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Plasmodium berghei NK65 in the Inbred A/J Mouse: Age Immunity
in the Female Retired Breeder A/J Mouse *

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Running title: Age Immunity in mice

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SYNOPSIS

Death rates of A/J and Carworth Farms (CF₁) female mice 4 weeks and 6 months of age were compared after the mice were infected with Plasmodium berghei NK65C deme (population) and NK65RR deme. Death rates were also compared when female A/J retired breeder mice were infected with blood passages 18 and 40 of NK65C.

NK65C was found to be less virulent than NK65RR. The 40th blood passage of NK65C was more virulent than the 18th passage, but still not as virulent as the NK65RR deme. A/J retired breeders were clearly more resistant to infection than 4 week old A/J mice, while little difference was found in the different age groups of the CF₁ mice.

Age immunity to Plasmodium berghei has been well established in rats (5, 6, 7). On the other hand, the course of infection in mice has usually been fatal at all ages with the exception of certain mouse strains, e.g. NMRI mice (2,3). Mehlitz and Schindler (4) correlated resistance to P. berghei in mice to stress and diet as well as to the genetic strain.

Unexpected recoveries of older A/J mice from the NK65 strain of P. berghei in our laboratory led us to suspect that age played a major role in the A/J mouse's ability to recover from the infection. The following experiments were undertaken to clarify the situation.

MATERIALS AND METHODS

General Procedures: All animals were A/J inbred female mice (Jackson Laboratories, Bar Harbor, Maine) or Carworth CF₁ female mice (Carworth Farms, Portage, Michigan). The 6-8 month old female retired breeder mice were used as standard colony breeding females. Although these females were healthy and could be used for longer periods as breeders, the suppliers have set time limits to insure the uniform high quality of their stock.

The mice for each age group were randomized by weight and placed in standard stainless steel mouse boxes with the same conditions of food, light, water and temperature. All animals were always handled in the same way at the same time. Parasite demes (indicated by a letter following NK65) were normally blood passed on a 3-4 day schedule, with the exception of passage at each of the following intervals: 16, 17, 18, 24, 27 and 28 days, in the NK65C₄₀ (subscript refers to the number of blood passages since a mosquito passage) series of passages (1). No animals were given drugs at any time by us or by the breeders.

Inoculum: Demes (populations) of NK65 were derived by mosquito passage and indicated by a letter following NK65. The blood passage of that deme is designated by a subscript number. Two demes of P. berghei, NK65C and NK65RR, were used in these experiments, both of which were derived by mosquito passage from NK65, but the NK65C deme was less virulent. All female Carworth (CF₁) and A/J mice regardless of age succumbed to NK65RR. About 5% of the 3-7 week old A/J mice recovered from NK65C. All A/J mice were inoculated interperitoneally (IP) with infected cells from inbred mice of the same A/J genetic strain and CF₁ mice were inoculated IP with infected blood from CF₁ mice. Both demes of NK65 were prepared for each experiment by blood-passing the deme 3 times at 3 day intervals before the inoculum was collected for injection.

Experiment 1. Twenty 4 week old female A/J mice and 17 A/J retired breeder female mice (6-8 months old) were given 25,000 NK65C₃₅ parasitized RBC's. Deaths were checked daily and Giemsa stained blood films were made every second or third day on all animals in all experiments.

Experiment 2. The same procedure was used for the second experiment, (20 4 week old female A/J mice and 18 retired breeders) but the mice were infected with the more virulent NK65RR₂₀.

Experiment 3. All mice were A/J female inbred retired breeders (6-8 months). Fourteen were inoculated with 1.2×10^4 NK65C₄₀ parasitized RBC's and 18 with 1.2×10^4 NK65C₁₈ parasitized RBC's.

Experiment 4. Twenty 4 week old CF₁ (Carworth Farms) female mice and 20 6-8 month old CF₁ retired breeders were challenged with 1.2×10^4 NK65C₁₇ parasitized RBC's.

Experiment 5. Nineteen 4 week old CF₁ mice and 18 CF₁ retired breeders (6-8 month) were challenged with 1.2×10^4 NK65RR₇.

RESULTS

Fig. 1. Is a graphic representation of the first two experiments described above. Experiment 1, is represented by the 2 lines to the right of the graph. Sixty-four percent of the retired breeder A/J mice infected with NK65C recovered, while only 5% of the 4 week old A/J mice recovered.

In experiment 2, by day 14, 70% of 4 week old A/J mice were already dead, while only 18% of retired breeders had died. By day 25, 100% of 4 week old mice had died, while only 70% of retired breeders had died.

