The Nude Mouse

A summary

By *Carl O. Povlsen & Jørgen Rygaard*

Introduction

The nude mouse is born without a thymus.
In most vertebrates the thymus constitutes a vital part of the immune system. If the thymus is surgically removed from adult mice, no immediate changes can be observed in immune responses, — even if present day refined laboratory techniques can demonstrate alterations in the function of lymphocytes of such animals. If the thymus is surgically removed from mice within 24 hours of birth, however, there is rapid marked impairment in the animals' cell-mediated immune responses, and in the formation of humoral antibodies to a number of antigens.
Now, the nude mouse is born without a thymus. This would make one suppose that the nude mouse does represent a base line for study of thymic function in the immune system. And furthermore, that the absence of the thymus could be exploited in studies of other biological systems, e.g. malignant tumour tissue, transplanted in the nude mouse.
In the following we summarize some of the evidence in favour of this.

The immune system

The immune system reaction to antigen can take one of two distinct forms:
1) Antibodies can be formed by certain cells. These antibodies circulate in the vascular and lymphatic systems, and react with the antigen that stimulated their formation.
2) Sensitized lymphocytes may be produced, which carry a specific antibody on the cell membrane. These lymphocytes react directly with the corresponding antigen in the so-called cell-mediated reaction. These distinct modes of reaction suggest that the immune system in fact comprises two functionally separate units, the one responsible for the humoral response, and termed the bursa-dependent system (from the avian Bursa of Fabricius) — and the other responsible for the cell-mediated response, and termed the thymus-dependent system.
The cells of the two systems are called B- and T-lymphocytes, respectively. They may function independently in response to some antigens, and collaborate in reaction to others.
The two groups of lymphocytes may be distinguished by their cell membrane antigens, — demonstrable antibodies in B-lymphocytes, and e.g. the theta-antigen marker in T-lymphocytes of the mouse.

The nude mouse

The nude mouse was first described by Flanagan in 1966. The absence

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of hair — or very sparse, occasional hair growth, is the most striking phenotypical characteristic of the nude mouse. Flanagan found that the absence of hair was due to an autosomal, recessive gene. He gave the name *nude*, genetic symbol *nu*, to the mutant.
It was not until 1968 that Pantelouris found that the homozygous nude mouse also lacked a thymus. Phenotypically normal littermates — homozygous +/+ and heterozygous +/*nu* — all had a normal thymus.

Genetical considerations

To work with a laboratory animal it is absolutely necessary to have access to reasonable numbers of well defined, identical animals. The direct attainment of an inbred nude strain, by mating of homozygous *nu/nu* males and females is not practical, because only few females are fertile in conventional or spf-conditions.
Even supposing this fertility could be improved, the resultant inbred mice would be less well suited for research purposes because of the lack of normal controls.
The alternative to be preferred is that of a gene transfer to known inbred strains of mice. Such a gene transfer can be conducted by constant back-crossing to the inbred strain for the number of generations required, using male heterozygotes checked by control matings to known heterozygous females in addition to transfer matings to homozygous +/+ females of the inbred strain. In this way, the *nu* gene has been transferred to a number of inbred strains, cfr. list enclosed.

Husbandry of the nude mouse

The immune deficiency of the nude mouse directly entails extreme sensitivity to microbiological stress of the milieu. This is well illustrated by the fact that life span of the nude mouse varies with the measures of isolation employed. In bad — or ordinary — conventional conditions the nude mouse will survive for only 2—3 months. Taking the mouse to good conventional conditions, particularly if the contamination from other conventional mice can be avoided, will increase life span to 4—6 months. In specified pathogen free conditions the life span is further extended, to 8—10 months for the nude of *BALB/c* background in our laboratory, but well 3—5 months longer for nude mice of *C3H* background. In germ free isolators we have observed life spans up to 18 months. This is our longest observation period, but a life expectancy as for normal mice should be obtainable in germ free conditions.
Due to the absence of hair, nude mice need a higher room temperature than normal mice, — we recommend 24—27 centigrades, which is well tolerated by normal haired mice also. Humidity should be 50—60 rel. %. Nude mice thrive well on normal feed pellets. Food intake is roughly as for normal mice, while the water consumption of nude mice is about 30 % higher than that of normal mice, probably due to increased evaporation from the nude skin.

