

—Review—

Mouse NC/Jic strain provides novel insights into host genetic factors for malaria research

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Abstract: Malaria is caused by *Plasmodium* parasites and is one of the most life-threatening infectious diseases in humans. Infection can result in severe complications such as cerebral malaria, acute lung injury/acute respiratory distress syndrome, and acute renal injury. These complications are mainly caused by *P. falciparum* infection and are major causes of death associated with malaria. There are a few species of rodent-infective malaria parasites, and mice infected with such parasites are now widely used for screening candidate drugs and vaccines and for studying host immune responses and pathogenesis associated with disease-related complications. We found that mice of the NC/Jic strain infected with rodent malarial parasites exhibit distinctive disease-related complications such as cerebral malaria and nephrotic syndrome, in addition to a rapid increase in parasitemia. Here, we focus on the analysis of host genetic factors that affect malarial pathogenesis and describe the characteristic features, utility, and future prospects for exploitation of the NC/Jic strain as a novel mouse model for malaria research.

Key words: cerebral malaria, host susceptibility, malaria complications, mouse NC/Jic strain, rodent *Plasmodium* parasite

Introduction

Malaria is one of the most important infectious diseases affecting humanity. In 2016, approximately 216 million cases of malaria were recorded, and 445,000 deaths due to malaria were reported by the World Health

Organization [95]. The term malaria is a general one that includes diseases caused by five major human-infective *Plasmodium* species: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. *P. falciparum* and *P. vivax* are especially widespread [94]. The outcome of the disease varies with the infective species [10]. *P. vivax* infec-

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Table 1. Models of malaria complications using specific combinations of mouse inbred strains and rodent malaria strains

Organ	Disease	<i>P. berghei</i> ANKA	<i>P. berghei</i> NK65	<i>P. chabaudi</i> AS
Brain	CM	C57BL/6, CBA, NC		
Kidney	GN			NIH, NC
Lung	ALI/ARDS	DBA/2	C57BL/6	

CM, cerebral malaria; GN, glomerulonephritis; ALI/ARDS, acute lung injury/acute respiratory distress syndrome.

tion has a lower mortality rate, whereas *P. falciparum* is the most virulent species and is responsible for the majority of malaria-associated deaths. Severe malaria, which is most frequently caused by *P. falciparum* infection, is associated with life-threatening complications in several tissues and organs including the brain, kidney, and lung [56]. Disease only occurs when the malaria parasites invade and grow inside red blood cells [56]. The clinical outcome of malaria is influenced by complex interactions between parasite factors, host factors, and geographic and social factors [56]. Host susceptibility/resistance genes are important host factors that affect the progress of the disease. For example, several hereditary erythrocyte abnormalities have been found to confer protective effects against malaria [46, 89]; additionally, several gene loci that influence malaria susceptibility/resistance have been reported [34, 84, 89]. Identification of the host genetic factors is important for gaining new insights into the molecular mechanisms underpinning malaria outcome [37, 51, 89].

Plasmodium species that can infect rodents were initially identified in Central African regions. Subsequently, a few species, such as *P. berghei*, *P. chabaudi*, and *P. yoelii*, were investigated in laboratory mice [34, 37, 44, 51, 98]. Infected mice have proved valuable for malaria research, such as in screening candidate drugs and vaccines. Furthermore, mouse inbred strains vary in their susceptibility to rodent malaria infection, indicating the importance of genetic factors in host immune responses and disease pathogenesis [34, 37, 51, 84]. Strictly controlled experimental infection in laboratory mice is a productive approach for dissecting host genetic factors. Each rodent *Plasmodium* species possesses specific features and similarities to human malaria parasites [44, 78, 98]. Depending on the combination of mouse strain and rodent malaria species or strain, different syndromes and outcomes can be produced that mimic the pathological symptoms in human malaria [44, 50]. Establishment of novel distinctive mouse models for severe malaria and

identification of genes associated with specific pathologies by genetic and/or genomic approaches will provide a powerful and valuable means for identifying novel mechanisms and for constructing a platform for malaria pathogenesis [34, 37, 51, 84].

We investigated novel combinations of rodent malaria parasite strains and mouse strains to identify distinctive mouse models of severe malaria. We selected *P. yoelii* 17XL, which has been subjected to comparatively little analysis in pathological studies of infected mice [34], and infected various inbred mouse strains. Unexpectedly, we discovered that the mouse strain NC/Jic, which we obtained from CLEA Japan (Tokyo, Japan), showed the highest level of susceptibility to infection and displayed a rapid increase in parasitemia and a 100% mortality rate [62]. Subsequently, we found that the NC/Jic strain exhibited distinctive disease-related complications after *P. berghei* ANKA or *P. chabaudi chabaudi* AS infection (Table 1). In this review, we describe the characteristic features of NC/Jic mice after infection with a rodent malaria parasite and discuss the use of this strain for dissecting the relevance of host genetic factors to malaria infection.

