# Title and Subtitle
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# Abstract
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Plasmodium berghei NK65 in the Inbred A/J Mouse: Immunity in the A/J Mouse Naturally Recovered from NK65C and Challenged with NK65E*

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SYNOPSIS. Female retired breeder A/J mice were infected with Plasmodium berghei NK65C deme. Those animals which recovered were allowed to recrudesce and were inoculated again with NK65C. Twenty-one weeks after the original challenge, the mice were divided into 2 equal groups. One group was challenged with NK65C and the other with NK65E. Both demes of P. berghei were mosquito derived from NK65. NK65C appeared to give less protection to mice challenged with NK65E deme than to those challenged with the homologous NK65C deme. One mouse which had recovered from infection with NK65C deme died from the NK65E challenge. No definitive conclusions could be drawn regarding antigenic variation and virulence between demes E and C.

Index Key Words: Plasmodium berghei; NK65C deme; A/J mice; recovery from NK65C infection; challenge with demes NK65C and NK65E.

Plasmodium berghei is normally lethal for mice with the exception of the NMRI mouse strain (9, 10) and some inbred mouse strains (12). Although humoral and cellular mechanisms of acquired immunity come into play in mice, these hosts usually are overwhelmed and the infection is lethal (11). Suppression of the infection to low levels of parasitemia by drug treatment allows time for the effective reinforcement of immunity. Mice are then resistant to challenge infections, although the resistance is associated with repeated recrudescence (2, 3, 6).

Cox (6-8) has provided evidence that drug-induced relapse strains vary in virulence and their susceptibility to latency-inducing treatments. His work suggests also that there might be differences in immunogenicity between the parent and relapse strains. Relapse strains of Plasmodium knowlesi, also obtained by drug suppression, were found to differ antigenically from the parent strain (4, 5).

The loss of virulence of some of our NK65 mosquito passed demes (1) suggested that this biologic difference might also be accompanied by antigenic differences. The following experiments were performed to examine this hypothesis.

MATERIALS AND METHODS

Inoculum.—The inoculum for each experiment was prepared by blood passages at 3 day intervals, and all injections were intraperitoneal (IP) (e.g., blood passage was made on Monday, Thursday, Sunday and experimental animals were infected on Wednesday). Blood films were made and parasitemias determined as previously described (1).

Experiment 1.—Fifty-two A/J 3-4 month old female mice were infected in groups with inocula varying from 1.25 x 10⁴ parasitized cells of the low virulence deme (population) NK65C. Twenty-one of these mice recovered and 4 weeks after all mice had become negative, as determined by blood smear examination, they were reinoculated with 1 x 10⁴ NK65C. Twenty-seven days after reinfection and 21 weeks from the initial infection, the animals were divided into 2 groups. Eleven mice were inoculated with 10⁷ NK65C parasitized red cells and 10 were inoculated with 10⁸ NK65E parasitized cells from pooled blood of A/J mice. Four A/J and 4 CF, normal mice of the same age as the experimental mice were inoculated with 10⁶ NK65C infected red cells. The NK65C line was in the 32nd blood passage and the NK65E in the 31st blood passage at the time of final inoculations.

Experiment 2.—This experiment was a duplicate of the 1st with the following exceptions: (a) the mice were 6-7 months old; (b) all mice were infected initially with 12,500 parasitized red cells of the 58th blood passage; (c) final inoculation was made from the 17th blood passage of NK65C and the 10th blood passage of NK65E; (d) the total number of initially infected mice was 100; (e) 2 groups of 8 control A/J mice of the same age were given the final inoculum.

RESULTS

The results of the 2 experiments are summarized in Tables 1 and 2. Comparison of the data indicates that the NK65C immunized mice were more resistant to challenge with the homologous deme than to challenge with the heterologous NK65E. In the 2nd experiment (Table 2), 1 animal was not protected against the heterologous deme (NK65E) and died. Its parasitemia was 15% 2 days before death. The NK65E controls' parasitemia ranged from 21-53% infection 2 days before death. The unprotected immunized animal in experiment 2 died on the 10th day after challenge, as did the 1st control animal. The NK65E controls died on 10-16 postchallenge days. The median day of recovery (no parasites found in 60 fields) for the NK65C-challenged group was 8 and for the NK65E-challenged group 12, or a difference of 4 days. Two animals in the former group remained negative.

In this group 7 animals returned to negative on day 6 (the 1st day any animal returned to normal), and in the NK65E-challenged group 15 animals returned to normal on day 10 (the earliest any animal returned to normal), or a difference of 4 days. In the NK65C-challenged group all animals returned to normal by day 12, and in the NK65E-challenged group all animals but 2 returned to normal on day 14. One animal did not recover until day 20 and 1 died.