Breeding

For the production of nude mice for experiments we hold one hetero-

TRANSFER OF nu ALLELE TO STANDARD INBRED STRAINS

List prepared by I. Lefkovits, Basel, based on a questionnaire

Breeding carried out by:		Nudes originally obtained (date/from)	*nu* allele transferred to:	Breeding system (a)	Back-cross (b)
W. M. Farrow	Life Sciences, Inc., St. Petersburg, Fl., USA.	June 1973 Veterinary Research Branch, NIII, Bethesda, USA.	BALB/c	DB	1
			NIH-SW	DB	2
M. Festing	MRC Laboratory, Animals Centre Carshalton, Surrey, GB.	Dec. 1970 D. S. Falconer, Edinburgh, GB.	AKR	B—I	2
			BALB/c	B—I	2
			CBA	B—I	2
			C57BL	B—I	2
			NZB	B—I	3
C. W. Friis	Bomholtgard, Breeding and Research Centre, 8680 Ry, DK.	Dec. 1969 J. Rygaard, Copenhagen, DK.	BALB/c/A/BOM	DB	7
			CBA/7/Cr/BOB	DB	2
			C_3H/Tif/BOM	DB	4
			C57BL/6/J/BOM	DB	4
			C57BL/10/ScCr/BOM	DB	2
E. B. Jacobson	Basel Institute for Immunology, Basel, CH. (present address: Schering, Cor., Bloomfield, NJ, USA).	Dec. 1970 C. W. Friis, Ry, DK.	DBA/2	DB	6
			SJL	DB	5
B. Kindred	Basel Institute for Immunology, Basel, CH.	Nov. 1969 D. S. Falconer, Edinburgh, GB.	BALB/c/AnK/Cr/Kb	DB	9
L. Kinnen	Institut für bioolgisch-medizinische Forschung, Füllinsdorf, CH.	Dec. 1970 C. W. Friis, Ry, DK.	BALB/cJ	DB	4
			C57BL/6J	DB	4
I. Lefkovits	Basel Institute for Immunology, Basel, CH.	Dec. 1970 C. W. Friis, Ry, DK.	C57BL/6	DB	9
R. Moutier	Centre de Selection et d'Elevage d'Animaux Laboratoire, CNRS, Orleans-Cedex, F.	June 1972 R. C. Roberts, Edinburgh, GB.	C57BL/6J	B—I	3
S. M. Poiley	National Cancer Institute, Bethesda, Md., USA.	Jan. 1973 C. W. Friis, Ry, DK.	BALB/c/ArCr	B—I	3
N. D. Reed	Montana State University, Bozeman, Mont., USA.	Jan. 1970 D. S. Falconer, Edinburgh, GB.	BALB/c	B—I	4
J. C. J. van Vliet	Central Institute for the Breeding of Laboratory Animals, Zeist, Netherland.	Jan. 1972 MRC Laboratory, Animals Centre, Carshalton, Surrey, GB.	B10.LP/JCsaCpb	B—I	3

(a) DB: direct backcross
B—I: backcross-intercross

(b) completed backcross until end of 1973.

Proceedings of The First International Workshop on Nude Mice, Gustav Fischer Verlag, Stuttgart, 1974.

zygous female and one homozygous (*nu/nu*) male per cage. Nude male breeders are routinely thymusgrafted to increase their life span. Heterozygous young are discarded by day 2, identified by their absence of whiskers — or small, curly whiskers. Nude young are weaned by day 21.

Humoral immune responses of nude mice reactions to mitogens

Studies of humoral immune responses of nude mice have confirmed the idea that the immune deficiency of the mutant is limited to the thymus-dependent responses. Reactions to thymus-independent antigens are normal.

