures were approved by the Animal Experiment Committee at the Graduate School of Medicine, Nagoya University, and were conducted according to the Regulations on Animal Experiments of Nagoya University.

Malaria parasites, infection and animal experimental protocol

Rodent malaria parasites *P. chabaudi* AS and *P. yoelii* 17X were stored as frozen stock at -80°C. Freshly thawed parasites were passaged once in mice, and 8-week-old female mice were infected by intraperitoneal injection of 1 $\times 10^6$ parasitized erythrocytes from passaged mice. Mice were sacrificed at 21 days post-infection to examine renal pathology and/or laboratory data. Since *P. chabaudi* AS-infected NC mice developed glomerular damage (described in the Results section below), the present study focused on this parasite-mouse model. To analyze the

temporal course of renal damage, *P. chabaudi* AS-infected NC mice were sacrificed at 4 days (n=4), 6 days (n=4), 8 days (n=4), 10 days (n=4), 14 days (n=5) or 21 days (n=3) post-infection for pathology and samples from the same mice at 4 days, 6 days, 8 days and 10 days post-infection were used for immunohistochemistry. Samples were taken from the same animals to be examined for pathology at 4 days, 6 days, 8 days, and 10 days post-infection for analysis of blood and urine biochemistry. Parasitemia (measured as the percentage of parasite-infected erythrocytes) was counted on methanol-fixed Giemsa-stained tail-blood smears on glass-slides. For control data, results from 5 untreated NC mice were used.

Analysis of blood and urine biochemistry

Urine samples were collected on culture dishes by gentle digital pressure to the caudal abdomen of *P. chabaudi* AS-infected NC mice. Blood samples from the right atrium were collected in EDTA-tubes under deep anesthesia. Erythrocyte count, the levels of serum albumin (Alb), creatinine (Cre), C3, blood urea nitrogen (BUN), total cholesterol (T-cho) and triglyceride (TG) in tail-blood samples and of urine Cre and total protein were measured by Mitsubishi Pharma (Nagoya, Japan). Protein excretion in urine was estimated as urine protein (UP)/urine Cre.

Histology, immunofluorescence (IF) and electron microscopy (EM)

For analyses under light microscopy, kidney tissues were fixed in 10% buffered formalin overnight and embedded in paraffin. Sections of 2-μm thickness were stained with periodic acid Schiff or periodic acid-methenamine silver. To investigate glomerular pathological changes such as mesangial proliferation, mesangiolysis, formation of adhesions and/or crescents, and formation of thrombosis in glomerular capillaries, we observed 20 glomeruli in a horizontal slice of each mouse kidney and calculated the ratios for individual pathological changes. Mesangial proliferation was defined as >4 cells in a mesangial area in the glomerulus. To

estimate segmental sclerosis in 20 glomeruli from each mouse under ×630 magnification, a semi-quantitative scale was used: 0, none; 1, sclerosed area comprising <25%/glomeruli; 2, 25-50%; 3, 50-75%; and 4, >75%. The mean value for from 20 glomeruli was used for mesangial sclerosis. To analyze tubular injuries such as loss of proximal tubules, vacuolation and/or detachment of tubular epithelial cells, interstitial pathologies were semi-quantitatively divided from 0 (no change) to 4 (diffuse pathological changes under a field), with observation of 10 random fields under ×400 magnification. The mean value was used for interstitial pathologies in each animal.

For studies using IF, kidney tissues were embedded in OCT compound, snap-frozen in liquid nitrogen, cryostat-sectioned at a thickness of 2 μm, and fixed with acetone for 10 min at room temperature. To examine IgG or C3 deposition, tissue sections were incubated with FITC-labeled goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, Philadelphia, PA) or FITC-labeled goat anti-rat C3 cross-reacted against mouse (Cappel), respectively.

Sections for EM were fixed with glutaraldehyde, postfixed with osmium tetraoxide, and embedded in Epon 812 (Nisshin EM, Tokyo, Japan). Ultrathin sections were viewed using a H7100 electron microscope (Hitachi, Ibaraki, Japan).