NC/Jic is a substrain of the NC/Nga strain, which was established as an inbred strain by Dr. Kondo in 1955 at the School of Agricultural Sciences, Nagoya University. The NC/Nga strain has been used as an atopic dermatitis model [54]. After challenge with various environmental allergens, NC/Nga mice display a range of characteristic phenotypes including itching, erythema and hemorrhage, and high titers of immunoglobulin E [41, 54]. Massive infiltration of CD4⁺ T cells and degranulation of mast cells and eosinophils have been observed in skin lesions of NC/Nga mice [54]. These effects in NC/Nga mice closely mimic human atopic dermatitis [41, 54, 55, 79]. This strain has been widely used for development of therapeutic agents against atopic dermatitis. The NC/Nga strain was passed to the Central Institute for Experimental Animals (Kawasaki, Japan) in 1966 and

Table 2. List of loci controlling parasitemia after infection with rodent malaria parasites

Locus	Chromosome	Mouse strain		Strong candidate gene	Rodent malaria strains	References
		Susceptible	Resistant			
<i>Char1</i>	9	C3H/He, SJL	C57BL/6	-	<i>P. c. adami</i> DS	23
<i>Char1</i>	9	C3H/He	C57BL/6	-	<i>P. c. adami</i> DS	8
<i>Char1</i>	9	A/J ^b	C57BL/6J ^b	-	<i>P. c. chabaudi</i> AS	26
<i>Char1</i>	9	SM/J	C57BL/6J	-	<i>P. c. chabaudi</i> AS	45
<i>Pymr</i>	9	NC/Jic	129X1/SvJ	-	<i>P. y.</i> 17XL	62
<i>Char2</i>	8	C3H/He	C57BL/6	-	<i>P. c. adami</i> DS	23
<i>Char2</i>	8	A/J	C57BL/6J	-	<i>P. c. chabaudi</i> AS	24
<i>Char2</i>	8	A/J ^c	C57BL/6 ^c	-	<i>P. c. chabaudi</i> 54X	32
<i>Char3</i>	17	C3H/He	C57BL/6	-	<i>P. c. adami</i> DS	8
<i>Char4</i>	3	AcB54 (A/J) ^b	AcB55 (C57BL/6J) ^b	<i>Pklr</i>	<i>P. c. chabaudi</i> AS	25, 58
<i>Char5</i>	5 ^a	A/J ^c	C57BL/6J ^c	-	<i>P. c. chabaudi</i> 54X	32
<i>Char6</i>	5 ^a	A/J ^c	C57BL/6J ^c	-	<i>P. c. chabaudi</i> 54X	32
<i>Char7</i>	17	A/J ^c	C57BL/6J ^c	-	<i>P. c. chabaudi</i> 54X	32
<i>Char8</i>	11	A/J ^c	C57BL/6J ^c	-	<i>P. c. chabaudi</i> 54X	33
<i>Char9</i>	10	AcB54 (A/J) ^b	AcB55 (C57BL/6J) ^b	<i>Vnn1, Vnn3</i>	<i>P. c. chabaudi</i> AS	25, 58

^a*Char5* and *Char6* are located at different positions on Chr 5. ^bRecombinant congenic strains developed from A/J and C57BL/6J.

^cAdvance intercross line developed from A/J and C57BL/6J.

maintained there as the NC/Jic strain. Later, the strain was passed to CLEA Japan (Tokyo, Japan), and it has been provided from there since 1998. In preliminary analyses, no significant genetic differences or phenotypic differences for atopic dermatitis and susceptibility to malaria have been observed between the NC/Nga and NC/Jic strains.

Parasitemia (Rate of Malaria Parasites in Erythrocytes in the Blood)

Laboratory mice strains show different susceptibilities to parasitemia after rodent *Plasmodium* infection [34, 37, 51]. After infection with lethal types of rodent malaria parasite, such as *P. yoelii* 17XL and *P. chabaudi chabaudi* AS, mice of susceptible strains die due to a high level of parasitemia and severe anemia; in contrast, mice of resistant strains survive infection and finally eliminate the parasites from their bloodstream [37]. Determination of the genes associated with this inter-strain difference in susceptibility to malaria infection may lead to the identification of novel key genes involved in host defense mechanisms. With respect to parasitemia, susceptibility has been shown to have a polygenic mode of inheritance, and it is likely to be complex [26, 34, 37, 51]. Quantitative trait loci (QTL) analyses using *P. chabaudi* and *P. yoelii* infection identified nine loci associ-

ated with parasitemia [8, 23–25, 32, 33, 45, 58, 62]; these loci are summarized in Table 2. A few strong candidate genes have been detected in the *Char4* (*chabaudi* resistance locus 4) and *Char9* (*chabaudi* resistance locus 9) loci [57, 58]; however, causative genes have not been identified for all QTLs.

