Thymus dysgenesis in nude (nu nu) mice

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SUMMARY

The thymic rudiment in mice homozygous for the recessive mutation nude (nu) has been compared to the normal at two stages: the 14- to 15-day foetus and the 1- to 2-day-old young. In the former, the normal thymus already comprises two adjacent lobes but in the nude it is represented by a pair of thin strands of tissue, each with a narrow central lumen. In further development these strands become thicker and vesiculated. They progress no further and are found in the 1- to 2-day young as small vestiges that are never populated with lymphoid cells.

The thyroid, parathyroids and mandible of homozygous nude mice are normal, indicating that the complex of structures derived from the third branchial arch is not affected as a whole.

INTRODUCTION

Research over the last ten years or so has amply demonstrated the central role of the thymus in the establishment of immunological competence, particularly in reactions to grafts of foreign tissue. The pioneering findings of Miller (1961) as well as much subsequent work were based on the use of laboratory animals, usually mice, thymectomized at birth (see Metcalf, 1966).

It would be obviously desirable to be able to thymectomize the foetus or, better still, to have animals born without a thymus. Hence the interest of the mouse mutant nude (nu), described in the first instance by Flanagan (1966). Recently, the homozygous nu nu animals have been described as thymusless, in the sense that no thymus can be found in them by dissection and macroscopic examination at any stage after birth (Pantelouris, 1968). It may be noted that there are in men at least three types of congenital thymus aplasia or deficiency, all leading to syndromes of the utmost gravity.

MATERIAL AND METHODS

Foetuses at the 14 to 15th day of pregnancy (timed by the vaginal plug method) and 1- to 2-day-old animals were obtained by crossing heterozygous +nu, phenotypically normal, mice. The homozygous nu nu foetuses were identified on dissection from the uterus by the piliferous colliculi which are much less prominent than in the normal. Homozygous nu nu young were identified by their distorted, irregularly curled vibrissae. The histological findings confirmed these
identifications. Five foetuses, from three pregnant females, were identified as homozygous $nu\ nu$ and, together with an equal number of normal litter-mates, were fixed in Bouin's solution, serially sectioned at $5\ \mu$ and stained with Delafield's haematoxylin. Four 1- to 2-day-old $nu\ nu$ and four normal litter-mates were also fixed, cut in two to facilitate processing.

Fig. 1. Part of a section through a thymic lobe of a 1- to 2-day-old mouse. Note the two types of cells, namely the reticular cells with faintly staining nuclei and the lymphoid cells with deeply staining nuclei. Delafield's haematoxylin. $\times 500$.

RESULTS

Observations on the 1- to 2-day-old specimens

In the normal animal, the thymus at this stage weighs about 50–70 mg against a body weight of 2–3 g. The two lobes are adjacent and rest on the anterior surface of the auricles and large vessels of the heart. A transverse section is shown in Fig. 1. Fibrous septa are beginning to divide the lobes into lobules, and cortical and medullary zones can be distinguished. Blood vessels are more abundant in the cortical areas, which are seeded with lymphoid cells. Such cells can also be seen free in blood vessels.

In the nude individuals no structure similar to the above is seen, but a thymic rudiment is found reaching half-way between thyroid and heart. In section, it is seen to comprise many vesicles or canaliculi delimited by epithelial-like cells, with no trace of lymphoid cells (Figs. 2–5).
Fig. 2. Part of a longitudinal section through the cervical and upper thoracic region of a 1- to 2-day-old nude (nu nu) mouse. A, Auricle of the heart; Ao, aorta; N, spinal cord; T, thymus rudiment; Th, thyroid. ×40.
Fig. 3. Part of a section similar to that of Fig. 2 to show at a higher magnification (×160) the thymic rudiment in the 1- to 2-day-old nude mouse. T, Thymic rudiment.
**Fig. 4.** Dorsoventral section through the cervical region of a 1- to 2-day-old *nude* mouse. *L*, Trachea; *O*, oesophagus; *T*, the paired thymic rudiments. ×200.

**Fig. 5.** The left thymic rudiment from the same specimen as Fig. 4, enlarged to show the vesicles or canaliculi, and the absence of lymphoid cells. Compare with the normal, Fig. 1. ×1200.
Fig. 6. An approximately transverse section through a normal 15-day foetus in the upper thoracic region. C, Clavicle; L, trachea; N, spinal cord; O, oesophagus; T, the two lobes of the thymus. × 200.

Fig. 7. Transverse section through the upper thoracic cavity of a 14- to 15-day nude (nu nu) foetus. L, Trachea; N, spinal cord; O, oesophagus; S = sternum; T, paired thymic rudiment. × 200.
Observations on the 14- to 15-day foetus

In the normal foetus the two lobes of the thymus are already formed, but there is no evidence of differentiation into medullary and cortical regions. Blood vessels are few. There are no lymphoid cells but some large cells present may be precursors (thymoblasts).