Statistical analysis

Results are provided as the mean ± SD. Statistical analysis was performed using the Kruskal-Wallis test and unpaired t-test. P values less than 0.05 (5%) were considered significant.

Results

Histology of *P. chabaudi* AS- and *P. yoelii* 17X-infected NC and B6 mice

At 21 days post-infection, *P. chabaudi* AS-infected NC mice showed diffuse mesangial proliferation with focal rupture of glomerular capillaries (Fig. 1A), but *P. chabaudi* AS infection did not induce any pathological changes in the kidneys of B6 mice (Fig. 1B). At 21 days

post-infection, no pathological changes were observed in the kidneys of *P. yoelii* 17X-infected NC and B6 mice (Fig. 1C and 1D). Therefore, this study focused on *P. chabaudi* AS infection in NC mice.

Temporal course of pathological changes in *P. chabaudi* AS-infected NC mice

At 4 days post-infection, a slight segmental and focal increase in mesangial cells became apparent in *P. chabaudi* AS-infected NC mice (Fig. 2D to 2F), compared with untreated NC mice (Fig. 2A to 2C). Diffuse and global mesangial proliferation was clearly seen at 8 days post-infection (Fig. 2G). At 14 days post-infection, segmental and focal mesangiolysis with thrombi or fibrin exudation in glomerular capillaries (Fig. 2M to 2O) and an increase in epithelial cells were observed (Fig. 2M). At 21 days post-infection, focal mesangiolysis expanded to segmental glomeruli, epithelial cell proliferation was still seen, and double contours of glomerular capillary walls were observed accompanied by fibrin exudation in glomerular capillaries (Fig. 2P to 2R, and 3E). Fig. 3A provides a summary of the temporal course of pathological changes in glomeruli as semi-quantified estimations.

On days 4 and 6 after infection, minimal detachment of the tubular brush border of tubular epithelial cells was observed (Fig. 2E and 2H). The temporal course of ~~the~~ interstitial injures is shown as semi-quantitative estimations in Fig. 3F. The appearance of tubular injuries peaked on day 10 after infection (Fig. 3F). During our observations, cast formation by erythrocytes infected with the parasite could not be clearly observed in the tubules.

Deposition of IgG and C3 in *P. chabaudi* AS-infected NC mice

Although mesangial deposition of IgG was observed in most glomeruli in untreated NC mice (Fig. 4A), slight C3 deposition was ~~fairly~~ recognized in the mesangium of glomeruli (Fig. 4B). The most significant depositions of both IgG and C3 w~~as~~ere observed in the mesangial area of *P. chabaudi* AS-infected NC mice at 4 days post-infection (Fig. 4C and 4D). At 6, 8 and 10

days post-infection, degree of ~~the~~ mesangial IgG deposition was slightly decreased (Fig. 3E, 3G and 3I). The C3 deposition pattern decreased in a ~~similarly~~ similar manner to the mesangial IgG pattern on day 10 after *P. chabaudi* AS-infection (Fig.4F, 4H and 4I) [6].

EM findings in P. *chabaudi* AS-infected NC mice

Glomeruli of *P. chabaudi* AS-infected NC mice were examined ~~by~~ under EM at 6 and 21 days post-infection (Fig. 5). Mesangial deposition was clearly seen on day~~s~~ 6 (Fig. 5C) and day 21 (Fig. 5~~C and 5~~E~~, respectively~~). Extensive effacement of podocyte foot process~~es~~ was also observed along the glomerular basement membrane. Of note, ~~small~~ many small, thick, dense deposits were observed along glomerular subepithelial cells on day 6 (Fig. 5A and 5B) and ~~they~~ were decreased on day 21 (Fig. 5D). These dense granular deposits might ~~be~~ represent ~~dense~~granules ~~which were~~ derived from the malaria parasites [7, 8]. ~~Red blood cells~~Erythrocytes filled with malaria parasites were seen inside the glomerular capillaries on day 6 (Fig. 5A). Because *P. chabaudi* AS-infected ~~red blood cell~~erythrocyte~~s~~ had already ~~were~~ decreased to very few or ~~under~~ below the limit of detection in the systemic circulation ~~on~~ by day 10 (Fig. 6A), with ~~and~~ no detection on day 21 (data not shown), we could not observe ~~the~~ parasite infected ~~red blood cell~~erythrocyte~~s~~ in glomeruli under EM ~~findings~~.

