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Emphasis on peanut lectin as a marker for granular cells

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Summary. Peanut lectin (PNL) binding to a total of 13 granular cell tumours was examined by means of the peroxidase antiperoxidase technique. The tumours included six tumourettes of the neurohypophysis, one malignant granular cell tumour of the brain, and six peripheral tumours of distinct locations. Every tumour studied showed intracytoplasmic fine granular PNL binding; after pretreatment with neuraminidase, the weakly positive reaction was enhanced to a great extent. In all tumours simultaneous examination for the detection of lysozyme and glial fibrillary acidic protein (GFAP) was also carried out. Lysozyme was negative in all cases, whereas GFAP expression could be demonstrated at the periphery of the malignant granular cell tumour of the brain.

The data presented clearly demonstrate that PNL can be used as a histochemical marker for granular cells regardless of their location. The fact that the presence of lysozyme could not be proved does not support the view of a histiocytic origin for granular cells, whereas the expression of GFAP in some immature granular cells of the brain tumour examined is considered to be an argument in favor of its glial origin.

Key words: Granular cell tumour – Peanut lectin – Lysozyme – Glial fibrillary acidic protein (GFAP) – Immunohistochemistry

Lectins are proteins of plant and animal origin which specifically bind with an antibody-like affinity to certain individual sugar residues of glycoconjugates (Sharon and Lis 1972; Köttgen et al. 1979; Köttgen et al. 1979). A purified peanut lectin was first isolated by Lotan et al. (1975). It was shown to possess carbohydrate binding sites complementary to beta-D-galactose(1-3)N-acetyl-D-galactosamin residues (Novogrodsky et al. 1975; Irle 1977; Skutelsky 1977; Suton et al. 1977). Recently; Müller et al. (1980) described PNL binding to granular cells of the neurohypophysis. Examination of a large number of human brain tumours by means of PAP technique on paraffin sections revealed intracytoplasmic PNL receptors in

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different tumours such as meningiomas, gliomas and especially neurinomas (Schwechheimer et al. 1982). Sian and Ryan (1981) demonstrated the ultrastructural identity between these cells and granular cell tumours.

The present study was undertaken to characterize the granular cells and their respective tumours further by histochemical methods, using peanut lectin as a marker. Additional examination of lysozyme and glial fibrillary acidic protein (GFAP) was carried out in order to contribute to the still controversal discussion on the origin of granular cells.

Material and methods

Thirteen specimens from different patients were selected from our autopsy material or biopsy files. They included six tumourettes of the neurohypophysis, one malignant granular cell tumour of the brain (reported in detail by Ule et al. 1975), 4 granular cell tumour of the skin, 1 tumour of the laryngeal mucosa, and of the muscle, respectively (Table 1). 2–5 µm sections of formalin-fixed and paraffin embedded tissue were done using a microtome with disposable blades (Feather Japan).

Sera. PNL and rabbit anti-PNL were purchased from E.Y. Lab. Inc. San Mateo Ca 94 401 USA. Swine anti-rabbit serum, rabbit PAP complex and antiserum to human lysozyme (muraminidase, Code A 099) were obtained from Dako, Denmark; neuraminidase from Behringwerke AG, Marburg, FRG. Rabbit anti-GFAP was a kind gift of D. Dahl, Veterans Administration Medical Center, West Roxbury, USA, and was used at a dilution of 1:200. Peroxidase reaction was visualized using 3,3′-diaminobenzidine (DAB; Fa. Merck, Darmstadt, FRG) as a chromogen.

