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# HOST DEFENSES IN EXPERIMENTAL RICKETTSIALPOX: RESISTANCE OF C3H MOUSE SUBLINES

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*Summary.* — Eight sublines of C3H mice were tested for their resistance to lethal infection with *Rickettsia akari*, strain Kaplan. C3H/HeJ mice were unique in their susceptibility to approximately one plaque-forming unit of rickettsiae. This lack of resistance is apparently due to a mutation in the mouse strain which occurred after 1950.

*Key words: rickettsiae; inbred mice; mouse genetics*

The closely related C3H strains of inbred mice have been of interest in the study of infectious diseases since the observation that the C3H/HeJ strain was resistant to the lethal effect of bacterial endotoxins (Sultzer, 1968). This endotoxin resistance is associated with a B lymphocyte defect (Glode *et al.*, 1976) and has been genetically characterized as an inherited trait controlled by a single gene on the fourth mouse chromosome (Watson *et al.*, 1977, 1978). More recently, a genetic linkage has been established between the lack of lymphocyte responsiveness to lipopolysaccharide (LPS) and an aberration in the activation of C3H/HeJ macrophages for tumor cytotoxicity (Ruco and Meltzer, 1978; Ruco *et al.*, 1978).

The potential usefulness of these C3H strain in studying the effect of rickettsial endotoxin on pathogenesis of disease was recognized in a recent study of scrub typhus rickettsiae (Groves and Osterman, 1978). However, inoculation of endotoxin-sensitive and endotoxin-resistant strains of C3H mice with *Rickettsia tsutsugamushi* produced no difference in the response pattern and all animals experienced a lethal infection. More recently, we have examined the susceptibility of inbred mouse strains and inbred hybrids to infection with *Rickettsia akari* (Anderson and Osterman, 1980), a species distinct from scrub typhus organisms and antigenically grouped with the spotted fever rickettsiae. Among the 24 mouse strains and hybrids examined, only the C3H/HeJ mice were susceptible to lethal infection with small doses of *R. akari*, strain Kaplan. The 50% mouse lethal dose ($MLD_{50}$) was approximately one plaque forming unit (PFU) of rickettsiae in the susceptible strain, but exceeded $10^5$ PFU in C3H/HeN mice. This vast difference in

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susceptibility between closely related inbred strains of mice suggested that further examination of C3H sublines was warranted to determine if the C3H/HeJ strain was unique in its susceptibility to lethal infection with *R. akari*, strain Kaplan.

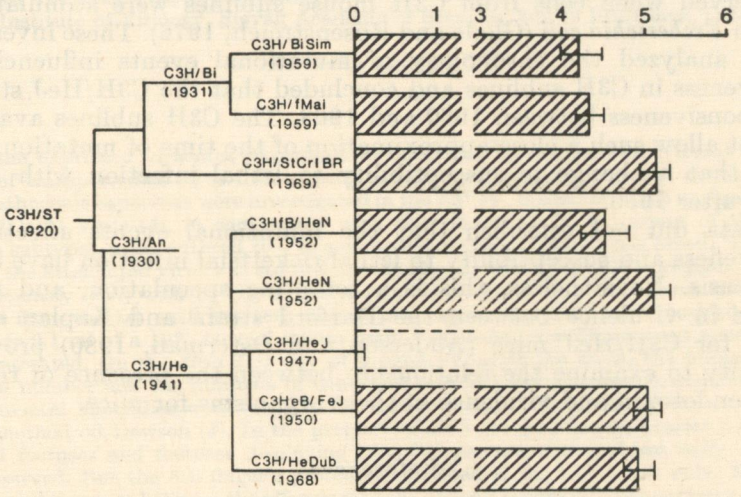


**Fig. 1.**
Susceptibility of C3H sublines to lethal infection with *R. akari*, strain Kaplan
Bars indicate the $MLD_{50}$, $\pm$ one standard deviation, for each mouse strain (expressed in $\log_{10}$ PFU values). The genealogy of mouse strains is shown in the left part of the figure. Numbers in parentheses indicate the date each subline was established.

Inbred strains of mice* were obtained from the following sources: C3H/HeJ and C3HeB/FeJ from Jackson Memorial Laboratory, Bar Harbor, ME; C3H/StCr1BR from Charles River Breeding Laboratories, Inc., Wilmington, MA; C3H/BiSim from Simonsen Laboratories, Inc., Gilroy, CA; C3H/HeDub from Flow Laboratories, Dublin, VA; C3H/fMai from Microbiological Associates, Walkersville, MD; and C3HfB/HeN and C3H/HeN were generously provided by the Division of Research Services, NIH, Bethesda, MD. The Kaplan strain of *R. akari* was purified by plaque isolation (Wike *et al.*, 1972), then subsequently propataged in embryonated chicken eggs, harvested, and stored employing standard methods (Smadel and Jackson, 1964). The $MLD_{50}$ was determined by infecting groups of five mice intraperitoneally with 0.2 ml each of 10-fold dilutions of a standard 20% yolk sac suspension of rickettsiae. At the time of each experiment, the number of viable rickettsiae in the standard suspesion was also determined by plague assay in chicken embryo fibroblast cells (Wike *et al.*, 1972). The $\log_{10}$ $MLD_{50}$ values and standard deviations were calculated by the method of Spearman and Karber (Finney, 1971) and were expressed as PFU.