The *Char1* (*chabaudi* resistance locus 1) locus located on chromosome 9 (Chr 9) is of particular interest. This locus was first reported in QTL analyses of the control of peak parasitemia and survival after *P. chabaudi adami* DS infection of (C3H/He×C57BL/6) F₂ mice and (SJL×C57BL/6) F₂ mice [23]. Another study also detected a major QTL in the same region as *Char1* for peak parasitemia after *P. chabaudi chabaudi* AS infection in (SM/J×C57BL/6J) F₂ mice [45]. A major locus named *Pymr* (*Plasmodium yoelii* malaria resistance), which regulates parasitemia and survival after *P. yoelii* 17XL infection, has been mapped to the *Char1* locus using mice from a (NC/Jic×129X1/SvJ)×NC/Jic backcross [62]. These various findings indicate that *Char1/Pymr* is the major locus controlling parasitemia regardless of rodent malaria species or strain. To evaluate the effect of this locus on parasitemia after infection with different rodent malaria species, we developed the NC.129X1-(D9Mit208-D9Mit279) congenic strain (common name: NC.129X1-*Char1/Pymr*) in which the *Char1/Pymr* locus from 129X1/SvJ strain was introduced into

Table 3. Comparison of mean parasitemia in NC/Jic and NC.129X1-*Char1/Pymr* congenic strains after infection with three rodent malaria parasites

Parasite species and strain	Infection dose ^a	Measured days after infection	NC/Jic (n) Mean ± SD	NC.129X1- <i>Char1/Pymr</i> (n) Mean ± SD
<i>P. yoelii</i> 17XL	1×10 ⁵	5	(11) 38.1 ± 11.4	(13) 18.7 ± 5.2 ^b
<i>P. chabaudi chabaudi</i> AS	1×10 ⁶	6	(9) 21.3 ± 9.2	(9) 8.7 ± 2.9 ^b
<i>P. berghei</i> ANKA	1×10 ⁶	6	(7) 11.9 ± 3.6	(7) 3.0 ± 1.7 ^b

^aNumber of intraperitoneally infected parasitized erythrocytes. ^bSignificantly different ($P < 0.05$) vs NC/Jic (t -test).

the NC/Jic strain. A comparison of the levels of parasitemia after infection with *P. yoelii* 17XL, *P. chabaudi chabaudi* AS, or *P. berghei* ANKA between NC/Jic and the congenic mouse strain (Supplementary Table 1) showed that significantly lower levels were present in the congenic strain (Table 3). Thus, this locus appears to control parasitemia after infection with three rodent malaria species. Each rodent malaria parasite has specific features: *P. yoelii* and *P. berghei* preferentially invade reticulocytes rather than mature red blood cells, and *P. chabaudi* shows no preference for the type of red blood cell, similar to the behavior of the erythrocytic stage of human malaria *P. falciparum* [37]. To date, no other locus has been identified that controls parasitemia after infection with a range of rodent malaria species (Table 2). It has been proposed that the *Char1/Pymr* locus is the essential regulator of host response to parasitic growth. Furthermore, the *Char1/Pymr* locus has been detected in all mouse strains used in QTL analyses (Table 2). This suggests that the susceptibility/resistance allele of the *Char1/Pymr* locus is present not only in specific mouse strains but is also ubiquitously distributed in mouse inbred strains. The causative gene(s) in the *Char1/Pymr* locus remains to be identified; the NC/Jic and NC.129X1-*Char1/Pymr* congenic strain will be of value for studies to identify genes within the *Char1/Pymr* locus and for subsequent functional analyses.

Malaria Complications in the Brain

Cerebral malaria (CM) is the most detrimental complication of *P. falciparum* infection and is the main cause of death in malaria [36, 91]. The clinical symptoms of CM are headache, delirium, seizures, impaired consciousness, and coma [36, 59]. The pathogenesis of CM is multifactorial and complex. The initial event of CM is thought to be the sequestration of infected red blood cells by endothelial cells of the brain microvasculature; several subsequent events, such as inflammation, blood-

brain barrier disruption, and endothelial cell apoptosis, are likely to be involved in CM progression [36, 92]. Since human CM studies are severely limited because of the difficulty of time-course investigation and the impossibility of biopsy, experimental mouse models have been employed to investigate the detailed pathogenesis of CM [16]. Fatal CM occurs in C57BL/6 and CBA mice infected with *P. berghei* ANKA; as a consequence, this system has been widely used as an experimental model of CM [12, 16, 20, 51, 84]. Infected C57BL/6 and CBA mice typically exhibit clinical signs, such as ataxia, paraplegia, seizures, and coma leading to uniform lethality with relatively low parasitemia, by 1 to 2 weeks after infection. By contrast, resistant strains, including BALB/c and DBA/2, do not develop CM but die by 2 to 4 weeks after infection due to severe anemia and hyper-parasitemia [20, 51]. Although human CM and experimental CM mouse models have several aspects in common with regard to disease pathogenesis and host immune responses, they also show a few differences in CM features [38, 84]. C57BL/6 and CBA strain mice infected with *P. berghei* ANKA provide the best and most easily available models for human CM [12, 84]. The existence of CM-susceptible and CM-resistant mouse strains shows that host genetic factors are important in CM pathogenesis. Mouse CM models are valuable for dissecting these host genetic factors in relation to CM outcome.