Procedures. The Sternberger PAP technique was employed with slight modifications for each of the three markers tested. For detecting peanut lectin binding, the extended PAP method as described by Möller (1982) was used; control experiments eliminating the specific staining product were performed by omitting PNL or by addition of β-D-galactose which is known

Table 1. Granular cell tumours[a] examined

No.	Biopsy No. (EN)/ Autopsy No. (SN)	Sex	Age (years)	Location
1	SN 861/74	M	51	Neurohyophysis
2	SN 916/74	M	66	Neurohyophysis
3	SN 951/74	F	68	Neurohyophysis
4	SN 1123/74	F	59	Neurohyophysis
5	SN 1131/74	F	72	Neurohyophysis
6	SN 1147/74	M	67	Neurohyophysis
7	SN 681/74	M	55	Brain
8	EN 52652/77	F	60	Skin
9	EN 35875/78	M	31	Skin, breast
10	EN 8655/79	F	32	Laryngeal mucosa
11	EN 24874/79	M	78	Skin, neck
12	EN 13716/80	M	27	Muscle
13	EN 25980/82	M	48	Skin, back

[a] Synonyms: Abrikossoff tumour (1), granular neuroma Feyrter (5), myoblast myoma; "tumourette" (21) in the neurohypophysis

to be a specific inhibitor of PNL binding at a final concentration of 0.8 M. Lysozyme at a dilution of 1:20 was tested according to the method described by Pinkus and Said (1977); in control experiments the first antibody was substituted by PBS. For the demonstration of GFAP technique as modified by Taylor and Burns (1974) and described in detail by Deck et al. (1978) was employed. Anti-GFAP was diluted 1:200; controls substituting the first antibody by PBS or normal rabbit serum yielded absolutely negative results.

Results

In all cases examined intracytoplasmic PNL binding can be demonstrated in varying degrees and number of cells. However, in no case are all the granular cells stained (Fig. 1a). After pretreatment with neuraminidase, PNL staining is very strong in every tumour regardless of its location, resulting in a fine granular structure in the cytoplasm of the tumour cells (Fig. 1b–d). All control experiments are negative. No lysozyme can be detected in the granular cells of the 13 tumours examined. As intrinsic positive controls tissue histiocytes as well as polymorphs and monocytes in the blood vessel show a strong reaction (Fig. 2).

The possible demonstration of GFAP might be of general interest in tumourettes and in the malignant granular cell tumour of the brain, in terms of the controversal discussion about their derivation. The granular cells of the neurohypophysis do not express GFAP. At the periphery of the malignant granular cell tumour of the brain, however, oval-shaped cells with eccentric nuclei resembling immature granular cells contain GFAP, whereas the vast majority of cells in the centre of the tumour is GFAP-negative. These GFAP-positive cells are easy to distinguish from dark brown coloured reactive astrocytes with multiple delicate processes (Fig. 3a–c). We note a shift in GFAP expression from strongly positive normal and reactive astrocytes in the environment of the tumour, GFAP-positive "immature" granular cells in the periphery and typical GFAP-negative but strongly PNL-positive granular cells in the centre of the tumour.

Discussion

Tumours which are composed of large uniform cell with eccentric nuclei and PAS-positive fine granular cytoplasm are called granular cell tumours because of their appearence in the light microscope. Paraffin section of 13 granular cell tumours of different sites were tested with regard to their PNL affinity, using an extended PAP technique. After pretratment with neuraminidase, a strongly positive PNL binding in the cytoplasm of all tumour cells occured. When sections were not treated with the enzyme, a variable weak or strong reaction was observed in a few cells. The enhanced PNL binding behaviour after neuramindase pretreatment indicates that the vast majority of PNL receptors is masked by silaic acid.

In contrast to this, intracytoplasmic PNL binding found in granular cells of different human brain tumours (Schwechheimer et al. 1982) was very strong even if sections were not pretreated with neuraminidase. Using immunohistochemical methods only, we are not able to demonstrate identity

