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THY 1 Expression in The Brain of Nude Mice

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Summary

The expression and cell distribution of Thy 1 antigen was studied in the brain of both normal and athymic (nude) young adult mice of the BALB/c strain by immunochemistry. In nude animals Thy 1 fluorescence was less intense and less regularly distributed in the molecular layer of the cerebellum and hippocampus. Thy 1 content determined by ELISA was lower by 10–16 % in the cerebellum and 20–25 % in the olfactory bulbs of nude mice. The total wet weight of the brain was lower by 16 % than in control animals; the deficit in body weight ranged from 34–45 %. It is supposed that the changes in Thy 1 expression in nude animals are caused mainly by the underdevelopment of late developing brain regions due to thermoregulatory problems and other postnatal strains occurring in the mutants.

Key words:
Thy 1 – Nude mice – Brain development – Glycoproteins

Introduction

Thy 1 is a small cell surface glycoprotein (m.w. 17.500, Kuchel *et al.* 1978) in thymocytes and some other types of cells including neurones of the central nervous system (Barclay and Hyden 1978, Mirsky and Thompson 1975, Moore *et al.* 1971, Schnitzer and Schachner 1981, Stohl and Gonatas 1977). It has a domain structure analogous to a group of proteins endowed with cell and molecule recognition abilities, such as immunoglobulins, histocompatibility antigens, beta-2 microglobulin and seemingly also the Neuronal Communication Adhesion Molecule (Williams and Gagnon 1982). Function of Thy 1 in the brain is not clear enough. It has been proposed to participate in cell recognition and interactions during development (Bolin and Rouse 1986) and in transmission of nerve cell impulses on synapses (Williams *et al.* 1980). It may act *via* ligand-receptor interactions or stabilization of high molecular weight glycoproteins on the surface of brain cells (Morris 1985, French *et al.* 1987).

In adult mice or rats, Thy 1 molecule is present mainly on the surface of large neurones rich in synaptic contacts. It is well expressed in the neuropil of forebrain hemispheres, some parts of the brain stem, molecular layer and synaptic glomeruli of the internal granular layer of the cerebellum, hippocampus, etc. (Barclay and Hyden 1978, Schnitzer and Schachner 1981, Morris and Barber 1983).

The nude mouse is a mutant characterized by thymic dysgenesis, hypoplasia of the T cell system and hairlessness (Holub 1989). There are also some minor structural abnormalities in the brain, for instance, in the shape of Purkinje cell dendrites (Henderson *et al.* 1981) and in the proportion of astrocytes and oligodendroglia in the spinal cord gray matter (Kerns and Frank 1981). Fewer oligodendrocytes, together with a slight volume deficit, have recently been reported in the occipital cortex of nude mice (−2 % in area 18, Diamond *et al.* 1986).

Expression of Thy 1 antigen in the brain of nude animals has not yet been studied. Similarly as in lymphocytes (Cantor *et al.* 1975), it may be influenced directly by the *nu* allele, or indirectly, by dysplasia of the thymus and T cell system, hairlessness and resulting metabolic disbalances. Therefore, we have screened the content and distribution of this molecule in the brain of young adult nude mice by enzyme linked-immunoassay and immunocytochemistry.

Material and Methods

Animals

BALB/c mice of both sexes aged 45−131 days were used. Nude homozygote mice (10th backcross generation) and *nu/+* mice were obtained from *nu/nu* x *nu/+* matings. As controls +/+ animals of the respective strain were used. All mice were reared in a closed colony under SPF conditions of the 3rd category under personal barrier and fed with sterilized ST 1 pellets (VELAZ, Prague). Litters were kept together without genotype selection. After weaning at postnatal day 25, four to five mice of both phenotypes were kept in a cage (temperature 28 °C, relative humidity 55 % and 12/12 light/dark cycle). The animals were killed by exsanguination under short-term ether anaesthesia.

