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The Nude Mouse—Characteristics, Breeding and Husbandry

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Since its discovery in 1966 ([2]), and the subsequent recognition that this virtually hairless mutant mouse is congenitally athymic ([10]), the nude mouse has rapidly assumed an important role in biomedical research. The nude mouse is currently the most used mutant mouse; most of its applications involve exploitation of the athymia to study basic immunologic mechanisms, or to study the growth of infectious agents and tumors. It is of particular interest to the leprosy researcher, because of its extraordinary susceptibility to infection by *Mycobacterium leprae,* and because the *M. leprae*-infected nude mouse represents a potentially most useful model system for the experimental chemotherapy of leprosy.

Characteristics. Nude mice are particularly susceptible to viruses commonly found (as non-pathogens) in colonies of euthymic mice. The two most important are murine hepatitis virus (MHV) and Sendai virus, probably the most important causes of wasting syndrome and decreased life span among nude mice. Infection of nude mice with MHV results in death of the mice within a few weeks, whereas heterozygous littermates remain free of apparent disease. At autopsy, characteristic necrotic lesions are found in the livers of the nude mice. Sendai virus is a common respiratory pathogen of mice. Euthymic mice may become ill during an outbreak of Sendai virus infection, but the infection is not fatal. On the other hand, Sendai virus infection of nude mice usually results in death of the mice.

Although susceptibility to viral infections often poses the major threat to the colony, nude mice are also vulnerable to a variety of bacterial, fungal and parasitic infections. A particularly common problem is infection with *Staphylococcus* sp., which may be introduced into the colony by the animal caretakers. Although these organisms are commensal in man and normal mice, in nude mice they cause formation of abscesses, particularly around the mouth and eyes, wasting and death.

One might expect nude mice to exhibit a higher frequency of spontaneous tumors than is encountered among normal mice; however, the situation is not clear, perhaps because nude mice have been observed for long periods in only a very few studies. Some reports have suggested that nude mice experience a higher than usual incidence of certain tumors, especially lymphoid and mammary tumors. On the other hand, the development of spontaneous tumors is not recognized to be a major problem in colonies of nude mice.

A large number of articles have been published on various aspects of the immunological status of nude mice, too many to be reviewed here. In general, nude mice lack functional T cells, although small numbers of cells carrying phenotypic T-cell markers have been detected. T-cell-mediated functions—e.g., rejection of organ allografts, and resistance to infection by intracellular organisms—are deficient or absent, and non-T-cell-mediated, cellular-immune mechanisms may be altered. Levels of macrophage activation and natural killer (NK) cell activity are elevated in nude mice, the extent of the elevation depending upon the mouse strain and the microbiological status of the mice.

The *nu* gene, that responsible in homozygous mice for congenital athymia, has been bred into mice together with other mutant genes associated with immune deficiencies. Some of these double mutants are: 1) the "LASAT" mouse, which is both athymic and congenitally asplenic ([8, 9]). This mouse

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is more profoundly immunodeficient than is the nude mouse, although NK-cell activity may be greater than that in either euthymic or athymic mice. Nakamura and Yogi observed enhanced *M. leprae*-infection of LASAT mice ([8, 9]); 2) the mouse possessing both *nu/nu* and *Xid/Xid* (if female, or *Xid* if male) genotypes. This mouse is characterized by deficiencies of both T-cell and B-cell function. Mice possessing the *Xid/Xid* genotype alone have been shown to respond to infection with *M. leprae* or *M. marinum* as do genotypically normal mice ([6]); 3) the mouse possessing both *nu/nu* and "beige" genotypes. The beige mouse is characterized by deficiencies of both macrophage and NK-cell function. This double mutant has recently been bred at the National Institute for Medical Research, and its responses to intracellular infection, including that with *M. leprae,* are currently being investigated; 4) the congenitally obese (*ob/ob*) mouse has a defective thermoregulatory mechanism, resulting in a core temperature that is significantly lower than that of normal mice. Although these mice do not develop a heavy infection after inoculation with *M. leprae,* the double mutant—*ob/ob, nu/nu*—develops disseminated *M. marinum* disease involving internal organs ([5]). Because of the difficulty of obtaining and maintaining these mice, *M. leprae* infection of the double mutant *ob/ob, nu/nu* mouse has not been studied.