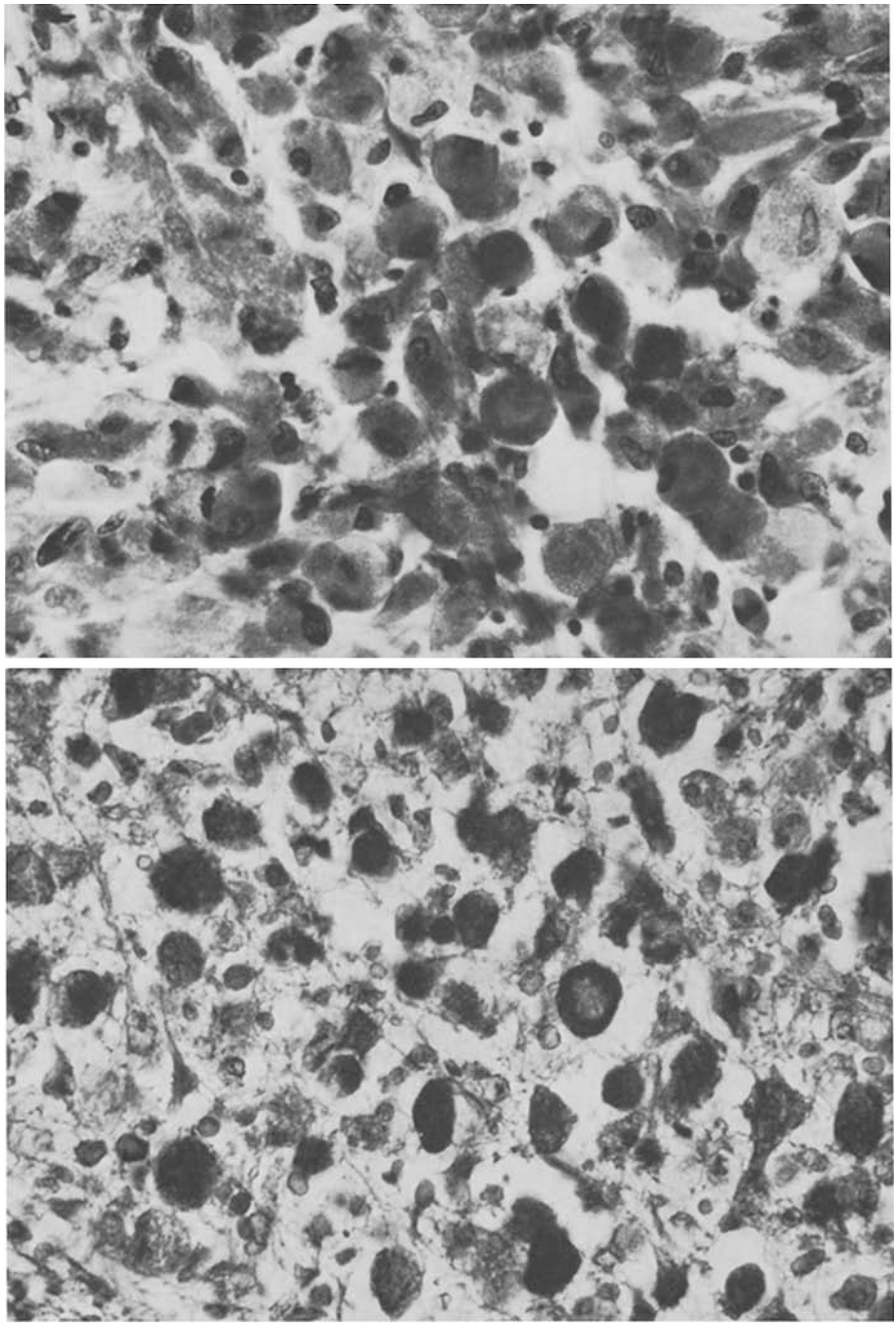
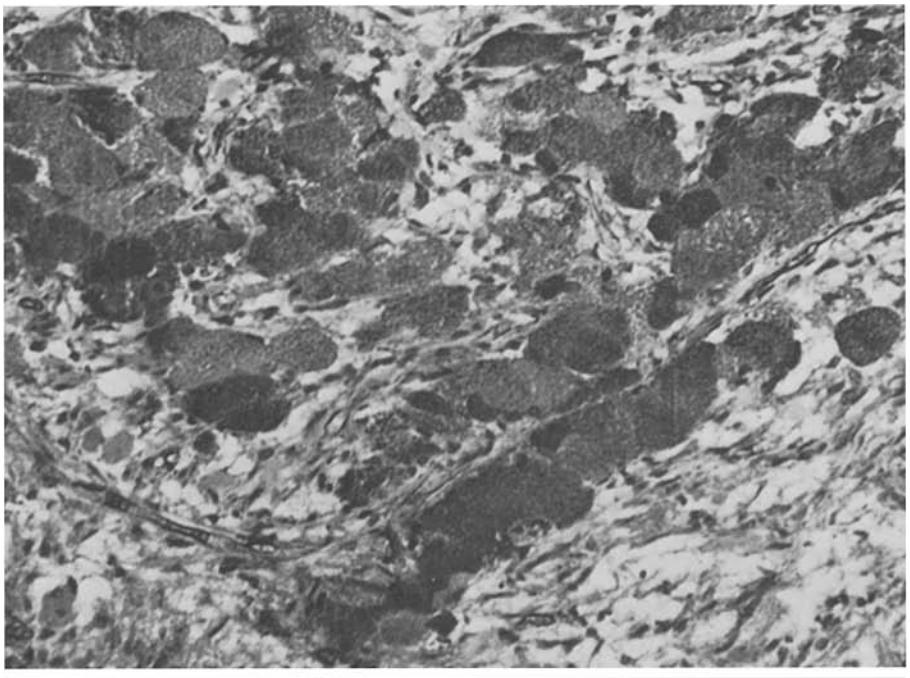