We infected several inbred mouse strains with *P. berghei* ANKA to screen for experimental CM susceptibility. Infected NC/Jic mice showed neurological symptoms including ataxia, paralysis, and coma at 6 days after infection, and all infected mice were dead by 10 days after infection (Table 4). Blood-brain barrier disruption and brain microvascular congestion were observed at autopsy in the mice (Fig. 1). The clinical and pathological symptoms observed in the infected NC/Jic mice were very similar to those displayed by the established experimental CM models, C57BL/6 and CBA mice. The

Table 4. Mortality at 14 days after infection and mean survival days after *P. berghei* ANKA infection in experimental cerebral malaria (CM)-susceptible and CM-resistant strains

Strain	Type	n	Mortality (%)	Survival days (Mean \pm SD)
NC/Jic	Susceptible	20	100	7.4 \pm 0.9
C57BL/6J	Susceptible	12	100	8.5 \pm 1.0
CBA/N	Susceptible	17	100	9.3 \pm 1.0
NC.129X1-Char1/Pymr	Intermediate	23	69.6 ^b	8.5 \pm 2.0
AKR/N ^a	Resistant	7	14.3 ^b	–
C3H/HeJ ^a	Resistant	8	12.5 ^b	–
A/J	Resistant	10	10 ^b	–
129X1/SvJ	Resistant	18	5.6 ^b	–
BALB/c	Resistant	10	0 ^b	–
DBA/2 ^a	Resistant	17	0 ^b	–

^aData cited from our previous report [63]. ^bSignificantly different ($P < 0.05$) vs NC/Jic (chi-square test).

availability of this additional experimental CM model will provide increased opportunities for comparing pathophysiology among CM models and will extend opportunities for identifying genes or regulators associated with susceptibility to experimental CM.

Approximately ten loci for CM susceptibility after *P. berghei* ANKA infection have been identified [1, 4, 6, 61, 63, 83]. Linkage analyses of four independent crosses between C57BL/6 (CM susceptible strain) and four CM-resistant strains (DBA/2, WLA, BALB/c, and 129S1) have been carried out, and eight loci associated with CM susceptibility have been identified [1, 4, 61, 83]. However, these were all unique loci, in contrast to the situation for parasitemia [84]. Similar linkage analyses using other CM-susceptible strains, such as CBA and FVB, have also been performed [4, 63], and the mapped loci in these studies do not overlap with those identified in the C57BL/6 analysis [84]. Although we conducted a QTL analysis to detect NC/Jic-derived CM-susceptible loci, we did not identify any statistically significant QTLs (data not shown). These findings may indicate that susceptibility to experimental CM is not controlled by strong and ubiquitous loci but rather is under the control of strain-specific loci with relatively small effects. Currently, no causative genes or strong candidate genes for experimental CM have been identified by conventional approaches based on forward genetics [84]. Consequently, it may be difficult to identify the causative genes for experimental CM by this method.

C57BL/6, a strain used in a wide range of experimental investigations, is CM susceptible following infection with *P. berghei* ANKA. Many spontaneous mutations and gene knockouts have been established on the C57BL/6 genetic background. Comparison of survival

periods after *P. berghei* ANKA infection between C57BL/6 mice carrying gene mutations or modifications and normal C57BL/6 mice can be used to identify critical genes for the pathogenesis of CM. This reverse genetics approach has been exploited in various studies, and approximately 80 genes associated with CM after *P. berghei* ANKA infection have been detected [84]. The majority of these genes are involved in immune and/or inflammatory cell functions. Early proinflammatory responses, including Th1-type immune responses, have been demonstrated to be an important component of experimental CM pathogenesis [5, 84]. The accumulation of knowledge by this approach will provide valuable insights into the pathways of experimental CM pathogenesis. However, it is possible that the information on experimental CM from the C57BL/6 strain may include findings that are unique to this strain and therefore are not generally applicable to experimental CM in mice. Therefore, it will be important to confirm these findings using other CM models such as the NC/Jic strain.

