ATHYMIC MICE OF INBRED STRAIN 101/HY WITH A NEW ALLELE AT THE NUDE LOCUS

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UDC 57.082.262

KEY WORDS: nude mutants; mice: immunodeficiency.

Spontaneous autosomal recessive mutation of nudeness was discovered at the Research Laboratory of the Experimental and Biological Models in mice of the 101/HY strain in 1986. Homozygous mutants have no hair color, and anatomical investigations of them show absence of the thymus. The allelism of the mutant gene with the nude gene was verified by crossing a heterozygous mutant 101/HY male with a female heterozygous for the nude gene, from a closed colony of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, generously provided for this purpose by E. S. Revazova. Among the progeny obtained from this crossing, three nude hybrid compounds were obtained. Consequently, the new mutation took place in the nude locus of the 11th chromosome of the mouse. The new gene was designated nu^Y. Possible differences between the nu^Y gene and the known nu gene were the subject of the present investigation.

Most 101/HY-nu-nu mutants will die before reaching maturity. The viability of nu/nu males can be maintained by transplantation of an embryonic thymus into them or by injections of a suspension of the thymus of normal mice [5]. However, the fertility of the survivors, as a result of this treatment of nu/nu males was only partially restored in this line (only one litter each was obtained from two males of eight receiving transplantation of embryonic thymus). For that reason, since 1987 the mutation has been maintained by the more effective method, for conventional conditions, of ovarian transplantation from homozygous mutants aged 1 month into recipients of the same line with normal phenotype [1].

These nu/nu mutants are a natural model with which to study the role of the thymus in the development and functioning of the immune system, for they are characterized by a weak immune reaction, due to the absence of mature forms of T lymphocytes (although their precursors are present among the bone marrow cells [6, 10]. The embryonic thymus of nu/nu mice consists of thin bands, and after birth only traces of its abnormal elements remain [7]. The histology of the rudimentary thymus in the postnatal period in nu/nu mice has been described in detail by previous investigators on the example of noninbred BALB/c mice carrying the nude gene [4]. In this paper we described a histological study of the rudimentary thymus in inbred 101/HY – nu/nu mice in the postnatal period, which was undertaken to determine the degree of development of this organ in mutants of this particular strain.

EXPERIMENTAL METHOD

Experiments were carried out on mice of the 101/HYo-nu/+ strain, reared under conventional conditions in the Department of Genetics, Research Laboratory of Experimental Biological Models. The animals were kept in T2 cages (from VELA2) and fed on PK-120-3 granulated combined feed (Kombikorm). To obtain a homozygous nu/nu progeny, ovaries of nu/nu females were transplanted into females of normal phenotype [9], which were mated with males of normal phenotype and, depending on the male genotype (+/nu or +/+), nu/nu were obtained in the F1 or F2 generations.

The isolated apical region of the mediastinum, fixed in Bouin's fluid, from nu/nu mice on the 11th day and at the 8th week after birth was subjected to histological treatment. Mice of normal phenotype of the same age and line were used

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Krasnogorsk, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 76-79, January, 1992. Original article submitted July 16, 1991.

