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BRIEF COMMUNICATION

Observations on the Immunobiology of 'Nude' Mice

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Summary. Mice homozygous for the mutation *nude* (*nu*), which are born without a thymus, accept allogeneic skin grafts. Prior intraperitoneal injection of a thymic cell suspension, or implantation of a thymus on the first day after birth, enables the 'nude' mice to reject skin grafts.

γ-Globulins are generally present in 'nude' mice. Haemolysin activity in response to sheep red cell injection is detectable but much lower than in normal animals.

The similarity of the 'nude' syndrome to certain human primary immunological deficiencies is briefly considered in the light of these experiments and of embryological evidence.

INTRODUCTION

This report deals with the immunobiology of mice homozygous for the mutation, *nude* (*nu*). Such animals have no thymus; there is a thymic anlage present in the foetus, but already on the 14th day of pregnancy it is found to be abnormal. It develops no further and at no time does it produce or acquire lymphoid-like cells (Pantelouris and Hair, 1970).

The lymph nodes of homozygous *nu nu* (to be described as 'nude') mice lack lymphoid cells in the region between the medullary cords and the cortex of the node (de Sousa, Parrott and Pantelouris, 1969). These are areas that were described on other evidence as 'thymus dependent' (Parrott, de Sousa and East, 1966). The number of circulating lymphocytes also is reduced in 'nude' mice (Pantelouris, 1968), and there is a reduction of lymphocytes positive for the theta antigen in lymph nodes and spleen (Raff, 1969).

MATERIALS AND METHODS

Mice

The mutation *nude* is maintained in this laboratory in a closed colony derived from a breeding nucleus donated by Professor D. S. Falconer and Dr S. P. Flanagan of Edinburgh University. We have not yet been able to type this stock for histocompatibility alleles or to make it isogenic. Animals of the inbred lines, A and C 57Bl have also been used in the experiments reported here.

Skin transplants

Skin grafting was carried out by the method of Billingham and Medawar (1951) under Nembutal anaesthesia. The operated area was protected with a Gypsona bandage, which was removed after 7 or 13 days for examination of the graft.

Thymic cell injection

One donor thymus was used per recipient. The thymus was macerated with scissors and by passing it through a fine hypodermic needle in a little saline. The resulting suspension of cells and cell clumps was injected intraperitoneally into the recipient.

Thymus implants

A whole thymus freshly dissected from the donor was inserted through a skin incision into the axillary region of the recipient.

Isoelectrofocusing

γ-Globulins in plasma were demonstrated on acrylamide gel by the isoelectrofocusing method of Awdeh, Williamson and Askonas (1968).

Haemolysin titres

The presence and titre of haemolysin activity in plasma was established by the standard procedures, as described for example by Sinclair and Millican (1967), using 'Microtiter' (Cooke Engineering Co.) equipment.

EXPERIMENTS AND RESULTS

1. *Skin grafts onto 'nude' recipients*

In a preliminary experiment, thirteen 'nude' mice aged 6–8 weeks were given a skin graft from normal animals of the same stock. In every case the graft was accepted and retained for the rest of the recipient's life. 'Nude' mice in our colony die by the time they reach the age of 3 months, with very rare exceptions. The mean survival time (MST) of the grafts could not therefore be measured in our tests, but there was no obvious sign of rejection in any of the thirteen recipients up to their death. This agrees with the experience of Dr H. H. Wortis (personal communication).

In the tests that follow, the success or failure of the graft at 2 weeks was scored, and the animals were killed soon after to permit collection of lymphoid organs for cytological examinations that are now in progress. In all instances, the state of the graft at 2 weeks showed a clear contrast between 'nude' and control recipients. The data for the grafts included under Experiments 1–4 are brought together in Table 1.

In one test eight 'nude' animals were given a graft from normal littermates (*nu*/+ or +/+) in genotype, to be described as +/?). For comparison, six normal littermates of the donors and six mice of the unrelated line, C57Bl, were also given similar grafts.

On day 13 after the operation it was found that all 'nude' recipients had accepted their grafts whilst all other hosts had rejected them. The test was repeated with eight 'nude' mice receiving skin from donors of another line, albino A. Again all grafts were accepted.

2. *Skin grafts from 'nude' donors*

Two groups of recipients were given skin grafts from a 'nude' donor. The first consisted of four 'nude' and the second of five normal littermates of the donors. All 'nude' accepted the graft, but four of the five normal recipients rejected them.

3. *Injection of thymus cells*

Six 'nude' mice received an intraperitoneal injection of cells from the thymus of normal

animals of the same colony. Whilst the recipients were 2 months old, the donors in this test were 2 weeks old.

After another 10 days, skin was transferred to the pretreated 'nude' animals from a donor of line A. All grafts were found to be rejected when the bandage was removed 13 days later.