Fig. 2. Is a graphic representation of a comparison of the virulence of the 18th and 40th blood passages of NK65C (Expt. 3). At the 18th blood passage 93% of the retired breeders recovered, while only 57% of the mice recovered from the 40th blood passage.

Fig. 3. Is a graphic representation of a comparison of NK65C₁₇ infection in 4 week old CF₁ and retired breeder CF₁ mice (Expt. 4). All mice died. Fifty percent of the retired breeders had died by day 18, and 25% of the 4-week old mice had also died. By day 22, 69% and 65% of the respective groups had died. All were dead by day 36.

Fig. 4. Is a similar representation of NK65RR₇ in CF₁ mice (Expt. 5). By day 12, 47% of four week old and 50% of retired breeders had died. All mice were dead by day 28.

Fig. 5. Is a graphic comparison of part of the data from experiments 4 and 5. By day 12, 50% of mice infected with NK65RR₇ had died, but only 5% of those mice infected with NK65C₁₇ had died. By day 21, when the deaths rate for NK65C₁₇ reached 50%, 73% of the NK65RR₇ mice had died.

Examination of blood films for concomitant bacterial infections were negative.

DISCUSSION

We have had no recoveries in any A/J mice infected with our more virulent or typical demes of NK65 (1), and the death curves shown here are typical of these demes in A/J mice in similar experiments over a 2 year period (Table 1). Our results here are also typical of the NK65C deme in A/J mice over a 2 year period (unpublished data). No significant difference was found in death rates between groups of 10 female A/J 4 week old mice injected with 12,000; 25,000 and 1×10^6 NK65C₃₅ parasitized cells.

Since, except for variables of parasite deme and mouse age, conditions were standardized (heat, food, light, handling, sex and mouse genetics) in experiments 1 and 2, the results of this experiment suggest that the recovery or death of mice depends on a balance between the virulence of the parasite demes and the ability of the mouse strain to resist. The resistance of mice in this case is dependent on age. Using the combination of A/J mice and NK65RR, in which all animals die, age immunity might easily be overlooked (Fig. 1.). Age immunity is very clearly established, however, using the combination of A/J and NK65C (Fig. 1).

On the other hand, the data for CF₁ mice (Figs. 3, 4, 5) fail to establish age immunity for either NK65RR or NK65C, but they do establish the difference in virulence between NK65RR and NK65C in the CF₁ mouse infected with NK65C. Although recoveries have been as high as 20% in a single experiment with five animals, the overall recovery rate is more nearly \approx 5% for 4-5 week old female A/J mice.

It would appear to us that the parasite ordinarily overcomes the resistance of mice so quickly that no differences are seen between young and old animals, and that only in optimal combinations of less virulent

demes of parasite and resistant strains of mice will such age resistance be apparent, e.g. deme and A/J mouse. Nevertheless, the possibility of age resistance must be kept in mind in evaluating the results of immunization procedures.

Comparison of the 18th and 40th blood passages (Fig. 2) indicates an increase in virulence of NK65C with continued passage. Comparison of any passage of NK65C (18, 35, 40) to the NK65RR₂₀ in the same age mouse (Figs. 1 and 2), however, again demonstrates the contrast in virulence of the virulent and less virulent NK65 demes (1). Increases in the virulence of a line of malaria transferred by blood passages after a mosquito passage is the rule (1). Nevertheless, the most virulent 40th passage of NK65C still results in 75% recovery in the retired breeders, while no mice of any age have as yet recovered from our more virulent demes.

TABLE I

P. berghei deme of NK65	Number of Parasitized Cells Inoculated	Numbers and age of A/J mice	Day of 50% Mortality	Percent Recovery
Less Virulent Demes				
C ₁₉	1.2 × 10 ⁴	18 Retired Breeders (RB) *	-----	83
C ₃₅	2.5 × 10 ⁴	17 RB	-----	64
C ₃₆	1.2 × 10 ⁴	8 RB	-----	75
C ₃₆	2.5 × 10 ⁴	9 RB	-----	56
C ₄₁	1.2 × 10 ⁴	14 RB	-----	64
C ₃₂	1.2 × 10 ⁴	20 10 wk	-----	85
C ₃₅	2.5 × 10 ⁴	20 4 wk	34	5
C ₃₆	1.2 × 10 ⁴	10 4 wk	25	0
C ₃₆	2.5 × 10 ⁴	10 4 wk	45	10
D ₉	1.2 × 10 ⁴	20 RB	51	30
I ₁₆	1.2 × 10 ⁴	20 RB	-----	60
BB ₈	1.2 × 10 ⁴	20 RB	-----	55
More Virulent Demes				
E ₈	1.2 × 10 ⁴	20 RB	21	0
E ₇	5.0 × 10 ⁴	9 3-4 wk	8	0
EE ₈	1.2 × 10 ⁴	20 RB	19	0
EE ₈	1.0 × 10 ⁴	8 7 wk	16	0
RR ₂₀	2.5 × 10 ⁴	18 RB	24	0
RR ₂₀	2.5 × 10 ⁴	20 4 wk	11	0
RR ₂₁	1.2 × 10 ⁴	8 RB	22	0