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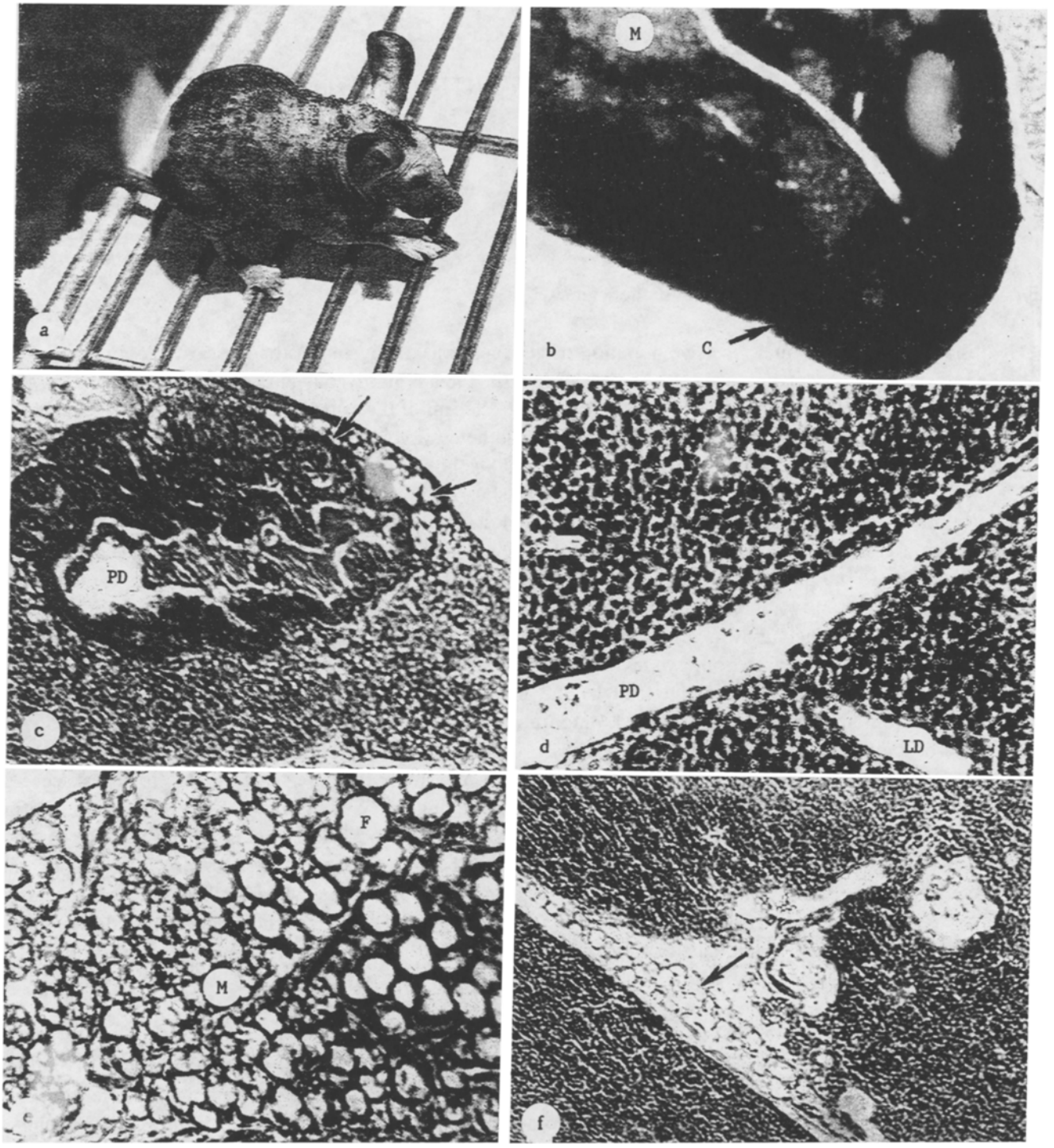

Fig. 1. Nude athymic mutants in mice of the 101/HY inbred line: a) pi/pi male, reaching maturity after subcutaneous transplantation of a normal embryonic thymus, with characteristic skin pigmentation; b, d) fragments of lobule of thymus in male with normal phenotype on 11th day after birth, C) cortex, M) medulla. PD) Principal duct of gland, LD) lateral duct of gland. Magnification: 58 and 330; c) rudiment of lobule of thymus in pi/pi male on 11th day after birth; PD) principal duct of rudiment of gland, arrows indicate mature fat cells. Magnification: 148; e) fatty infiltration at site of rudimentary thymus in pi/pi male at 8th week after birth; F) fat cell, M) mucoid cell. Magnification 330; f) fragment of lobule of thymus in male of normal phenotype at 8th week after birth, arrow indicates fatty infiltration of interlobular zone of thymus. Magnification: 148.

as the control. The material was embedded in paraffin was and serial frontal sections were stained with Ehrlich's hematoxylin and counterstained with eosin.