TABLE 1
SKIN GRAFTING EXPERIMENTS

Experiment	Recipients	Pre-treatment of recipients	Donors	Graft survival after 2 weeks +	−
1. a	13 *nu nu*	—	+/?	13*	0
b	8 *nu nu*	—	+/?	8	0
c	6 +/?	—	+/?	0	6
d	6 C57B1	—	+/?	0	6
e	8 *nu nu*	—	++ (A)	8	0
2. a	4 *nu nu*	—	*nu nu*	4	0
b	5 +/?	—	*nu nu*	1	4
3. a	6 *nu nu*	Thymic cells	++ (A)	0	6
4. a	4 *nu nu*	Thymus implant	++ (A)	0	4
b	6 *nu nu*	—	++ (A)	6	0

* These were followed, until they died, for another 3–6 weeks. No sign of rejection of the graft appeared.

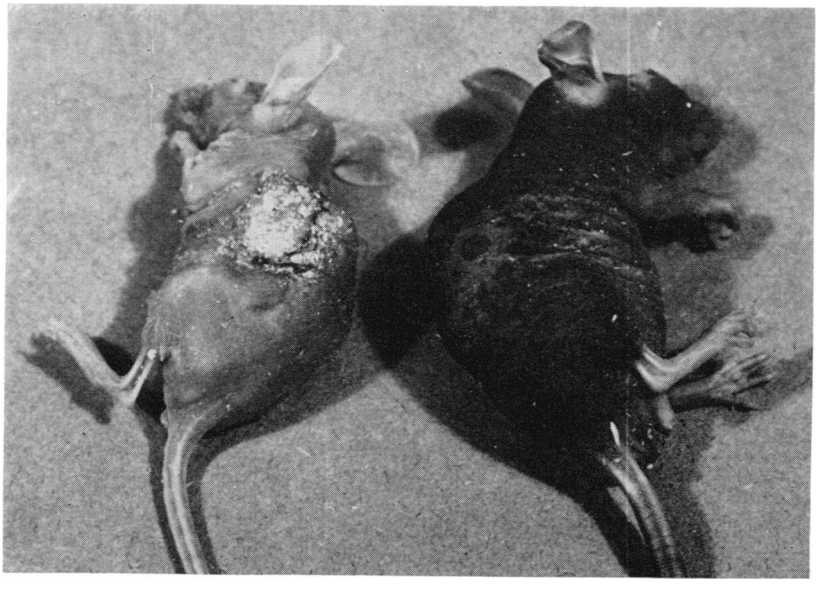

FIG. 1. Two *nu nu* mice of the same age (6 weeks), 7 days after the grafting of a piece of skin from a donor of the inbred line, A. Left: 'Nude' mouse that had not been pretreated in any way retains the graft. Right: 'Nude' mouse that had received, when 1 day old, a thymus implant from a +/? littermate, rejected the graft, wound is healing.

4. *Implantation of whole thymus*

Eight newborn mice were identified as 'nude' by the absence of vibrissae. Each one of these animals received a thymus implant from a normal littermate. Thus, all donors and hosts were at the most 30 hours old. Only four of the operated animals survived, the others

falling victims of cannibalism within 2 days of the operation. The survivors were raised to the age of 5 weeks during which period they grew much better than other 'nude' mice. They were then given a skin graft from an albino (line A) donor. Another six 'nude' animals without a thymus plant were also given similar grafts, to serve as controls.

The bandage was removed 7 days after the skin grafting. The mice with a thymus implant had rejected the graft and the wound was healing, as shown in Fig. 1. The other recipients had retained their grafts, and continued observation for another week confirmed that these grafts were accepted (Table 1, Exp. 4).

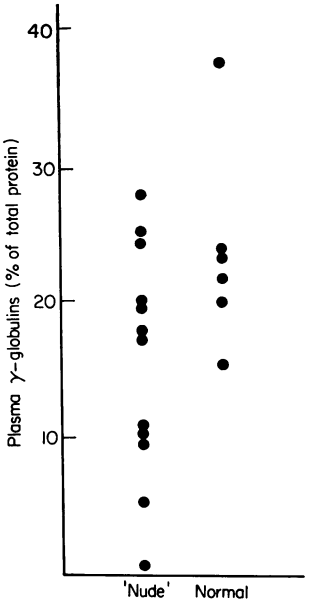

FIG. 2. Relative amounts of γ-globulins in plasma of 'nude' and normal (+/?) nice. Estimated as percentage of total plasma protein from densitometric records of acrylamide gels after isoelectrofocusing. Each dot represents one mouse. γ-Globulins extending on the electropherogram from point at which the gel pH is 8 to that where the pH is 6·5.