TABLE I (continued)

RR ₂₁	2.5 × 10 ⁴	9 RB	25	0
RR ₂₁	1.2 × 10 ⁴	10 4 wk	14	0
RR ₂₁	2.5 × 10 ⁴	10 4 wk	14	0

* Retired breeders are discarded from the colonies of our suppliers between 6 and 8 months of age. The animals are healthy and fertile. Young breeders assure uniform quality of off-spring.

Fig. 1. Experiment 1. Comparison of the cumulative death rates of twenty 4 week old A/J mice and 17 retired breeder A/J mice infected with 25,000 erythrocytes parasitized with NK65C₃₅. (Right side of the graph).

Experiment 2. Similar comparison of cumulative death rates of twenty 4 week old A/J mice and 18 retired breeder A/J mice infected with 25,000 erythrocytes parasitized with NK65RR₂₀. The term breeder in the figure is equivalent to the retired breeder of the text.

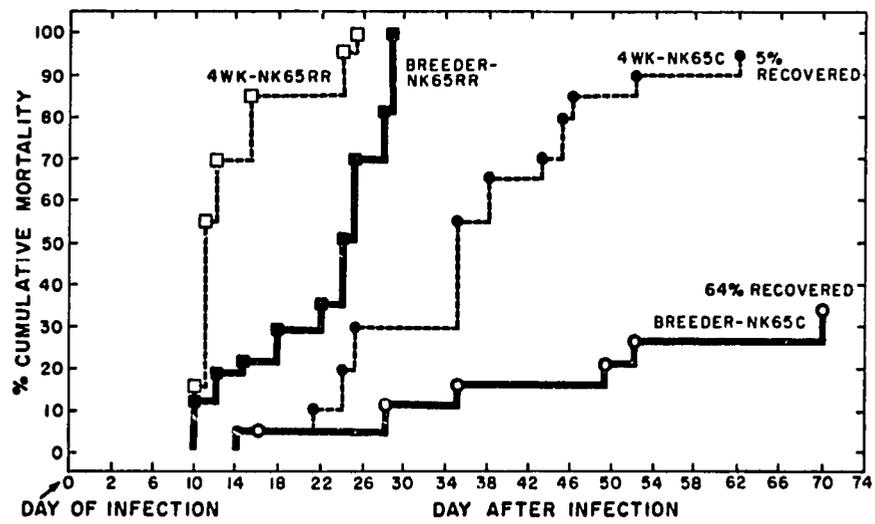


Figure 1

Fig. 2. Comparison of death rates of female retired breeder A/J mice infected with 1.2×10^4 RBC's parasitized with NK65C blood passages 18 and 40.

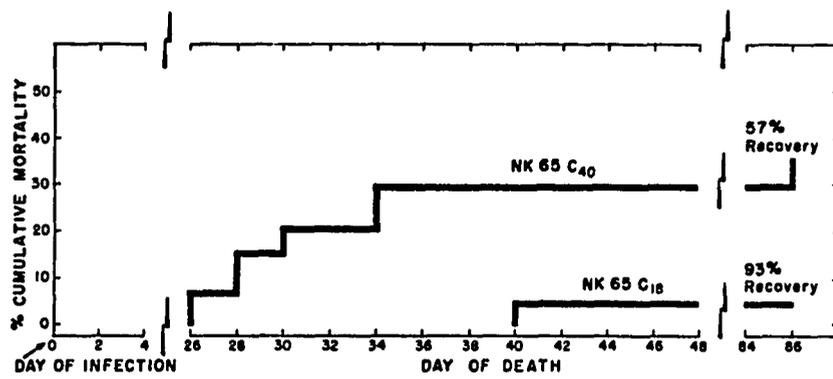


Figure 2

Fig. 3. Comparison of the death rates of 4 week old female and 6 month old retired breeder female Carworth Farms (CF₁) mice infected with 1.2×10^4 RBC's parasitized with NK65C₁₇.