The number of antigen binding cells, reactive with a number of antigens tested, are similar to values found in normal mice.

Nude spleen cells will not perform in the Jerne hæmolytic plaque assay, but on addition of appropriate numbers of T-cells a normal number of plaque forming cells of nude origin can be demonstrated.

Following immunization of nude mice with SRBC in vivo low plaque counts are found, and solely of the IgM type. A switch to indirect plaque formation (IgG plaques) demands reconstitution with thymus graft or T-cell suspensions.

Studies of serum immunoglobulin values in nude mice are conflicting, but generally demonstrate normal IgM levels and low levels of other immunoglobulin classes.

Nude cell suspensions will react to B-cell mitogens, but not to T-cell mitogens.

Skin grafting in nude mice

The standard measure of cell-mediated response is reaction to foreign tissue transplantation.

The nude mouse will accept grafts of normal skin of allogeneic and heterogeneic origin, including skin from widely disparate species such as aves and amphibiæ.

Normal human foetal organs are accepted and will differentiate further in the nude host.

Grafts of endocrine organs, such as the rat pancreas, have been accepted and will reconstitute nude mice suffering from streptozotocin-induced diabetes.

The skin grafting technique used in our laboratory is described.

Grafting technique

Propanidid (Epontol®, Bayer AG, BRD), 0,5 mg/g BW was administered to recipients intraperitoneally, and the animals were laid on sterile cloths, on their stomachs, with no form of restraint. 12 × 12 mm sections of skin were removed from the back, centred at about the 10th thoracic vertebra, more cranial if the animals were destined for second set grafts. The thus prepared graft beds were covered with sterile gauze. Bleeding was, as a rule, minimal. Donors were placed in a refrigerator at +4°C for approximately 10 minutes, and, thereafter, decapitated high up the neck. Two grafts can be obtained from the back of each donor.

The free skin graft is turned over a finger tip and scraped clean of loose connective tissue and muscle with the sharp blade of a fine pair of

straight scissors. Care is taken to free the dermis completely from underlying tissues, while avoiding perforation. The graft is then immediately set in the graft bed. With experience, it is practically always possible to achieve continuous contact of graft edge and recipient skin edge. If a graft is too large, it is cut to size, if too small, it is discarded. Immediately on completion of matching graft with graft bed, the graft is fixed in place with Histoacryl® (Braun Melsungen, BRD), an absorbable neutral tissue adhesive. No bandages are used, and animals are not muzzled. Recipients remain anæsthetized for approximately seven minutes, which allows ample time for the procedure. They are observed until spontaneous walking movements begin, and then placed in individual cages. To obviate risk of aspiration, the bottoms of cages are covered with paper napkins for the first day, whereafter these are replaced by usual wood granulate. Animals are kept in individual cages over the whole of the observation period to avoid trauma to grafts which might occur during combat.

The human tumour/nude mice system in cancer chemotherapeutic studies.

A simple *in vivo* method for testing the sensitivity of human tumours to various anticancer agents is of great potential value. The human tumour/nude mice system fulfils a number of critera which are necessary for the exploitation of the model in cancer chemotherapeutic studies.

1. A high percentage of human solid tumours are accepted and can be serially grown in nude mice.
2. Tumours have a constant growth pattern.
3. The local mode of growth will allow easy direct observation.
4. Human characteristics are preserved (histology, cytology, chromosome pattern, isozymes, antigens).
5. Nude mice are bred relatively easy, allowing large scale investigations.
6. No host conditioning is needed.

Early investigations have shown that growth of an epidermoid carcinoma transplanted to nude mice could be retarded by bleamycin in dosis below the LD_{10}.

The wellknown clinical effect of cyclophosphamide on Burkitt's lymphoma could be reproduced when the tumour had been transplanted in nude mice.