5. *Demonstration of γ-globulins*

Plasma samples from twelve 'nude' mice and a large number of normal samples were examined by the isoelectrofocusing method of acrylamide gels. The 'nude' animals displayed in the electropherogram a wide range of γ-globulin content. Densitometer records were taken of these electropherograms, and from them the proportion of γ-globulin to whole plasma protein was calculated (Fig. 2). More detailed studies of γ-globulins are in progress.

6. *Haemolysin titres*

A group of 2-month-old 'nude' mice and normal controls received an injection of 0·15 ml of a 15 per cent sheep red cell suspension. Blood was collected from some injected animals (which were then killed) 7, 10 or 14 days after the injection. Uninjected mice served as controls.

The numbers of animals used and the results of the tests for haemolysin activity in their plasma are shown in Table 2. 'Nude' animals showed some haemolysin activity, but much less than normal animals. Controls displayed no haemolysin activity.

TABLE 2

HAEMOLYSIN RESPONSES OF NUDE AND NORMAL MICE

Plasma sample No.	Phenotype of mouse	Days from injection of SRBC	Titre ($\log_2$ of dilution)
1	+/?	7	3
2	+/?	7	2
3	+/?	7	4
4	*nu nu*	7	5
5	*nu nu*	7	1
6	*nu nu*	7	2
7	+/?	10	10
8	+/?	10	10
9	+/?	10	10
10	*nu nu*	10	3
11	*nu nu*	10	3
12	*nu nu*	10	3
13	+/?	14	8
14	+/?	14	8
15	+/?	14	8
16	*nu nu*	14	1
17	*nu nu*	14	1
18	*nu nu*	14	1

DISCUSSION

The experiments described were carried out on a relatively small number of animals and cover a range of questions in a qualitative rather than quantitative way. They are preliminary experiments which provide a baseline for further analysis on the immunobiology of 'nude' congenitally thymusless mice.

Thymectomy carried out on the newborn mouse, i.e. during the first day of life, results in animals which accept allogeneic skin grafts from genetically dissimilar members of the same species. The 'nude' serve as a parallel 'experiment of nature' with thymectomy at a much earlier stage, and, as expected, also accept foreign skin grafts.

Subcutaneous implantation of a thymus into neonatally thymectomized mice has been used by Miller (1961, 1962) and many other workers to restore the ability to reject grafts. The implant used in such experiments was from donors less than 1 week old. It is accepted that both implanted thymus and host lymphoid tissues contain, some time after the operation, lymphoid cells of host origin rather than cells derived from the implant (Miller, 1963). Our Experiments 3 and 4 (Table 1) extent this concept to the 'nude' animals. Histological examination of lymphoid tissues after thymus implantation is now being carried out. It should be noted that in Experiment 4 the skin was grafted 4 weeks after the implantation of a thymus; and this thymus was taken from 1-day-old donors.

Fig. 2 demonstrates that agammaglobulinaemia, although it may occur in some, is not constant or general among 'nude' mice. In this again there is agreement with experiments involving thymectomy (Parrott and East, 1964).

Haemolysins are produced by 'nude' mice but they never reach the level found in normal mice after the injection of sheep red cells. The time course of haemolysin production is being investigated.

The results shown in Table 2 may be relevant to discussion of the methods of antibody production. Current theories postulate a synergistic action of two cell lines for the production of an antibody. It is suggested that thymic, or at least thymus-influenced cells, constitute one of these lines (Mitchison, 1969; Taylor, 1969). The production of some

haemolysins by 'nude' mice implies that antibodies *can* be produced without a thymus, but that the rate of their production is amplified by some influence from the thymus. It is also known that the thymus exerts a hormonal influence stimulating mitosis of lymphoid cells (Goldstein, Slater and White, 1966). In general, the preliminary observations reported here are confirmatory of work with experimentally thymectomized animals, and suggest that further analysis would be worthwhile.

The absence of thymus and the immunological defects of 'nude' mice raise the question as to whether this syndrome parallels any of the primary immunological deficiencies known in man. Seligman, Fudenberg and Good (1968) proposed a classification of these deficiencies taking into account cellular and immunological defects, pathology, etc. Two of these groups show great similarity to the situation in 'nude' mice.

The first group (Di George's syndrome) combines absence of the thymus with scarcity of lymphocytes in paracortical regions of lymph nodes and absence of cellular immunity responses. These symptoms occur in 'nude' mice also, but these differ from the human syndrome in having parathyroids and showing no tetany of the newborn. There is no general involvement of all derivatives of the III and IV pharyngeal pouches (Pantelouris and Hair, 1970).

The second comparable syndrome observed in man is Nézelof's autosomal recessive lymphopenia, where the thymus is hypoplastic. In 'nude' mice, however, it can be said that there is a more extreme hypoplasia of the thymic rudiment. It is obvious that there are many points of similarity as well as dissimilarity between human syndromes, in particular Di George's and Nézelof's, and the condition in 'nude' mice. They reflect the timing and the degree of deviation from normal embryogenesis and later development.

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