Fig. 1. a Malignant granular cell tumour of the brain. Clearly stained granular cells without neuraminidase pretreatment. (PNL; PaP technique; diaminobenzidine (DAB)/hemalaun; ×320) **b** Strong staining of the tumour cells after pretreatment with neuraminidase (PNL; PaP technique; DAB/hemalaun; ×320) **c** Tumourette of the neurohypophysis. Fine granular



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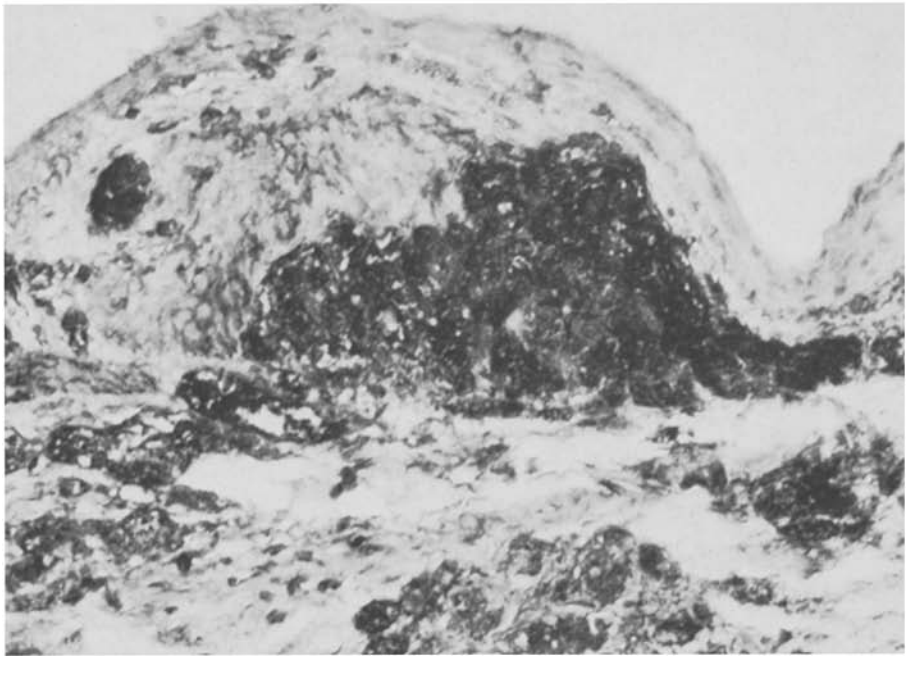

d

PNL-binding in the cytoplasm of the tumour cells. (neuraminidase; PNL; PaP technique; DAB/hemanlaun; ×210) **d** Granular cell tumour of the laryngeal mucosa. Nodular arrangement of PNL-positive cells. (neuraminidase; PNL; PaP technique; DAB/hemalaun; ×210)