Complement activation is consistently observed in severe malarial infections in humans; consequently, the role of complement in CM pathogenesis in experimental models has been explored [75]. Infection of mice with *P. berghei* ANKA has been shown to be a powerful tool for defining the role of complement in CM pathogenesis. C5 (complement component 5) deficiency is observed in 40% of inbred strains [9]; this deficiency results from a 2-bp deletion in exon 6 of the C5 (*Hc*) gene, which causes the creation of a stop codon four bases from the deletion and the production of a nonfunctional, truncated polypeptide that is not secreted [64, 93]. Several CM-resistant inbred strains, such as A/J, AKR, and DBA/2, are C5 deficient; by contrast, the CM-suscepti-

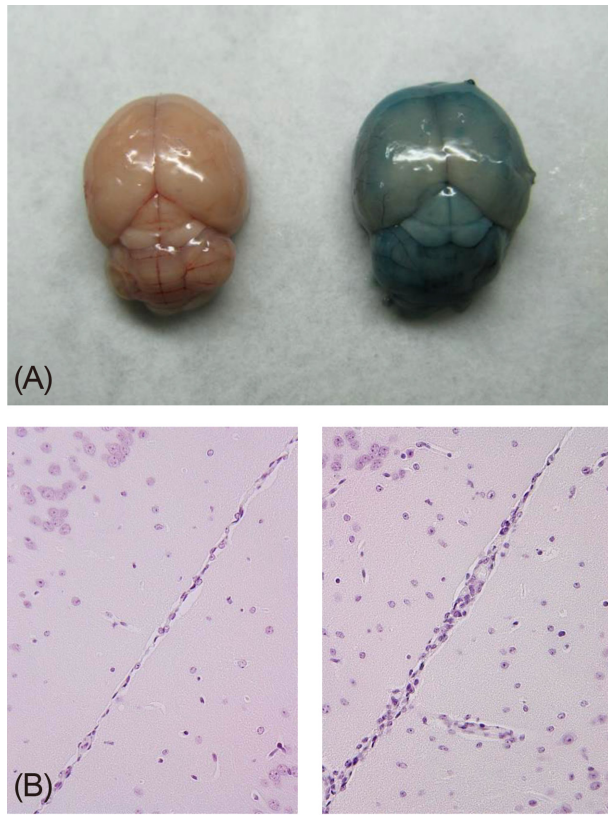


Fig. 1. (A) Brains of NC/Jic mice that were intravenously injected with Evans blue dye one hour before sacrifice. Breakdown of the blood-brain barrier results in a “bluish brain” and was observed NC/Jic with experimental CM after *P. berghei* ANKA infection (right side), but it was not observed in noninfected NC/Jic mice. (B) Histopathological analyses of *P. berghei* ANKA-infected NC/Jic mice (right side) and noninfected mice. Brain microvascular congestion was analyzed in infected mice after hematoxylin and eosin staining.

ble C57BL/6 and CBA strains have sufficient C5 [66]. The association of C5 status with CM susceptibility in recombinant congenic strains between deficient-type A/J ($C5^{-/-}$) and sufficient-type B6 ($C5^{+/+}$) strains indicates that C5 might be an important contributor to host responses in CM [66]. The resistance of the B6.D2- $C5^{-/-}$ congenic strain to experimentally induced CM provides further support for the role of C5 [69]. Interestingly, the NC/Nga strain is classified as a C5 deficient-type strain [9]. The NC/Jic strain possesses the same causative variant as in C5 deficient-type strains (data not shown). Experimentally induced CM is associated with an increase in the number of peripheral $CD8^{+}$ T cells that have T cell receptors using V β 8.1 and V β 8.2 segments [7, 71]; mortality was reduced in V β 8.1 and V β 8.2 antibody-