Immunochemistry

Thy 1 was detected in Tween 40 extracts of freshly dissected brain regions stored at −25 °C. Microwells were precoated by affinity purified swine-anti-mouse IgG on which Thy 1 monoclonal antibody (1aG4/c5/IgG3 class, Dráber *et al.* 1980) was adsorbed. Bound Thy 1 from tissue extracts was detected by polyclonal rabbit anti-Thy 1.2 IgG (adsorbed by mouse liver tissue powder) and by swine anti-rabbit peroxidase labelled IgG. After reaction with o-phenylendiamide the results were recorded by Multiscan MCC reader (Labsystems) at 492 nm. The values (i.e. absorbances per wet weight tissue equivalents) are expressed as the ratio (given in percentage) of data obtained in nude and control littermates.

For immunocytochemistry the brains were frozen in a dry-ice cooled isopentan bath and then cut in the middle sagital plane at 10 μm. The slices were incubated with monoclonal anti Thy-1 F7D5 antibody (IgM class, Lake *et al.* 1979, kindly provided by Dr. I. Hilgert, Inst. Mol. Genetics, Czechoslov. Acad. Sci., Prague) and FITC-labelled swine antimouse Ig conjugate (ÚSOL, Prague) for 60 min for each step. Slides were counterstained by 0.1 % Evans blue.

Results

a) *General observations*

In both normal and nude animals there was a relatively high interindividual variability in the degree of "convexity" of the frontal poles of the forebrain hemisphere and in the size of olfactory bulbs. In some animals, left-to-right asymmetry was apparent in the latter region. The brains of adult *nu/nu* mice were often smaller in size. The differences concerned mainly olfactory bulbs and the cerebellum. In 6-week-old animals the wet weights of the brain and the body in nude animals were lower by 16 % and 45 %, respectively. The difference in body weight

was smaller at 8 weeks (−25 %) while that of the brain was unchanged (−16.5 %, Tab. 1).

Table 1

Brain and body weight of young adult normal and nude mice

Age	Haired animals		Nude animals	
(days)	Body weight (g)	Brain weight (mg)	Body weight (g)	Brain weight (mg)
42 n=10	19.7 ± 0.8	413 ± 3.9	10.8 ± 0.8* (−45.4%)	346 ± 5.9* (−16.3%)
57 n=7	21.6 ± 0.7	437 ± 5.5	16.1 ± 0.9 (−25.0%)	365 ± 6.9* (−16.5%)

*Means ± S.E.M.; * = P<0.01 (Student's t-test)*

Table 2

Relative content of Thy 1 antigen in tissue extracts of nude mouse brain

Thy 1 relative amount in nude animals is expressed in percentage of absorbance values measured in normal animals as estimated by ELISA

Age/Region (n)	45 days (4)	57 days (4)	73 days (8)	131 days (2)	45–131 days (18)
Olfactory bulbs	74.3	74.8	86.5	80.5	78.8 ± 2.6*
Cerebellum	89.4	83.9	88.8	87.8	87.5 ± 1.1*
Forebrain	96.0	n.s.	n.s.	121.0	108.5 ± 8.9
Brain stem	102.1	n.s.	n.s.	94.0	95.7 ± 2.9

*(n) = number of animals used; means ± S.E.M.; * = p<0.05 (one sample t-test); n.s. = not studied*

b) *Thy 1 antigen content and distribution within the brain*

aa) The values found in four gross anatomical regions of the brain by ELISA in 45- to 131-day-old animals are shown in Table 2. In nude animals, there is a deficit in Thy 1 content in the olfactory bulbs of all age groups (ranging from −19.5

to −26.4 %) and also in the cerebellum (from −10.6 to −16.1 %). In the brain stem and the cerebrum either no differences were found (45-day-old animals) or the values were non-significantly higher than in normal littermates (131-day-old animals, Tab. 2).

ab) Immunocytochemistry revealed a dot-like pattern of fluorescence in several brain regions in normal animals. This was most apparent in the neuropil of the cerebral cortex, molecular layer of the cerebellum, the dentate gyrus of the hippocampus, mesencephalic tectum and some other minor areas of the brain stem. In olfactory bulbs, the fluorescence was more abundant in the mitral and inframitral layers. Large commissures (e.g. corpus callosum, commissura hippocampi) were almost unstained.