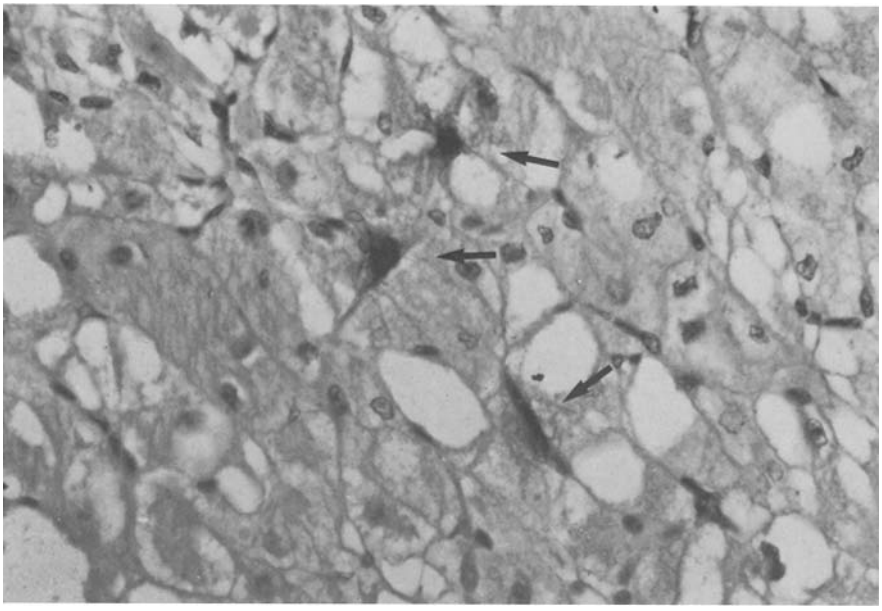

Fig. 2. Granular cell tumour of the skin. Granular cells do not contain lysozyme; *arrows* indicate strongly positive histiocytes. (lysozyme; PaP technique; DAB/hemalaun; ×160)

between these cells and those of the granular cell tumours as suggested by ultrastructural examination (Sian and Ryan 1981).

The intracellular binding sides for PNL are unknown. Histochemically granular cells contain proteins, lipids and glycol groups (Pearse 1950) which may be the target for peanut lectin. Ultrastructural investigation, however, will be necessary to determine the exact location of the lectin acceptor molecules.

Schwann cells, histiocytes and myoblasts were claimed to be the original cells for the peripheral granular cell tumours (Haisken and Langer 1962; Weiser 1978), whereas pituicytes were regarded as "starter cells" for the tumourettes of the neurohypophysis. In the case of malignant granular cell tumour of the brain, Ule et al. (1975) favored the view of an astrocytic origin.

By our methods this controversy is far from being solved; the fact that we were not able to detect lysozyme which is regarded as a marker for histiocytes in the tumor cells, neither supports nor rejects the hypothesis of the histiocytic derivation of granular cells. We must bear in mind, however, that only some kinds of histicytes contain lysozyme (Pinkus and Said 1977).

In order to clarify the possible astrocytic derivation of granular cells we examined the expression of GFAP, which is widely accepted as the most specific glial marker (Eng et al. 1978; Bignami et al. 1980). Although of glial origin pituicytes of the neurohypophysis do not express GFAP. For that reason it was not surprising that the intermediate-sized filament