A mouse grown human malignant melanoma responded differently to three anticancer agents. 5-FU was without effect, a single dosis of CCNU caused temporary retardation of tumour growth whereas DTIC had a marked effect. The results of this sensitivity test and the results with bleomycin treatment of epidermoid carcinoma and cyclophosphamide treatment of Burkitt's lymphoma correspond with clinical experience with these drugs.

Heterotransplantation of human malignant tumours to the mouse mutant nude.

The immune deficiency of nude mice entails that they accept trans-

plants of foreign tissue — both normal and tumour tissue. 44 of 102 different human malignant tumours were successfully transplanted directly to nude mice. These include adenocarcinomas of colon and rectum (14 takes in 19 transplantation attempts), malignant melanomas (14/32), epidermoid carcinoma (7/12). Negative results were obtained in attempts to transplant human leukaemia (9 cases) and Hodgkin's disease (5 cases). 26 of accepted tumours have been serially transplanted for 2 to 56 passages so far in new nude recipients in a period of maximally $5^1/_2$ years.

Tumours grow locally at the site of inoculation and metastases to lymph nodes or organs have never been observed. The growth pattern of the individual tumours have been constant. The microscopic appearance of the mouse grown tumours is unchanged even after numerous serial transplantations and is in accordance with the human donor material.

Chromosome analysis of early and late passages of human tumours grown in nude mice have only shown cells with human karyotype. The human character of the mouse grown Burkitt's lymphoma has further been confirmed by isozyme studies and by demonstration of antihuman antibodies in sera of tumour bearing mice. All investigations so far has confirmed the human character of the mouse grown tumours.

It is essential that the pattern of drug susceptibility of the tumour is preserved after transplantation to nude mice if the system should be of value as a screening test for the sensitivity of human tumours to various anticancer agents.

The short life span (4—6 months) of nude mice under conventional conditions represents a limitation for the practical application of this model. However, life span of nude mice can be extended under specified pathogen free or germfree conditions. This model is of great potential value in screening the sensitivity of the individual human tumours to various anticancer agents. When more data are collected the role of the human tumour/nude mice system in primary and secondary screening studies of anticancer agents can be evaluated.

Normal and pathological anatomy of nude mice. Effect of thymus-grafting. Spontaneous and induced tumours in nude mice.

Normal anatomy.

Nude mice have several developmental defects. Apart from being hairless nude mice suffer from congenital absence of the thymus. Light and electron microscopic examinations have revealed an epithelial partly cystic organ in the superior mediastinum of nude mice. This structure never becomes populated with lymphocytes. It has been claimed to be a thymic rudiment, but the precise origin and function of this rudimentary organ is unknown. The so-called thymus dependent areas of lymph nodes, spleen and Peyer patches are depleted of lymphocytes. Total and differential counts of leukocytes in peripheral blood shows a marked lymphopenia — with lymphocyte counts about 1400/µl. Differential

counts of bone marrow preparations revealed a significant lower lymphocyte count. Morphological investigations indicate that nude mice lack thymus dependent lymphocytes (T-lymphocytes) whereas other parts of the hæmatopoietic system including bone marrow derived lymphocytes (B-lymphocytes) are normal.

Pathological anatomy

Due to the immune deficiency nude mice are more susceptible to infections than normal mice. In a detailed study of 24 nude mice reared under SPF conditions pathological changes were found in one or more organs in 11 out of 24 animals. This included infection of salivary glands, Harderian glands, uterine horns and seminal vesicles. A common feature of these infections is spread by the canalicular route. The causative agent of these infections have in some cases shown to be normally non-pathogenic microorganisms. The finding of pathological changes in about half of animals kept under conventional conditions calls for a redefinition of SPF milieu in relation to nude mice.

Nude mice kept under conventional conditions only live for 4—6 months. By improving the milieu life span can be extended to 8—10 months using SPF conditions and up to 18 months in germfree milieu.

Reconstitution of immunological competence by thymustransplantation.