The nude foetus at this stage has a thymic rudiment which, compared to the normal, is much smaller and is dorsoventrally flattened. It begins anteriorly at a level just below the thyroid as two separate strands, each of which has a narrow central lumen. Further back these strands are thicker and vesiculated, but always remain smaller than the normal. The length of these structures is about 360 µ (Figs. 6–8).

The thyroid–parathyroids complex shows no difference from the normal at either stage examined.

DISCUSSION

In examining our material we were guided by the description of thymus development in the mouse given by Crişan (1935). The thymic Anlage becomes patent on day 9–10 of intrauterine life and incorporates ectodermal as well as endodermal contributions. A small anterior portion, the ‘head’ of the thymus, is derived from endoderm of the lateral and dorsal sides of branchial pouch III, and from ectoderm of branchial arch III. The ‘head’ is followed by a narrow strand which thickens to form the ‘tail’ of the thymic rudiment. The whole
rudiment is paired. Subsequently the thymus ‘slides away’ from the cervical region to the thoracic cavity where it grows into the definitive organ.

The thyroid begins as a thickening of the ventral side of the pharyngeal endoderm at the level of branchial arch II. The mesobranchial plate so formed (becoming later the discus thyroideus) incorporates also three pairs of diverticula from aortic arches, and becomes detached from the pharynx. Whilst it remains thin at the midline (mesobranchial groove) it thickens at the lateral lobes. It also incorporates the paired ultimobranchial body, a product of branchial pouch V, and the paired epithelial body from pouch III. It is because of these shared connexions to the branchial arches, especially the third, that thymus, thyroid and parathyroids are viewed developmentally as one complex.

From our observations we conclude that at about day 14 or 15 of intra-uterine development the thymic rudiment in homozygous nu nu mice is already arrested at a stage more primitive than the corresponding normal rudiment, but has passed the stage of ‘sliding’ down into the thoracic cavity. The exact time, and nature of divergence from normal ontogenesis remains to be determined by further work. As a result of this developmental arrest, the animal is born, as described, with no effective thymus as a lymphoid organ.

The situation in nude mice differs from all three forms of thymic aplasia described in man so far. In the ‘Swiss type’ agammaglobulinemia it is not the thymus alone but all lymphoid tissues that fail to develop normally. In nude, the dysgenesis is restricted to the thymus, although the thymus-dependent areas of the lymph nodes are also affected secondarily (de Sousa, Parrott, & Pantelouris, 1969). The DiGiorge syndrome involves aplasia of the thymus as well as of the parathyroids, thyroid and mandible. There is no such generalized defect of derivatives of branchial arch III in our mouse mutation; thyroid, parathyroids and mandible remain normal. It may therefore be suggested that the basic defect in the nude occurs later than in DiGeorge’s syndrome. The third syndrome described in man combines aplasia of the thymus with generalized lymphopenia and imperfections of the lymph nodes (Nezelof et al. 1964). In fact, the three children described by Fulginiti et al. (1966) had rudimentary lymph nodes, quite difficult to find. Their vestigial thymus was described as a ‘small alveolar gland-like structure’, a description that would also fit the 1- to 2-day-old nude. In the latter, however, lymph nodes—although devoid of lymphocytes in the thymus-dependent areas—are normal as far as their cortical and medullary regions are concerned.

RÉSUMÉ

La dysgènèse du thymus chez des souris nudes (nu nu)

L’ébauche du thymus, chez des souris homozygotes pour la mutation recessive, nude (nu) a été comparée à celle d’animaux normaux témoins. Deux stades ont été étudiés: le stade correspondant aux 14 à 15e jours du développement fœtal et le stade du 1 au 2e jour après la naissance. Pour le premier stade considéré, il apparaît que le thymus est déjà constitué par
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deuX lobes adjacent chez les témoins, alors que chez le mutant nude il est représenté par une paire de fins cordons tissulaires présentant chacun une étroite lumière centrale. Au cours du développement ultérieur ces cordons s'épaississent et deviennent vésiculeux. Ils ne progressent pas davantage et, chez le jeune âgé de 1 à 2 jours, ils apparaissent sous une forme vestigiale. A ce stade ils ne sont jamais peuplés de cellules lymphoïdes.

La thyroïde, les parathyroïdes et la mandibule des souris homozygotes pour la mutation nude sont normales, ce qui indique que le complexe de structures qui dérive du troisième arc branchial n'a pas été affecté dans sa totalité.

REFERENCES


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