Breeding. Nude mice are available in which the *nu* gene has been bred onto an inbred or an outbred background. In general, outbred nude mice are more vigorous, live longer, and are more fertile than are inbred nude mice. On the other hand, if genetic uniformity together with a precisely defined genetic background are required, then one must use nude mice in which the *nu* gene has been bred onto a background of the desired inbred strain.

The nude female has poor fertility, and does not generally suckle her young ([12]). Therefore, the breeding of nude mice usually employs "families," in which the male is nude (*nu/nu*) and the female heterozygous (*nu/+*) for the *nu* gene. From such a cross, half of the offspring are expected to be nude, and the remaining half phenotypically normal (i.e., hairy), but heterozygous for the *nu* gene. Heterozygous males are generally more fertile than homozygous nude males, but breeding is not ordinarily carried out employing heterozygous males and females. Only one quarter of the offspring will be nude, on average; and the phenotypically normal offspring will include one normal (+/+) mouse for every two heterozygotes. Heterozygous females can be distinguished from normals only by laborious test breeding with nude males.

Nude males may be mated with one or more females. The most productive system appears to be that of pairing one nude male with one or two heterozygous females, who are left together throughout their breeding life. Alternatively, one nude male may be mated with three or more females (so-called harems). However, to prevent cannibalism, the male must be removed before the litters are born; because female mice are most fertile immediately post-partum, this system results in reduced fertility. On the other hand, the harem could include nude females, all of whose offspring would be nude; the heterozygous harem-mates would suckle the young. Finally, vigor and survival of the nude males may be improved by thymus transplantation ([4]).

The duration of the gestation period of mice is 21 days; when the continuous mating system is used, the young of the first litter are ready to be weaned just before the birth of the next litter.

The nude offspring are smaller and weaker than their heterozygous littermates, and, as a result, often do not compete successfully. Production of nude mice is improved by "culling" the heterozygotes from the litter. Shortly after birth, nudes can be distinguished from their heterozygous littermates by the absence of vibrissae (whiskers). Because disturbing the litter during the first 24 hr after birth may lead to cannibalism by the mother, it is the usual practice to remove most of the heterozygotes on the third or fourth day, keeping one or two heterozygote females to replace breeders when required. In any case, one should leave one heterozygote per litter, to stimulate the mother's lactation.

Finally, Fortmeyer advocates ([3]) weaning nude mice early—on the 15th or 16th day of life. By this time, lactation has diminished, and nude offspring, lacking hair, are more vulnerable to dehydration than are

normal mice. He has found that late mortality of the nude mice is reduced by separating them from the mother, and placing them in a cage containing an easily reached supply of wet mouse-meal.

Husbandry. Because nude mice are vulnerable to infection by organisms that are commensal in normal mice, it is essential to begin with clean breeding stock, to maintain the cleanliness of the colony, and to prevent contact with other mice. MHV, present in many mouse colonies, will decimate a colony of nude mice. Insects, wild rodents and man may all carry microorganisms pathogenic for nude mice. Thus, although workers in Thailand have successfully maintained large number of nude mice under conventional conditions ([11]), a conventional mouse room is generally unsatisfactory for the husbandry of nude mice.

Nude mice may be maintained successfully under specific-pathogen-free (SPF) conditions. These conditions include sterile food and bedding, autoclaved or acidified water, frequent sterilization of water bottles and cages, restricted access, use of protective foot-wear and clothing, scrupulous cleanliness, sealing of the rooms against access of wild rodents and vermin, closure of drains, screening of personnel for pathogenic organisms, and routine screening of the colony for potential pathogens, including viruses ([7]).

Small numbers of nude mice may be maintained in a laminar-flow hood or rack, and larger numbers can be maintained in a laminar-flow room ([1]). A laminar-flow room, which is expensive to purchase and install, should be equipped with entry and exit air locks; the entry air lock should include a shower, and workers should don clean clothes, cap and mask after emerging from the shower. Sterile materials are introduced through a dip tank or double-ended autoclave. Of course, the animals to be introduced into the laminar-flow facility should have been bred and maintained under SPF conditions, which should be maintained.

Further protection of the animals is provided by the use of filter boxes—cages that are equipped with tight-fitting filter caps. These should be maintained and opened only under SPF conditions.