In nude animals, the "dots" of fluorescence were less expressed and less finely distributed within the Thy 1 positive regions. As a consequence, micropatches of fluorescence occurred in some localities. This was well apparent in the molecular layer of the cerebellum and the hippocampus (Fig. 1,see Plate 3). There was, however, great variability in the intensity and the microdistribution of Thy 1 fluorescence both among the nude animals and also in individual brains.

Discussion

The basic pattern of Thy 1 distribution in the brain of normal animals is, with a few exceptions, in good agreement with earlier studies on normal adult mice and rats (Barclay and Hyden 1978, Schnitzer *et al.* 1981). The exceptions consist in our material in the absence, or very low amount, of Thy 1 in large fibre tracts (Stohl and Gonatas 1977, Granholm *et al.* 1986) and in the synaptic glomeruli-rich granular layers of the cerebellum and olfactory bulbs (Bolin and Rouse 1986). For explanation, differences in techniques of the preparation of brain tissue for immunochemistry, or in epitope specificity of the antibodies used in the above studies, should be taken into consideration (Bolin and Rouse 1986).

As revealed by ELISA, expression of Thy 1 in the olfactory bulbs, cerebellum and the hippocampus of nude mice is lower (Tab. 2). Immunocytochemistry showed that in the former two regions the defect is present mainly in the molecular layers. The implication is that the changes in Thy 1 are due to alterations in the morphology of dendrites and axons which are the main antigen-bearing structures in these regions. Nerve cell processes and their synaptic endings in the brain of small laboratory rodents are mainly formed postnatally (for review see Jacobson 1978). In nude animals, this occurs under increased levels of factors mediating non-shivering thermogenesis (Weihe 1984, Holub 1989), especially thyroxine which is known to affect cell growth and differentiation in the brain (Lauder 1984). The late developing regions, such as the cerebellum, hippocampus and olfactory bulbs (Jacobson 1978), are evidently more exposed to such influences and, consequently, also found to be almost selectively affected in our study. In some nude animals the impairment even resulted in a macroscopically evident deficit in the size of these regions of the brain. Changes in morphology of Purkinje cell dendrites and climbing fibres, i.e. the main Thy 1- bearing structures in the molecular layer of the cerebellum (Morris *et al.* 1985), have already been shown in nude mice by electron microscopy (Henderson *et al.* 1981). The morphology of nerve cell dendrites and synapses can, however, be influenced by many whole body acting factors, such as

undernutrition, hormonal disbalances, etc. (Jacobson 1978) which may also participate in the introduction of changes observed in nude mice in this study. This view is supported by the preferential affection of Thy 1 expression in the late developing parts of the brain. In addition to the above hormonal disbalances and thermal discomfort, the consequences of stress, resulting from interactions with haired littermates may also have influenced brain development in nude animals. The same reasons may explain the deficits in body weight (Tab. 1). Better growth of nude mice kept without haired littermates have, indeed, been observed by Rygaard and Friis (1974). A primary defect in Thy 1 gene expression is also less likely in view of the non-significant changes in the remaining parts of the brain (Tab. 2). Finally, it is of interest that the brain regions found to be affected in our study play an important role in behaviour and, therefore, may represent a structural basis for the defects in locomotor activity and social interactions reported in nude mice (Kršiak *et al.* 1987).

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Plate 3 – Mareš et al.

Fig. 1.
Distribution Thy 1 antigen in normal (a) and nude (b) BALB/c mouse cerebella. Indirect immunofluorescence, monoclonal anti Thy-1 F7D5 antibody and SwaM-FITC Ig conjugate, Orthomat, Leitz, 250x.

Fig. 1a, b