EXPERIMENTAL RESULTS

Postnatal 101/HY—nu/nu mutants are distinguished by delayed growth and reduced body weight: at the age of 3 weeks their body weight was 6.8 ± 0.4 g (12.3 ± 0.6 g in the control). Because of the early death of these two mutants the average size of the litter reared by the recipient was quite small (3.1 ± 0.3) compared with the size of the litter after transplantation of nude C57BL/10-hr^{rhY} mutants with a thymus (4.7 ± 0.5) [1], but this was sufficient to maintain the breeding nucleus. The fraction of nu/nu homozygotes in the progeny after mating of the recipients with +/nu males was 21.9 ± 4.9%, but in the F2 generation, i.e., after crossing of heterzygous pairs, it was 10.6 ± 1.4%.

At autopsy on nu/nu mutants at the 11th day athymia was discovered, for the size of the rudimentary thymus was an order of magnitude smaller than the usual size for the thymus. In control mice of the same age, in median sections of the lobule of the thymus the principal duct with lateral branches, lined by pseudo-simple epithelium, could be seen, and whole stroma of the organ, in which cortical and medullary zones could be distinguished, was infiltrated with lymphocytes, particularly densely in the cortical zone (Fig. 1b, d). In nu/nu mutants at this age, rudimentary structures of the two lobules of the thymus could be seen in the apical region of the mediastinum, partly transformed into a mature band of fat in the immediate vicinity of the rudiment (Fig. 1c). These structures lacked a developed stroma and lymphoid infiltration, in agreement with observations made by other workers [4, 7]. The rudiment of each lobule consisted of a convoluted duct with hypertrophically dilated lumen, filled with an amorphous mass and lined by epithelial cells.

At the age of 7.5 weeks the size of involution of the thymus in the control mice were expressed as an increased frequency of lumina, possibly connected with the development of Hassall's concentric corpuscles, and by only slight fatty infiltration in the region of the interlobular septum (Fig. 1i). In nu/nu mice at this age rudimentary structures were already virtually completely replaced by adipose and mucoid cells (Fig. 1e). This fact points to greater severity of the athymia in nu/nu mice of the 101/HY strain compared with athymia of mice described previously, in which ducts of rudimentary structures of the gland were still present at the age of 7 weeks [4]. It can accordingly be expected that the immune reaction would be weakened by the greatest degree in 101/HY—nu/nu mice. This conclusion is in agreement with the increased mortality of 101/HY—nu/nu mutants compared with BALB/c—nu/nu mutants under conventional conditions [8], where nu/nu males may survive without any form of treatment until the age of 70 weeks.

It is difficult at the moment to say whether the pathological picture observed is the result of the influence of the genetic environment of strain 101 or whether it is specific for its own nu^{Y} allele. However, whatever the case, the mutant form 101-nu^{Y} is a new and valuable model for several trends in biological research and, in particular, for antiviral and other antiinfectious preparations and for studying the possibility of correcting various kinds of organ under conditions characterized by the severest T-lymphocyte deficiency, and also to study the role of the gene (and, perhaps, of its different alleles) in the development of these pleiotropic effects. The new mutant form may possess interesting features, caused by the effect of the 101/HY strain genotype, from the standpoint of its use as a biological model This strain is being used to obtain 101.C3HF1 hybrids – the principal object in radiobiology and radiation genetics of mammals. The strain 101/HY was sent to our laboratory by the British Radiological Centre in 1969. Mice of the 101/HY strain have been found to be highly sensitive to the mutagenic effect of thiotepa [2], probably due to a defect in the genetic repair systems [3]. Thus 101/HY–nu mutants are defective in relation to two fundamental functions of the living organism: repair of genetic injuries to chromosomes and of the immune system.

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