Clinical parameters of *P. chabaudi* AS-infected NC mice

Parasitemia in *P. chabaudi* AS-infected NC mice increased~~,~~ until~~peaked at~~ 6 days post-infection, ~~and~~ then decreased (Fig. 6A). On day 21, ~~the~~ no parasitemia was ~~not observed~~evident in living animals (data not shown). As a consequence of the parasitemia, the count of circulating ~~red blood cell~~erythrocyte~~s~~ ~~of~~ in infected mice was decreased ~~on~~ at 6 days ~~of~~post-infection, ~~was~~ continuing at a low level~~s~~ up to 8 days ~~of~~ post-infection~~, and then began~~before starting ~~–~~to increase again (Fig. 6B). In contrast, serum levels of C3 decreased significantly but transiently to 6 days post-infection ~~and then~~before ~~recovered~~ recovering (Fig.

6C).

To investigate renal function and urinary protein excretion in *P. chabaudi* AS-infected NC mice, BUN was measured. A non-significant tendency toward ~~and found to begin to i~~increases was evident ~~at~~ from 10 days post-infection~~, but this increase was not statistically significant~~ (Fig. 7A). Likewise, s~~S~~erum Cre did not change significantly in infected mice up to 10 days (Fig. 7B). ~~There was also a~~A continual post-infection decrease in serum Alb was also seen ~~post-infection~~ (Fig. 7C). To examine urinary protein excretion, UP/U Cre was measured and was seen ~~found~~ to ~~begin to~~start ~~increase~~ increasing from ~~at~~ 8 days post-infection (Fig. 7D). On day 10, increased ~~of~~ serum levels of T-cho and TG supported ~~to~~ the existence of nephrotic conditions (Fig.7E and 7F).

Edema and ascites began to be seen in *P. chabaudi* AS-infected NC mice at 10 days post-infection and became distinct at 14 days post-infection (Fig. 8). A~~fter that, a~~nimals subsequently fell into a cachectic condition and some ~~of them were~~ died within 21 days (data not~~w~~ shown).

Discussion

Quartan malaria nephropathy due to *P. malariae* infection is generally caused by immune~~-~~ complex~~-~~mediated GN, typically leading to nephrotic syndrome [1, 2, 3]. As described in an~~o~~other report~~s~~, ~~a different~~various malaria parasite~~s can induce~~~~d~~ interstitial nephritis similar to the interstitial injuries seen with *P. falciparum* malaria [9-11]. However, animal models for each type of malaria ~~are~~ remain ~~very~~ quite limited~~, concerning with malaria parasite infection~~. Here, we have presented an animal model of mesangial proliferative GN-like glomerulopathy in ~~mouse~~ mice ~~which was~~ induced by infection ~~of one~~with the ~~of~~ malaria parasite species~~,~~ *P. chabaudi* AS~~-~~, and accompanied ~~with~~ by severe proteinuria and edema as a nephrotic syndrome. The~~ presentis~~ model ~~proceeded~~ eventually produced sclerotic lesions in glomeruli~~ later~~.

~~Light microscopic e~~Examination of kidney tissues ~~of~~ from *P. chabaudi* AS-infected NC

mice under light microscopy generally showed diffuse mesangial proliferation in the early stages (Fig.1 and 2). ~~Immunofluorescence~~ IF findings supported observations under light microscopy,~~LM study with~~showing severe deposition of IgG and C3 in the mesangial area (Fig. 4). These pathological findings in *P. chabaudi* AS-infected NC mice ~~are~~ essentially ~~identical to~~match those observed in human mesangial proliferative GN. On day 21, ~~electron microscopy~~EM demonstrated that the presence of mesangial deposits ~~were~~ was attenuated. Interestingly, electron-dense granules were observed along epithelial cells under EM. ~~It~~ These granules might be similar to the dense granules which were reported as derivatives of secretory organelles from malaria parasites (Fig. 5). Serum and urine parameters in *P. chabaudi* AS-infected NC mice ~~we~~resembled ~~similar to~~ those seen as ~~in~~ clinical manifestations of m~~m~~esangial proliferative GN. Therefore, *P. chabaudi* AS-infected NC mice ~~are~~ seem to offer a novel animal model ~~for~~ of mesangial proliferative GN ~~following~~ followed by focal segmental sclerotic GN, ~~later~~ and may be ~~an~~ expected to offer insights into some forms of malaria-related nephropathy. ~~For early stage of the present animal model, infected~~ The malaria parasites themselves might induce glomerular injuries in the early stages of the present animal model, because the ~~existence~~ presence of parasites ~~were~~ was confirmed ~~as~~ in infected ~~red blood cell~~erythrocytes under EM.