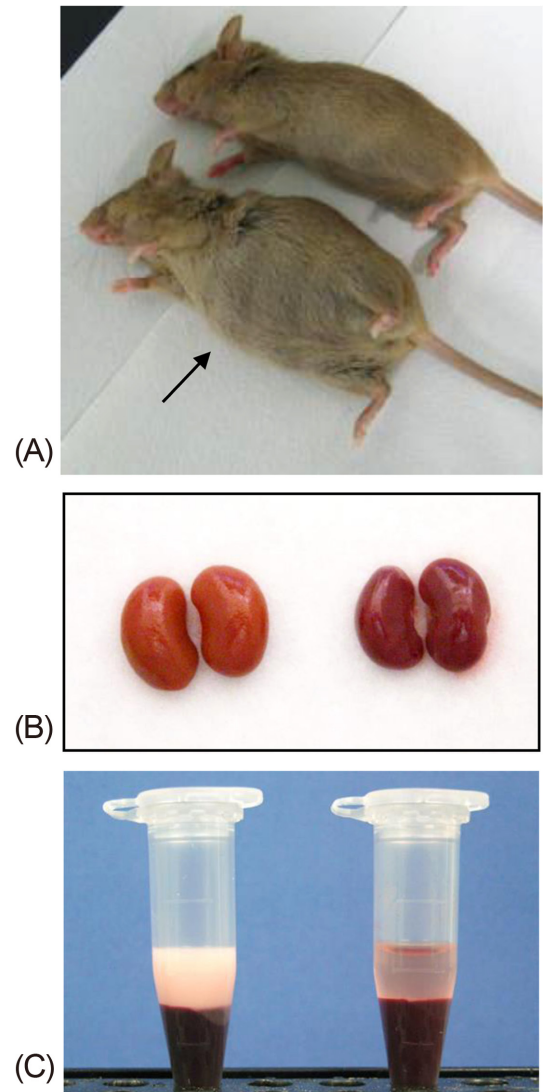


Fig. 2. (A) Appearance of NC/Jic mice (littermates). Noninfected and nephrotic (indicated with an arrow) mice at 2 weeks after *P. chabaudi chabaudi* AS infection. (B) Kidneys of noninfected NC/Jic and nephrotic NC/Jic mice after *P. chabaudi chabaudi* AS infection. The kidneys of nephrotic mice shows yellowing (left side). (C) Plasma of noninfected NC/Jic and nephrotic NC/Jic mice after *P. chabaudi chabaudi* AS infection. Chylemia was observed in nephrotic NC/Jic mice (left side).

treated mice and V β 8.1 segment-deficient mice [7, 85]. The T cell receptor V β 8 gene cluster of the NC/Nga strain lacks the V β 8.1 to V β 8.3 segments [27], similar to the NC/Jic strain (data not shown). These findings suggest that the NC/Jic strain possesses CM susceptibility genes and/or a metabolic pathway that can overcome the resistance effect of both C5 and T cell receptor V β 8 deficiency and that these factors are not relevant in

other CM-susceptible strains (C57BL/6 and CBA).

Microarray analyses have been performed on brain tissues from CM-susceptible (C57BL/6, CBA) and CM-resistant (BALB/c, DBA/2) mouse strains after *P. berghei* ANKA infection [13, 14, 52, 53, 72]. These analyses revealed that interferon (IFN) and IFN-regulating processes play a major role in experimental CM pathogenesis [13, 14, 42, 72]. Furthermore, it has been suggested that the C57BL/6 and CBA strains, which are predisposed towards Th1 immune responses, are susceptible to experimental CM, whereas BALB/c, which is predisposed toward Th2 responses, is resistant [28, 29]. Atopic dermatitis in the NC/Nga strain has been reported to involve Th2-dominant immune responses with a systemic deficiency in Th1 responses after microbial stimulation [27, 43]. The NC/Jic strain also shows atopic dermatitis, similar to the NC/Nga strain, indicating that although NC/Jic is assumed to be a Th2 dominant-type strain, it is also susceptible to experimentally induced CM. The NC/Jic strain, which has a different type of immune response from preexisting CM models, thus expands the range of experimental CM-susceptible strains. Comparative microarray analyses using CM-susceptible strains including NC/Jic will undoubtedly identify common gene networks in susceptible strains. This may lead to the identification of novel networks in CM pathogenesis and identify new targets for therapeutic intervention.

In addition to the genetic defects that influence the response to experimental CM, the NC/Jic strain also displays another characteristic feature in response to infection with *P. berghei* ANKA that is not found in other established experimental CM models. In most mouse strains, susceptibility to experimental CM is not linked to parasitemia [84], and death due to experimental CM occurs at a low level of parasitemia (less than 20%) [51]. However, parasitemia in infected NC/Jic mice frequently exceeds 20% just before death. Experimental CM with high parasitemia is a distinctive characteristic of this strain and is not observed in the C57BL/6 and CBA strains. Although experimental CM with typical clinical symptoms and death is observed in the NC.129X1-*Char1/Pymr* congenic strain, mortality at 14 days after infection is lower than in the NC/Jic strain (Table 4). There are two possible interpretations for experimental CM pathogenesis in this congenic strain in relation to parasitemia. First, the reduction of parasitemia due to the effect of the *Char1/Pymr* locus (Table 2) inhibits CM to some extent, although this locus is not di-

rectly involved in the onset of CM. Second, another locus conferring experimental CM susceptibility is colocalized with the *Char1/Pymr* locus in the region from *D9Mit208* to *D9Mit279*. The use of subcongenic strains fragmented this chromosomal region and congenic mapping for both experimental CM susceptibility and parasitemia after *P. berghei* ANKA infection might help to determine which of these two possibilities is correct.