The most rigorous protection of nude mice is provided by housing in a germ-free isolator, in which the animals may be maintained under germ-free or SPF conditions. Germ-free mice must be administered vitamin K, or they may be deliberately infected with a defined bacterial flora designed to provide vitamin K. Isolators are fabricated from clear plastic, which may be flexible, in which case the isolator must be suspended from a metal frame; or the plastic may be rigid, in which case no external support is required. Isolators are equipped with fans, which introduce air from the outside (positive pressure), or exhaust air from within the isolator (negative pressure); in either case, both incoming and outgoing air are conducted through high-efficiency filters. Positive pressure protects the animals from contamination, which results from leakage into the isolator of unsterile air. Negative pressure protects the operator, an important consideration in work with animals infected with human pathogens, but exposes the animals to possible contamination. Work with animals housed in isolators is technically very demanding; food, bedding and every item of equipment must be sterilized before entry into the isolator, and must be introduced while maintaining sterility. Space within the isolator is limited; soiled bedding and other unneeded equipment and supplies must be stored within the isolator, until they can be removed under sterile conditions. Finally, the animal caretaker stands outside the isolator, and with his hands and arms encased by heavy, puncture-resistant gloves, a situation markedly limiting manual dexterity.

However the nude mice are housed, certain environmental requirements must be met. Reference has already been made to the vulnerability of nude mice to dehydration; to minimize the loss of water through the hairless skin, the animals must be maintained in an atmosphere of about 60% humidity. Also because of its lack of hair, the nude mouse readily loses body heat and must be maintained at a temperature of 26–28°C. Especially for breeding, a uniform dark-light cycle of 10 hr of light and 14 hr of darkness, or 12 hr each of light and darkness, is recommended.

REFERENCES

1. Edinger, R. and Giovanella, B. C. Current knowledge of breeding and mass production of the

nude mouse. In "The nude mouse in experimental and clinical research". Eds. Fogh, J. and Giovanella, B. C. Academic Press, 1978.
2. FLANAGAN, S. P. Nude, a new hairless gene with pleiotropic effects in the mouse. Genet. Res. **8** (1966) 295.
3. FORTMEYER, H. P. and BASTERT, G. Breeding and maintenance of nu/nu mice and rnu/rnu rats. In: *Thymusaplastic nude mice and rats in clinical oncology*. Eds. Bastert, G., Fortmeyer, H. P. and Schmidt-Matthiesen, H. Gustav Fischer, Stuttgart, 1981, pp. 25–37.
4. HETHERINGTON, C. M. and HEGAN, M. A. Breeding nude (nu/nu) mice. Lab. Animals **9** (1975) 19.
5. LANCASTER, R. D., COLSTON, M. J., HILSON, G. R. F. and TURNER, S. M. The effect of body temperature and cell-mediated immunity on the growth of *Mycobacterium marinum* and *Mycobacterium leprae* in mice. J. Med. Microbiol. **14** (1981) 493.
6. LEVY, L., AIZER, F., BEJAR, C., LUTSKY, I. and MOR, N. Experimental mycobacterial infections of CBA/N mice. Israel J. Med. Sci. **20** (1984) 598.
7. LEWIN, L. and HANSEN, G. A simple, cheap barrier system to upgrade the health status of a conventional rat breeding colony. Animal Technol. **37** (1986) 93.
8. NAKAMURA, K. and YOGI, Y. The hereditary athymic asplenic (LASAT) mouse as an experimental lepromatous leprosy model (continued): role of the spleen in the formation of lepromatoid lesions. Int. J. Lepr. **50** (1982) 586.
9. NAKAMURA, K. and YOGI, Y. The NSF/N nude mouse, Lasat mouse and carrageenan-treated nude rat as a new model for experimental lepromatous leprosy. Proc. XII Int. Lepr. Cong. New Delhi, 20–25 Feb. 1984. Printaid.
10. PANTELOURIS, E. M. Absence of thymus in a mouse mutant. Nature **217** (1968) 370.
11. RUNGRUANG, S., RAMASOOTA, T. and SAMPATTAVANICH, S. Study of the use of nude mice in the cultivation of *M. leprae* in a normal, non-specific pathogen free room at a temperature of 30–35°C without air conditioning. Lepr. Rev. **54** (1983) 305.
12. RYGAARD, J. In "Thymus and self-immunobiology of the mouse mutant nude". F.A.D.L., Copenhagen, 1973, p. 48.