Transplantation of neonatal thymus to nude mice will reconstitute the animals immunologically. Reconstitution can be assessed by

1. weight gain and good general condition
2. lymphocyte population of thymus dependent areas of lymphnodes, spleen and Peyer patches
3. lymphocyte counts in peripheral blood of near normal values.

Transplantation of neonatal thymus to nude mice will reconstitute the animal's capability to reject the foreign skin graft.

Nude mice supposed to be fully immunologically competent following thymus grafting will reject transplants of human malignant tumours. Nude mice being in intermediate stages in the process of immunological reconstitution will also recognize transplants of human malignant tumours as "not self".

Spontaneous and induced tumours

According to the theory of immunological surveillance one of the functions of the cell mediated immune response is to eliminate malignant cells which are supposed to arise continuously by somatic mutation. Nude mice lack a cell mediated immune response and could be expected to have an increased incidence of spontaneous tumours. However, no spontaneous tumours were observed in 13000 nude mice studied. This striking observation suggests that the concept of immunological surveillance should be reconsidered. Nude mice represent a new model for testing the effects of absence of thymus dependent immune functions on tumour development. Nude mice and their normal littermates showed no differences in incidence of tu-

mours after administration of 3-methylcholantrene at birth. Latent periods for tumour development were also comparable, similar results were obtained with urethane.

Studies have shown that nude mice are highly sensitive to virus oncogenesis (polyoma virus) at an age when normal littermates are resistent, confirming the major role of T-lymphocytes in limiting virus oncogenesis.

References

Flanagan, S. P.: 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genet. Res. (Camb.)* 1966, *8*, 295.

Pantelouris, E. M.: Absence of thymus in a mouse mutant. *Nature* 1968, *217*, 370.

Rygaard, J. & C. O. Povlsen: Heterotransplantation of a human malignant tumour to "nude" mice. *Acta path. microbiol. scand.* 1969, 77, 758.

Rygaard, J.: Immunobiology of the mouse mutant "nude". Preliminary investigations. *Ibid.* 1969, 761.

Povlsen, C. O. & J. Rygaard: Heterotransplantation of human adenocarcinomas of the colon and rectum to the mouse mutant nude. A study of nine consecutive cases. *Ibid.*, Sect. A., 1971, *79*, 159.

Visfeldt, J., C. O. Povlsen & J. Rygaard: Chromosome analyses of human tumours following heterotransplantation to the mouse mutant nude. *Ibid.*, Sect. A, 1972, *80*, 169.

Povlsen, C. O., P. J. Fialkow, Eva Klein, G. Klein, J. Rygaard & F. Wiener: Growth and antigenic properties of a biopsy-derived Burkitt's lymphoma in thymusless (nude) mice. *Int. J. Cancer* 1973, *11*, 30.

Povlsen, C. O., Grete Krag Jacobsen & J. Rygaard: The mouse mutant nude as a model for testing of anicancer agents. 5th ICLA Symposium, G. Fischer Verlag, Stuttgart, 1973.

Rygaard, J. & C. O. Povlsen: Effects of homozygosity of the nude (nu) gene in three inbred strains of mice. *Acta path. microbiol. scand.*, Sect. A, 1974, *82*, 48.

— & —: The mouse mutant nude does not develop spontaneous tumours. An argument against immunological surveillance. *Ibid.*, Sect. B, 1974, *82*, 99.

Povlsen, C. O., N. E. Skakkebæk, J. Rygaard & Gerd Jensen: Heterotransplantation of human foetal organs to the mouse mutant nude. *Nature* 1974, *248*, 247.

Povlsen, C. O. & J. Rygaard: Effects of cyclophosphamide (Endoxan®) on a Burkitt's lymphoma grown in nude mice. Proc. First Internat. Workshop nu Nude Mice. (J. Rygaard & C. O. Povlsen, Eds). G. Fischer Verlag, Stuttgart 1974. p. 285.

Rygaard, J.: Skin grafts in nude mice. 1. Allografts in nude mice of three genetical backgrounds. *Acta path. microbiol. scand.*, Sect. A, 1974, *82*, 80.