Malaria Complications in the Kidney

The kidneys of malaria patients often display life-threatening complications [3, 17, 19, 74]. Clinically relevant renal dysfunction in humans is mainly caused by *P. falciparum* or *P. malariae* infection [3, 19, 74]. There are two major renal syndromes: acute renal injury (ARI) associated with *P. falciparum* infection and chronic and progressive glomerulopathy after *P. malariae* infection [3, 19, 74]. Although the characteristics of renal dysfunction in the former vary widely, the main histopathological finding is acute tubular necrosis [19, 74]. The pathogenesis of malaria-associated (MA) ARI (MA-ARI) is not fully understood; however, parasitized erythrocytes play a central role in all etiological factors [19]. Although no specific MA-ARI mouse model has been developed, studies of ARI have been undertaken after infection of C57BL/6 or BALB/c strains with *P. berghei* ANKA [18, 77, 98]. Further investigations are required to compare the pathophysiology of ARI in these mice and human patients. Infection with the benign malaria parasite *P. malariae* does not usually cause severe disease but is occasionally associated with nephrotic syndrome (quartan malaria nephropathy) [11, 31]. This latter type of renal complication is generally caused by immune complex-mediated mesangiocapillary glomerulonephritis, which typically leads to nephrotic syndrome [3, 19, 74]. It has been suggested that the Th2-type immune response after *P. malariae* infection induces immune processes that result in the deposition of immune complexes in the kidney, leading to glomerulonephritis [74]. Few studies on nephrotic syndrome in mice infected with rodent malaria have been reported. Quartan malaria nephropathy has been described in inbred NIH strain mice infected with *P. chabaudi chabaudi* AS; approximately 10% of the infected mice developed nephrosis [80].

As there is a clear need for better mouse models of malarial nephrosis, we infected several mouse strains with *P. chabaudi chabaudi* AS or *P. yoelii* 17XNL and

screened the mice for nephrosis. Nephropathy was only observed in NC/Jic mice infected with *P. chabaudi chabaudi* AS. Most of the NC/Jic mice infected with *P. chabaudi chabaudi* AS showed typical nephrotic syndrome (Fig. 2) accompanied by severe edema, proteinuria, hypoalbuminemia, and hyperlipidemia. These results suggested that malarial nephrosis was a consequence of interactions between a specific genetic factor in NC/Jic mice and *P. chabaudi chabaudi* AS (Table 1) [97]. In these mice, diffuse mesangial proliferation was clearly seen at 8 days post-infection [97]. Significant depositions of both IgG and C3 were also observed in the mesangial area [97]. However, malaria parasites were almost eliminated from infected mice at 10 days post-infection; the degree of segmental sclerosis, adhesion/crescent, formation of thrombi in glomeruli, and degree of tubular injuries were markedly increased at that time [97]. In concert with this pathological progress, the levels of urine protein/urine, blood urea nitrogen, serum total cholesterol, and serum triglyceride were elevated [97]. The clinical and pathological features NC/Jic strain mice infected with *P. chabaudi chabaudi* AS have a number of similarities to quartan malarial nephropathy [3, 19, 31]. As improved treatment of renal damage is important to reduce malaria mortality in humans, this model may be useful to increase our understanding of the underlying mechanisms and for developing novel therapeutic approaches to malarial nephropathy [97].

The NC.129X1-*Char1/Pymr* congenic strain also shows the same renal pathological symptoms after infection with *P. chabaudi chabaudi* AS, although the degree of clinical features is milder than in NC/Jic mice. This reduced effect might be caused by a decrease in immune complex deposition in association with considerable reduction of parasitemia due to the *Char1/Pymr* locus (Table 3). Notably, when the initial infection dose of *P. chabaudi chabaudi* AS parasite is increased, the congenic strain shows the same level of severe nephrotic damage as seen in NC/Jic. This may indicate that the *Char1/Pymr* locus is not directly involved in the pathogenesis of the nephrotic syndrome in NC/Jic mice infected with *P. chabaudi chabaudi* AS.

Malaria Complications in the Lung and Other Organs

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is another important clinical complication

of severe malaria caused by *P. falciparum* or *P. vivax* infection [60]. ALI/ARDS is thought to be involved in increased alveolar permeability, parasite sequestration, and host immune responses; however, the pathophysiological mechanisms are not fully understood [60, 81]. Mouse models of MA-ALI/ARDS show similar clinical symptoms as in humans, and two models have been developed (Table 1) [67, 87]. The first model is DBA/2 strain mice infected with *P. berghei* ANKA [21, 65, 88]. This strain is resistant to experimental CM, and about half of the infected mice develop MA-ALI with dyspnea, hypoxemia, reduced respiratory rate, and lung opacification [21, 65]. NC/Jic mice, which are susceptible to experimentally induced CM after *P. berghei* ANKA infection, die before they can be evaluated for the onset of MA-ALI/ARDS and thus cannot be utilized as an MA-ALI/ARDS model. The second model is C57BL/6 mice infected with *P. berghei* NK65; CM is not induced in these infected mice [67, 86–88]. This model is characterized by cellular infiltration, edema in lung interstitial tissue, alveolar edema, and hyaline membrane formation, which is typical of ALI/ARDS. In this model, approximately 90% of infected mice die from ALI/ARDS [86]. NC/Jic strain mice infected with *P. berghei* NK65 do not show lesions in their lungs, and this mouse strain is therefore unsuitable as an MA-ALI/ARDS model.