The lineage of eight C3H sublines (Glode and Rosenstreich, 1976; Altman and Katz, 1979) and their susceptibility to challenge with *R. akari*, strain Kaplan, is shown in Fig. 1. Seven of the sublines were resistant to at least

* In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals", as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

$10^4$ PFU of rickettsiae, and only the C3H/HeJ strain was killed by a very small dose. This decreased resistance of the C3H/HeJ strain appears to be a result of a genetic mutation occurring after establishment of the C3HeB/FeJ strain in 1950. The response of these sublines to infection with *R. akari*, Kaplan, is closely paralleled by the pattern of in vitro lymphocyte proliferation observed when cells from C3H mouse sublines were stimulated with LPS from *Escherichia coli* (Glode and Rosenstreich, 1976). These investigators carefully analyzed the chronology of mutational events influencing LPS responsiveness in C3H sublines and concluded that the C3H/HeJ strain lost LPS responsiveness between 1960 and 1968. The C3H sublines available to us did not allow such a close approximation of the time of mutation, but did indicate that a change in susceptibility to lethal infection with *R. akari* occurred after 1950.

Our data did not establish that the mutational events affecting LPS responsiveness and susceptibility to lethal rickettsial infection have a similar genetic basis. Nevertheless, this is a tempting speculation, and the vast difference in virulence between the Harford strain and Kaplan strain of *R. akari* for C3H/HeJ mice (Anderson and Osterman, 1980) provides an opportunity to examine the relationship between the presence of rickettsial cell wall endotoxin and virulence of these organisms for mice.

## References


Altman, P. L., and Katz, D. D. (Eds) (1979): *Inbred and genetically defined strains of laboratory animals*. Federation of American Societies for Experimental Biology, Bethesda, Maryland.

Anderson, G. W., Jr., and Osterman, J. V. (1980): Host defenses in experimental rickettsialpox: Genetics of natural resistance to infection. *Infect. Immun.* (in press).

Finney, D. J. (1971): *Statistical methods in biological assay*, 2nd ed., Charles Griffin and Co., Ltd., London.

Glode, L. M., and Rosenstreich, D. L. (1976): Genetic control of B cell activation by bacterial lipopolysaccharide is mediated by multiple distinct genes or alleles. *J. Immunol.* **117**, 2061–2066.

Glode, L. M., Scher, I., Osborne, B., and Rosenstreich, D. L. (1976): Cellular mechanism of endotoxin unresponsiveness in C3H/HeJ mice. *J. Immunol.* **116**, 454–461.

Groves, M. G., and Osterman, J. V. (1978): Host defenses in experimental scrub typhus: Genetic of natural resistance to infection. *Infect. Immun.* **19**, 583–588.

Ruco, L. P., and Meltzer, M. S. (1978): Macrophage activation for tumor cytotoxicity: Tumoricidal activity by macrophages from C3H/HeJ mice reguires at least two activation stimuli. *Cell. Immunol.* **41**, 35–51.

Ruco, L. P., Meltzer, M. S., and Rosenstreich, D. L. (1978): Macrophage activation for tumor cytotoxicity: Control of macrophage tumoricidal capacity by the LPS gene. *J. Immunol.* **121**, 543–548.

Smadel, J. E., and Jackson, E. B. (1964): Rickettsial infections, p. 743–770. In E. H. Lennette and N. J. Schmidt (Eds): *Diagnostic procedures for viral and rickettsial diseases*, 3rd ed. American Public Health Association, Inc., New York.

Sultzer, B. M. (1968): Genetic control of leucocyte responses to endotoxin. *Nature* (*Lond.*) **219**, 1253–1254.

Watson, J., Riblet, R., and Taylor, B. A. (1977): The response of recombinant inbred strains of mice to bacterial lipopolysaccharides. *J. Immunol.* **118**, 2088–2093.

Watson, J., Kelly, K., Largen, M., and Taylor, B. A. (1978): The genetic mapping of a defective LPS response gene in C3H/HeJ mice. *J. Immunol.* **120**, 422–424.

Wike, D. A., Tallent, G., Peacock, M. G., and Ormsbee, R. A. (1972): Studies of the rickettsial plaque assay technique. *Infect. Immun.* **5**, 715–722.