After a return to normal (no parasites found in 60 fields of a thin blood film) following the final challenge (Expt. 1), the parasitemia recrudesced at intervals which varied from animal to animal. The duration of the recrudescence was from 1-5 days, with only 1 animal having a recrudescence lasting longer than 2 days. The parasitemia was less than 1% in all but 1 animal which had recrudescent peak parasitemia of 3%. Half of the animals challenged with NK65E recrudesced, while only

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Induced suppression and recrudescence may not be comparable

to immune-induced (age-resistance) suppression and recrudescence. Our results seem to suggest that there may be antigenic differences between these mosquito derived demes, although there is strong cross immunity.

On the other hand, if one interprets the results on the basis of different innate virulence, one must still account for both the 1 vaccinated unprotected animal that died and the 4 day lag in median recovery day. Since the NK65C deme parasitemia normally increases more slowly (on day 6, virulent NK65E deme 13%, NK65C deme 1%) (1), it would be expected that this deme would have a lower percent parasitemia when the course of infection was interrupted by the immune reaction if interference took place on the same day. Yet there was a 4 day lag.

An increase in virulence in blood passed lines following isolation from the mosquito host has been found common by other investigators (1). Even so, these changes have not usually been correlated by the investigator with antigenicity. On the other hand, varying virulence in recrudescence lines has been noted and correlated with antigenic variation. According to Cox (8) the virulence of drug-induced relapse strains was both greater and lesser than that of the parent strain. His evidence supports the theory of differences in antigenicity in the recrudescence lines.

Although our evidence is not clear-cut as one might wish, we feel that there is some support for the hypothesis that antigenicity has varied with virulence and that the present results indicate cross protection between 2 antigenically different demes of NK65 P. berghei, both derived by mosquito passage.

REFERENCES

| Table 1. Rechallenge of female adult mice recovered from infection with Plasmodium berghei demes NK65C (Exp. 1). |
|---|---|---|---|---|---|
| Mice* | No. and strain of mice | No. infected or rechallenged with deme | No. mice with parasitemia (% parasitemia) | Range of parasitemia, % parasitemia | Mice dead or total infected |
| Recovered from | 11A/J NK65C | 11 | 0 | 1-3 | 3-22 | 0/11 |
| 10A/J NK65C** | 2 | 3 | 5 | 0/10 |
| Controls | 4A/J NK65C | 45-65 | 3/4 |
| 4CF NK65C | 5-15 | 4/4 |
| 4A/J NK65E | 20-40 | 4/4 |
| 4CF NK65E | 16-30 | 4/4 |

* Mice at 1st infection, 3-4 months old. † Control mice of the same age as experimental animals. ‡ Mice challenged 21 weeks after 1st infection and 4 weeks after 2nd infection. § All inocula were 10° parasitized cells. ‡ NK65C line in 32nd blood passage. ** NK65E line in 31st blood passage.

2 of those challenged with NK65C were found to do so over a 2 month period. Since only thin films were examined and those taken only every 2nd day, it is presumed that recrudescence lasting only 24 hr could have been missed. No attempt was made to determine whether any of these animals had established sterile immunity by the end of the experiment.

DISCUSSION

Although both NK65C and NK65E demes were derived by mosquito passage from NK65 (1), the results reported here appear to indicate that mice immunized by infection with NK65C and its subsequent recrudescences were still not as well protected against the heterologous, as against the homologous deme. All animals were allowed to recover naturally so that drug selection played no part in the results.

It can be argued that since NK65E is a more virulent deme (1), the differences seen on rechallenge are the result of natural differences in virulence rather than to immunogenetic variation. Until the mechanisms of virulence variations are fully understood, we cannot disregard this argument. On the other hand, the death of 1 mouse with a parasitemia and time of death similar to the unprotected controls suggests that this animal had received little or no protection from its previous exposure to NK65C. The abrupt recovery of the group at the time the controls died (day 12-15), however, indicates strong protection in this group as a whole. Parasitemia alone is not an accurate indication of virulence, since some animals may die at low parasitemias (control NK65E in Tables 1 and 2) while others recover from much higher parasitemias. In experiment 2 (Table 2), however, the higher parasitemias in both demes in challenged animals, when compared with those in experiment 1 (Table 1), suggest that all animals were less well protected in experiment 2 than in experiment 1. If this is so, we may have been on the borderline of sufficient protection against NK65E. Since experiment 2 was repeated in the same manner as experiment 1, except for the blood passage used, the reason for poorer protection against both demes is not clear. The reverse experiment is not possible since the NK65E deme kills all animals. Drug suppression could be used, but drug-induced suppression and recrudescence may not be comparable