Other malaria complications are seen in humans, such as liver injury, placental malaria, and severe anemia [94, 98]. Mouse malaria models for these complications have also been developed and exploited [15, 98]. However, to date, no specific combinations of mouse strains and rodent malaria parasite species/strains have been identified for these complications. Most mouse strains, including the NC/Jic strain, exhibit anemia and liver injury to a greater or lesser degree after infection with some rodent malaria strains. Further work is required to determine the usefulness of the NC/Jic strain for studying these types of malaria complication.

Conclusions and Future Perspectives

Malaria imposes an enormous disease burden on the human population, and currently no vaccine is available against the disease [94]. The problems posed by the disease are becoming more serious due to the emergence and spread of antimalarial drug-resistant parasites [94]. Our understanding of disease progression mechanisms in humans is also inadequate [98], and mouse models

will be of value for identifying the molecular and cellular basis of malaria pathogenesis and for identifying new clinical therapeutic targets [16, 34, 37, 51]. Since results in mouse models do not fully reflect human malaria outcome, experimental observations arising from mouse malaria models should be interpreted with caution [12]. However, it is undoubtedly true that use of mouse models has significantly contributed to elucidation of the mechanisms of malaria pathogenesis [16, 34, 37, 51, 98]. Mouse inbred strains exhibit various degrees of susceptibility to rodent malaria infection, as observed in human cases [34]. Several mouse malaria outcomes which mimic typical complications in severe malaria are observed depending on the combination of mouse strain and rodent malaria parasite strain [44, 50]. Therefore, mouse models provide a powerful tool for exploring and dissecting host genetic factors relevant to malaria pathogenesis [34, 37, 51, 84]. The NC/Jic strain, a substrain of the NC/Nga strain utilized as an atopic dermatitis model, shows a rapid increase of parasitemia when infected with different rodent malaria strains (*P. yoelii* 17XL, *P. chabaudi chabaudi* AS, and *P. berghei* ANKA). Furthermore, the NC/Jic strain also exhibits two characteristic malaria complications, that is, CM after *P. berghei* ANKA infection and malarial nephropathy after *P. chabaudi chabaudi* AS infection. Further investigations using this strain may provide novel insights into host genetic factors for malaria research.

The development of new gene editing technologies, such as the CRISPR/Cas9 system in mice [68, 96], will aid the identification of genes associated with host responses to infection with malaria parasites. Mutant mice generated by this system at *Sptb* (spectrin beta, erythrocytic) and *Atp2b4* (ATPase, Ca⁺⁺ transporting, plasma membrane 4) genes showed that these genes were involved in malaria susceptibility [47, 49]. Genome-wide association studies (GWAS) have identified several risk and protective genes for severe malaria [2, 40, 82]. The introduction of specific mutations into mouse strains by CRISPR/Cas9 will enable the validation of candidate genes identified by GWAS. One of the advantages of this innovative approach is that we can quickly and effectively introduce mutations into whichever strain we want. For instance, phenotypic comparison of experimental CM between C57BL/6 and NC/Jic strains carrying the same introduced mutation will help elucidate the commonality or specificity of effects of targeted genes or mutations depending on genetic background. Next-

generation sequencing technology can be used to extract mutations and variants in the whole genome of any mouse strain [22, 48, 76]. It will also be possible to generate modified NC/Jic strains in which candidate mutations identified by next-generation sequencing have been corrected. Comparison of various phenotypes, such as parasitemia after *P. yoelii* 17XL infection, experimental CM after *P. berghei* ANKA infection, and nephrotic syndrome after *P. chabaudi chabaudi* AS infection, between modified and normal NC/Jic mice will elucidate the roles of mutations and variants in each pathology.

In addition to atopic dermatitis, the NC/Nga strain shows high susceptibility to anaphylactic shock [54, 55], allergic asthma [39, 73], and atopic keratoconjunctivitis [30]. These characteristics are also observed in the NC/Jic strain (data not shown). This suggests that both strains have a predisposition to allergic responses due to immunological defects associated with Th2-dominant immune responses [27, 43, 90]. In humans, a few studies have demonstrated an association between susceptibility to malaria and asthma and atopic dermatitis [35, 70]. It will be of interest to identify causative gene mutations that are common to allergic diseases, susceptibility for malaria, and development of malaria complications using the NC/Jic strain.

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