Taylor-Robinson provided the~~The sole~~ only previous report of a mouse model ~~for~~ of quartan malaria nephropathy ~~was by Taylor Robinson~~ [12]. GN with nephrosis was induced by chronic *P. chabaudi* AS infection of NIH mice with artificially depleted $CD4^+$ lymphocytes, although only about 10% of infected mice ~~were~~ developed ~~nephrotic~~nephrosis. In contrast, all *P. chabaudi* AS-infected NC mice in the present study exhibited ~~a~~ nephrotic syndrome. Quartan malaria nephropathy proceeds to renal failure even after successful eradication of the malaria parasite. Glomerular damage increased in *P. chabaudi* AS-infected NC mice in the present study until 21 days post-infection, ~~although~~ despite almost complete elimination of the parasite ~~was almost eliminated~~. ~~Therefore,~~ *P. chabaudi* AS-infected NC mice thus appear~~ed~~ to ~~be~~ represent a

~~new~~ useful animal model with GN ~~and~~ for ~~the~~ malaria parasite ~~–~~infected nephropathy. In untreated NC ~~strain of~~ mice, ~~a little of~~slight focal and segmental mesangial proliferation might be observed, ~~as the back-ground of develop mesangial GN-like pathological changes~~ with IgG deposition in the mesangial area as a natural background renal pathology ~~of NC strain mice~~. However, significant C3 deposition was not observed in the mesangial area of untreated NC mice. ~~Therefore, c~~Complement activation might thus be related to the development of ~~the~~ mesangial proliferative GN ~~by the~~following parasite infection, because glomerular tissue injuries could ~~give~~ provide an opportunity ~~to~~ for ~~induce~~ induction of complement activation [13].

In the present study, *P. chabaudi* AS-infected NC mice exhibited mesangial proliferative GN. However, ~~a~~an~~yo~~ specific renal ~~injury~~ injuries w~~as~~ere ~~not~~ observed in *P. chabaudi* AS-infected B6 mice or in *P. yoelii* 17X-infected NC ~~and~~ or B6 mice. In a preliminary study, we infected several mouse strains other than NC and B6 with *P. chabaudi* AS, but no nephropathy was observed in ~~the~~ any of those ~~other~~ mouse strains. ~~These~~ Those results suggested that mesangial proliferative GN with nephrosis only occurred when NC mice were infected by *P. chabaudi* AS. NC mice ~~were~~ have been reported ~~to be~~as a model for atopic dermatitis [14]. *P. yoelii* 17XL-infected NC mice also exhibit ~~an~~ acute increases in parasitemia compared with other mouse strains [15]. ~~These~~ Such results have been suggested to be related to immunological defects in NC mice [15]. Mesangial proliferative GN may be a consequence of specific interactions between the immunological defects ~~of~~ in NC mice and the *P. chabaudi* AS parasite.

In human~~s~~ after severe malarial infection, ~~typically~~ renal injuries typically ~~occurs~~ arise in the form of~~as~~ acute renal failure related to ~~because of~~ multiple ~~–~~organ failure~~s~~ [45]. In fact, some animals ~~of the~~in other mouse strain~~s~~ ~~were lethal~~die within ~~two~~ 2 weeks after infection of by ~~the~~ malaria parasites [16]. On the other hand, ~~T~~~~there were~~ little evidence~~s~~ of chronic renal injuries related to infection ~~infected~~ with malaria parasites has been accumulated in humans.