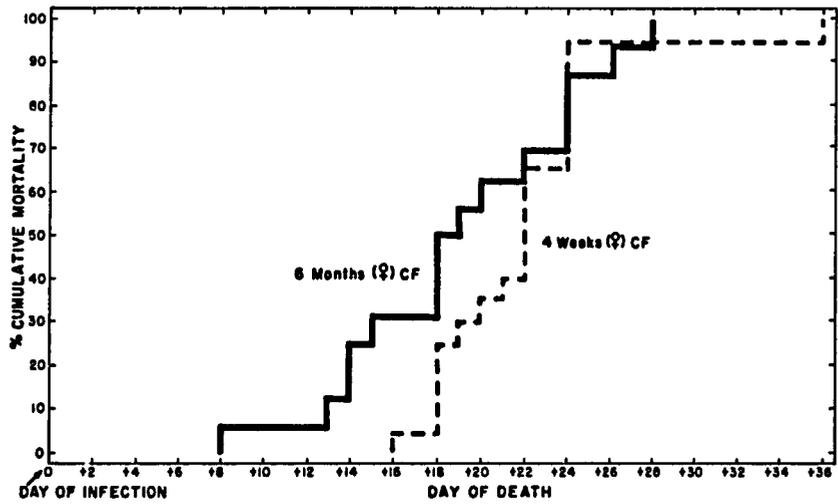


Figure 3

Fig. 4. Comparison of death rates of 4 week old female and 6 month old female retired breeder Carworth Farms (CF₁) mice infected with 1.2×10^4 RBC's parasitized with NK65RR₇.

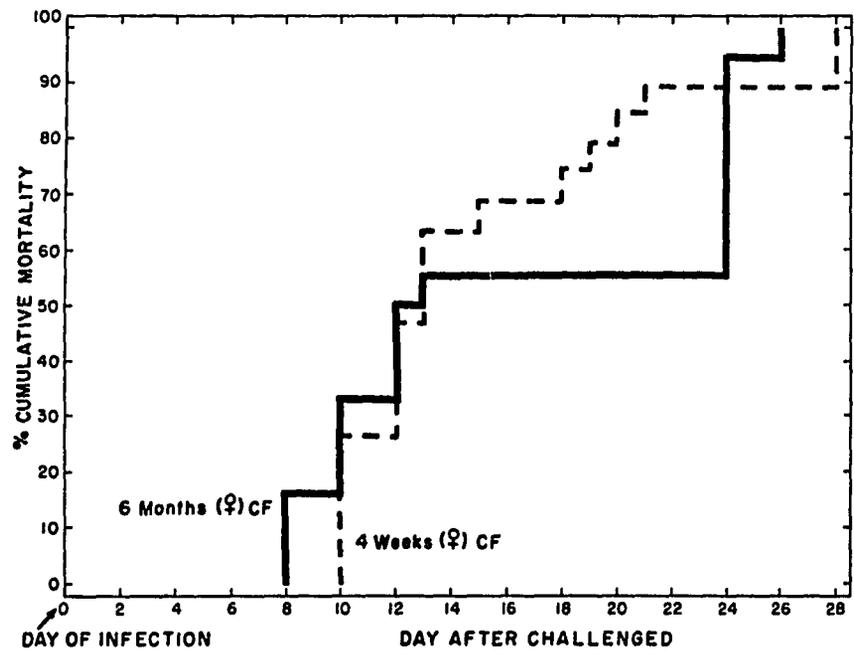


Figure 4

Fig. 5. Comparison of combined death rates of 4 week and 6 month old female retired breeder CF_1 mice infected with 1.2×10^4 RBC's parasitized with either NK65C₁₇ or NK65RR₇.

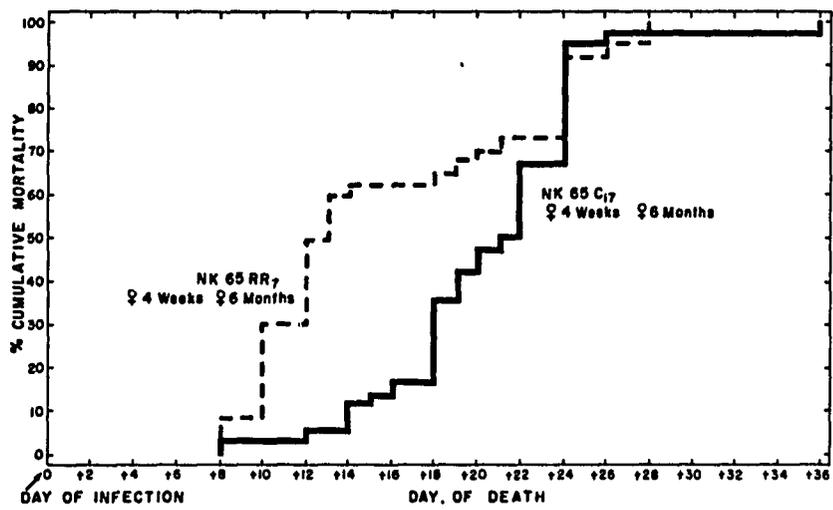


Figure 5

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