— : — —. 2. Rat skin grafts in nude mice of three genetic backgrounds. The effects after preparation by thymus grafts. *Ibid.*, Sect. A, 1974, *82*, 93.

— : — —. 3. Fate of grafts from man and donors of other taxonomic classes. *Ibid.*, Sect. A, 1974, *82*, 105.

Rygaard, J.: THYMUS & SELF. Immunobiology of the mouse mutant

nude. F.A.D.L. Publishers, Copenhagen 1973. Also published by: John Wiley and Sons, London, N.Y., Toronto, Sydney, 1975.

Rygaard, J. & C. O. Povlsen (Eds.): Proceedings of the First International Workshop on Nude Mice. G. Fischer Verlag, Stuttgart, 1974.

A bibliography on the nude mouse is under preparation and can be ordered from NUDE MOUSE SECRETARIAT, c/o Mrs. G. Samuelsen, Pathological Anatomical Institute, Kommunehospitalet, DK-1399 Copenhagen K, Denmark.

Siden sidst

Styrelsen har holdt møde den 8.–9. maj 1976 i Lellinge ved Køge (Danmark). Blandt de mange emner på dagsordenen skal nævnes:

Årsmøder 1977 og 1978:

Da Danmark står for tur, vil Scand-LAS næste årsmøde blive afholdt i København i april-maj 1977. Den endelige dato er endnu ikke fastsat. 1978 årsmøde vil finde sted i Norge (Oslo?).

Nordiske forskerkurser:

Som tidligere omtalt blev forsøgsdyrkurset i Uppsala i 1975 en stor succes. Mange ansøgte forgæves om deltagelse, men en ny chance kommer måske i 1977. Scand-LAS har ansøgt om midler til afholdelse af et nyt kursus.

Uddannelse af forskere i forsøgsdyrteknik:

En rapport om post-graduate uddannelse af doktorander i Sverige er under udarbejdelse af en arbejdsgruppe. Den vil blive tilstillet styrelsen og vil formentlig danne grundlag for en henvendelse til de respektive landes undervisningsministerier om nødvendigheden af uddannelse i forsøgsdyrkundskab.

AV-programmer om forsøgsdyr:

Indtil dato har Scand-LAS solgt 132 diasserier. Der er nu fremstillet kassettebånd med engelsk tekst og man vil derfor forsøge at sælge serierne udenfor Norden. Yderligere to serier om »Hund og Kat« er under forberedelse. Nærmere oplysninger om AV-programmerne kan fås ved henvendelse til Leif Schjerven (adresse på side 2).

Ny styrelse den 11. maj 1976:

Ved et styrelsesmøde i Lund den 11. maj 1976 har styrelsen konstitueret sig således:

President: Karl Johan Öbrink
Vice president: Hans Mogens Andersen
Generalsekr.: Lars Wass
Bitr. gen.sekr.: Hans Jørgen Skovgaard Jensen
Kassör: Gert Lindblad
Utan angiven funktion:
Kaj Bjondahl
Annelise Lyngset
Hely Oja
Leif Schjerven

Ny litteratur

The UFAW Handbook on the Caren and Management of Laboratory Animals väntas komma ut med sin 5ed hösten 1976.

Journal of the Comparative Pathology: Academic Press (London-New York-San Francisco) (utkommer kvartalsvis, Volym 86 år 1976 kostar 17,40 £ i England och 19,85 £ för de som bor på andra sidan vattnen).

General, Comparative and Clinical Endocrinology of the Adrenal Cortex (Volym 1) edited av I. Chester Jones och I. W. Henderson, Academic Press (London-New York -San Francisco) 14,50 £ (Volym 2 under framställning liksom vol 3).

Kidney Hormones edited av J. W. Fisher (Academic Press) 10,50 £.

Veterinary Microbiology – an International Journal (Elsevier Scientific Publishing Company, Amsterdam) 115 Holländska floriner/år).