However, the incidence of ~~it is predicted to spread~~ malarial infection is predicted to spread ~~in~~ around the world ~~because of~~due to global warming. In the ~~Near~~ near future, our model might ~~be~~ prove useful to analyze the underlying mechanisms and ~~to~~ develop new therapies for malarial renal injuries. ~~In future, s~~Similar pathological changes might be found in association with human renal injuries infected ~~with~~ resulting from infection with some malaria parasites.

The present results suggest~~ed~~ that *P. chabaudi* AS-induced mesangial proliferative GN in NC might ~~be~~ offer a unique model ~~for~~ of malaria nephropathy, although the detailed mechanisms ~~to~~ behind the development of the observed pathologies ~~we~~remain unclear ~~unknown~~. This model might be useful for analyzing the mechanisms of malaria nephropathy and developing new anti-malaria therapies.

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Conflict of interest

The authors have declared that no conflict of interest exists.

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Figure legends

Fig. 1 Glomeruli in mice 21 days after ~~murine~~ infection with two different species of malaria ~~infection~~parasite~~.~~

(A) Glomerulus of a *Plasmodium~~.~~ chabaudi* AS-infected NC mouse. **(B)** Glomerulus of a ~~*Plasmodium (*~~*P.~~)~~ chabaudi* AS-infected B6 mouse. **(C)** Glomerulus of a *P. yoelii* 17X-infected NC mouse. **(D)** Glomerulus of a *P. yoelii* 17X-infected B6 mouse. Original magnification, ×630.

Fig. 2 ~~Time~~ Temporal course of ~~glomeruli changes~~ renal pathology in *P. chabaudi* AS-infected NC mice under light ~~microscopic~~ microscopy~~findings.~~

(A-C) Untreated NC mice. **(D-F)** NC mice 4 days after infection with *P. chabaudi* AS. **(G-I)** NC mice 8 days after infection with *P. chabaudi* AS. **(J-L)** NC mice 10 days AS. (**M -O)** NCmice14 days after infection with *P. chabaudi* AS. **(P-R)**NC mice 21 days after infection with *P. chabaudi* AS. ~~Kidney tissue was examined in untreated NC mice; (A), (B) and (C), untreated, on 4 days; (D), (E) and (F), 8 days; (G), (H) and (I), 10 days; (J), (K) and (L), 14 days; (M), (N) and (O), and 21 days post infection; (P), (Q) and (R).~~ Arrows in (E) and (F) mark segmental and focal mesangiolysis. Arrowheads in (E) and (F) ~~mark~~ show epithelial cell increases. ~~**Frames**~~ (A), (B), (D), (E), (G), (H), (J), (K), (M), (N), **(P), (Q)** ~~are s~~Stained by periodic acid Schiff. **(C), (F), (I), (L), (O), (R)** ~~and the~~S~~ other frames are~~ tained by periodic acid-methenamine silver. Original magnifications: (A), (C), (D), (F), (G), (I), (J), (L), (M), (O), (P) and (R): ×630; ~~and~~ (B), (E), (F), (H), (K), (N) and (Q): ×200.

Fig. 3 Analysis of mesangial proliferation, mesangiolysis, degree of segmental sclerosis, adhesion/crescent, formation of thrombi in glomeruli and degree of tubular injuries

(A) Mesangial proliferation~~,~~; **(B)** mesangiolysis~~,~~; **(C)** degree of segmental sclerosis~~,~~; **(D)** adhesion/crescent~~,~~; **(E)** formation of thrombi in glomeruli ~~are shown in A, B, C, D and E, respectively.~~; **(F)** ~~D~~degree of tubular injuries ~~is shown in F~~. Data are shown as mean ±SD~~and~~

~~asterisks show significances for untreated~~. The n~~N~~umber of animals ~~are~~ was n=5 for untreated, n=4 for days 2, 6, 8 and 10 post-infection, n=5 for day 14 post-infection, and n=3 for day 21 post-infection. ~~*Asterisks show significances of~~ p<0.05 ~~for~~ vs. untreated mice.