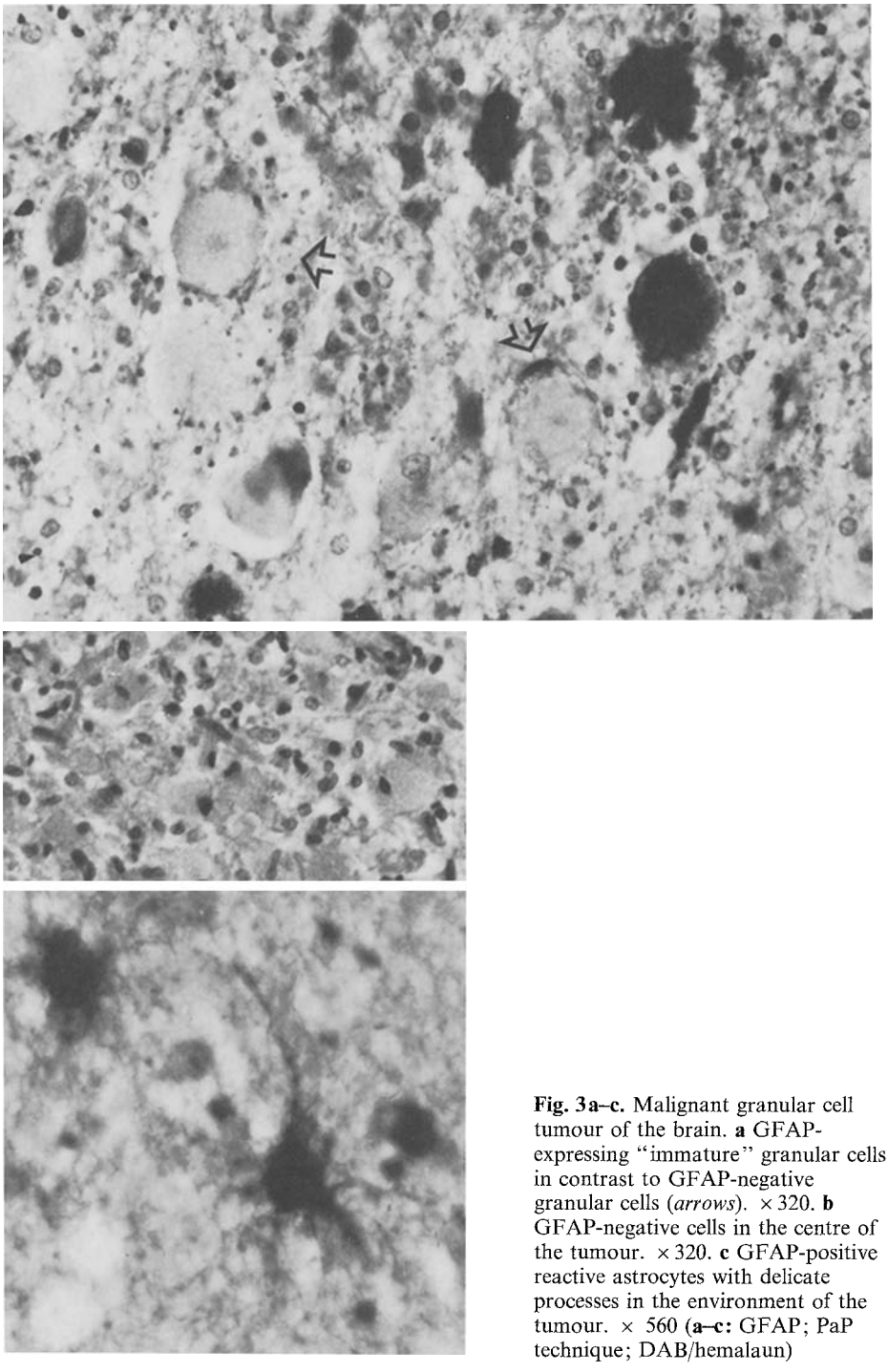

Fig. 3a–c. Malignant granular cell tumour of the brain. **a** GFAP-expressing "immature" granular cells in contrast to GFAP-negative granular cells (*arrows*). × 320. **b** GFAP-negative cells in the centre of the tumour. × 320. **c** GFAP-positive reactive astrocytes with delicate processes in the environment of the tumour. × 560 (**a–c:** GFAP; PaP technique; DAB/hemalaun)

was not present in the granular cells of the tumourettes. In contrast to this some "immature" granular cells at the periphery of the malignant granular cell tumour of the brain were GFAP-positive. This result is interpreted as a strong argument for the astrocytic origin of the granular cells in the brain.

In conclusion our data present PNL as a marker for granular cells regardless of their location; they are a further proof in favor of the view that the granular cell represents only a certain functional state independent of its origin.

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