Fig. 4 ~~Time~~ Temporal course of IgG and C3 deposition in glomeruli of *P. chabaudi* AS-infected NC mice

Tissues ~~were~~ examined in untreated mice (A and B), and in infected mice at 4 days (C and D), 6 days (E and F), 8 days (G and H), and 10 days (I and J) post-infection. (A), (C), (E), (G), ~~and~~ (I) show IgG deposition. (B), (D), (F), (H), ~~and~~ **(**J) ~~show~~ C3 deposition. Original magnification, ×400.

Fig. 5 ~~Electron microscopic changes~~ Changes to~~f~~ glomeruli ~~after infection of~~in *P. chabaudi* AS-infected ~~in~~ NC mice under EM~~.~~

~~Frames~~ (A) and (B) ~~show~~ gGlomeruli on day 6 ~~after~~ post-infection ~~of the parasites~~, ~~we~~ clearly ~~observed~~ showing fusion ~~of~~ with foot processes of podocytes and high electron dense deposits along epithelial cells (A) and mesangial deposition (B). ~~Frames~~ (C) and (**D)** ~~show~~ gGlomeruli on day 21 post-infection, ~~supporting~~ revealing decreased ~~of~~ de~~gree~~position of IgG and C3~~depositions~~. (A) shows ~~red blood cell~~erythrocytes infected by malaria parasites in glomerular capillaries (*). (B) ~~and (C)~~ shows basement membrane area of glomeruli. (C) shows ~~and~~mesangial areas of glomeruli~~, respectively~~. Black arrowheads mark high electron dense deposits along epithelial cells ~~which~~ that might ~~be~~ represent dense granules from the malaria parasites (see text). White arrowheads mark putative immune-complex deposits.

Fig. 6 ~~Time~~ Temporal courses ~~of~~ for parasitemia, anemia and ~~level~~ concentration of complement component 3 (C3) in *P. chabaudi* AS-infected NC mice~~.~~

(A) ~~Time Rcourse of r~~atio of parasite-infected ~~red blood cells (RBC)~~erythrocytes~~,~~. (**B)** C~~counts~~

of circulating ~~red blood cell~~erythrocyte count. **(C)** S~~(RBC) and s~~erum concentration of C3 ~~levels in NC mice after *P. chabaudi* AS infection are shown in (A), (B) and (C), respectively. Each d~~Data ~~is~~ are presented as ~~the~~ mean ~~values~~ ±SD, with ~~of~~ n=5 for untreated mice and n=4 for other groups. ~~Asterisks show significances of~~ *p<0.05 ~~for~~ vs. untreated mice.

Fig. 7 Temporal courses for ~~Laboratory~~ laboratory data after *P. chabaudi* AS infection

(A) ~~Time B~~course ~~of b~~lood urea nitrogen (BUN); **(B)** ~~,~~ serum creatinine (Cre); **(C)** serum albumin (Alb); **(D)** urine protein/urine Cre (UP/U Cre) ~~levels,~~; **(E)** serum total cholesterol (T-cho); ~~**and**~~ **(F)** serum triglyceride (TG) ~~in NC mice after *P. chabaudi* AS infection are shown in (A), (B), (C), (D), (E) and (F), respectively. Each d~~Data are presented ~~ai~~s the mean ~~values~~±SD, with ~~of~~ n=5 for untreated mice and n=4 for other groups. ~~Asterisks~~*~~show significances of~~ p<0.05 ~~for~~ vs. untreated mice.

Fig. 8 Appearances of NC mice after *P. chabaudi* AS infection or without infection

~~**Frames (**~~**A**~~**)**~~ Backs of NC mice. ~~**and (**~~**B**~~**)**~~ ~~show back and a~~Abdom~~inal~~ens ~~sides~~ of NC mice~~, repetitively~~. ~~No~~ Unmark~~sed~~ images ~~and arrow heads~~ show an uninfected NC mouse. ~~(no mark) and an~~Arrowheads show clear evidence of edema and ascites in the NC mouse on day 14 after *P. chabaudi* AS infection ~~(arrow heads), respectively~~. ~~The mouse with *P. chabaudi* AS infection was extremely expanded with edema and ascites (arrow heads).~~