Immunohistochemical Localization of S-100 Protein and Peripheral Nerve Myelin Proteins (P2 Protein, P0 Protein) in Granular Cell Tumors

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The presence and distribution of nervous-system-specific protein (S-100 protein), peripheral nerve myelin proteins (P2 protein and P0 protein) that have not been given any attention in the field of tumor pathology, and striated muscle-related proteins (myoglobin and myosin) were studied in 18 cases of granular cell tumor by the peroxidase-antiperoxidase method. The granular cells of all cases were negatively stained with anti-striated-muscle-related protein antiserums. On the other hand, they were positively stained with anti-S-100 protein, P2 protein, and P0 protein antiserums. The distribution of P2 protein and P0 protein corresponded with that of characteristic PAS-positive granules on serial sections. Angular bodies in the interstitial cells were also positively stained with anti-P2 protein antiserum and anti-P0 protein antiserum. These results further support the hypothesis that the granular cell tumor is derived from Schwann cells and also suggest that myelin proteins are major core proteins of the characteristic granules and angular bodies of interstitial cells. The biologic significance of these results in relation to myelinogenesis is also discussed (Am J Pathol 1983, 112:139-146)

EVER SINCE the report on myoblastomas by Abrikossoff[1] in 1926, the granular cell tumor has been known widely as a definite pathologic entity. This lesion is found in various organs, such as the tongue,[1-7] skin,[3,5] soft tissue,[8] breast,[5-7] and digestive organs.[9] Multiple granular cell tumor[10] and malignant variants[11,12] have also been reported. Many theories have been advocated concerning its histogenesis. Striated muscle cells,[14] phagocytes,[8] peripheral nervous tissue (Schwann cells),[3,6,10] endoneural fibroblasts, and perineural fibroblasts[5,13] have been cited as the origin of the lesion. Some[14,15] have advocated a multicell origin over a single-cell origin, while others[2,7] have indicated that the disease originates from undifferentiated mesenchymal cells. The neurogenic theory is the most widely accepted. Some immunohistochemical studies[16-18] supporting this theory have been reported recently demonstrating the localization of the nervous-system-specific protein (S-100 protein) in the granular cells. Some ultrastructural, histochemical, and biochemical studies[3,18,13,19] have been made of periodic acid-Schiff (PAS)-positive diastase-resistant characteristic granules in the granular cells.

The histologic origin and the true nature of the characteristic granules were elucidated by immunohistochemical studies of lesions found in various organs and sites in a total of 18 cases. Not only was the localization of S-100 protein in the granular cells demonstrated, but the localization of peripheral nerve myelin proteins (P2 protein and P0 protein) was also demonstrated. In addition, the angular bodies that are often detected in the interstitial cells in the periphery of the granular cells were studied.

Materials and Methods

As indicated in Table 1, 18 cases with lesions in various organs and sites were studied. All surgical specimens were fixed in 10% formalin and embedded in paraffin. For routine histologic study the speci-

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Table 1—Cases of Granular Cell Tumor

Case	Age	Sex		Location
1	35	F	Soft tissue	Left scapular region
2	42	F		Right leg
3	27	M		Left upper arm
4	45	F		Right leg
5	61	M		Right thigh
6	37	F	Skin	Right supraauricular region
7	19	F		Left neck
8	33	M		Left upper arm
9	28	M		Right forearm
10	44	F		Right upper arm
11	87	F		Breast (left)
12	35	F	Vulva	
13	24	F		
14	43	F	Vocal cord	
15	9	F		
16	10	F	Tongue	
17	59	M		
18	46	M		Esophagus

mens were stained with hematoxylin and eosin (H&E), PAS (with and without diastase digestion), and oil red O.

Immunohistochemical studies were performed by the peroxidase-antiperoxidase (PAP) technique, based on the method by Sternberger et al[20] (Table 2). Table 3 gives the 5 different types of antiserums used. Anti-myosin antiserum, against myosin in human skeletal muscle, was supplied by Dr. Hideo Sugita,[21,22] Department of Neurology, Institute of Brain Research, Faculty of Medicine, University of Tokyo. Anti-S-100 protein antiserum, anti-P2 protein antiserum, and anti-P0 protein antiserum were all supplied by Professor Keiichi Uyemura,[23-29] Department of Physiology, Saitama Medical School, prepared respectively from S-100 protein extracted from bovine brain and P2 protein and P0 protein extracted from bovine peripheral nerve. As controls, normal rabbit serum and antiserums absorbed with each of the specific antigens were used in the reaction. As further controls, 12 cases of schwannoma, 7 cases of neurofibroma, 5 cases of malignant fibrous histiocytoma, 15 cases of rhabdomyosarcoma, and 3 cases of malignant lymphoma were studied for similar responses.

Table 2—PAP Method for Demonstrating Specific Proteins

1. Cut 3-μ sections, deparaffinize in xylol, and wash in PBS.*
2. Block endogenous peroxidase with 1% $NaIO_4$ (10 minutes, room temperature); rinse in PBS.
3. Treat with normal swine serum,† 1:20 (30 minutes, room temperature); rinse in PBS.
4. Incubate with rabbit anti-specific protein antiserum, 1:200 (60 minutes, room temperature); rinse in PBS.
5. Incubate with anti-rabbit IgG,† 1:20 (30 minutes, room temperature); rinse in PBS.
6. Incubate with PAP reagent,‡ 1:20 (30 minutes, room temperature); rinse in PBS.
7. Add DAB-H_2O_2 solution§ (2-7 minutes, room temperature; rinse in PBS.
8. Counterstain, dehydrate, and mount.

* Phosphate-buffered saline, pH 7.4.
† DAKO immunoglobulins, Copenhagen.
‡ Soluble complex of horseradish peroxidase/rabbit anti-horseradish peroxidase.
§ 20 mg of 3,3'-diaminobenzidine-4HCl in 100 ml of Tris buffer (pH 7.6) containing 0.005% H_2O_2.

Table 3—Antiserums Used

Anti-striated muscle protein antiserums
Anti-myoglobin antiserum*†
Anti-myosin antiserum*
Anti-nervous tissue protein antiserums
Anti-S-100 protein antiserum*
Anti-P2 protein antiserum*
Anti-P0 protein antiserum*

* Prepared in rabbits.
† Behring Institute, Marburg.

Results

Light Microscopy

All 18 cases studied indicated histopathologic features typical of granular cell tumor, revealing strands, alveolar and sheetlike alignments of large spindle-shaped, spherical, and polygonal granular cells. The granular cytoplasm was eosinophilic to varying degrees, and the small spherical nuclei revealed one or two nucleoli. Different sizes of intracytoplasmic granules were present, but all were PAS-positive, ranging from intensely to weakly positive, and resistant to diastase. In 4 cases stained with oil red O (Cases 1, 3, 8, and 11) the granules were positive. Spindle-shaped interstitial cells filled with angular bodies were detected in 6 cases (Cases 2, 5, 9, 12, 16, and 18), and in these cases the angular bodies were found to be strongly PAS-positive and diastase-resistant.

Immunohistochemistry

The localization of S-100, P2, and P0 protein in peripheral nervous tissue was first studied by the PAP technique. Anti-S-100 protein antiserum revealed definitely positive findings only in the Schwann cells; but they were totally negative in the perineural cells, endoneural fibroblasts, and axons (Figures 1 and 2). For P2 protein and P0 protein positive findings were identified only on the sites of peripheral nerve myelin (Figures 7-9).

The localization of these three proteins in the granular cells was found in all 18 cases of granular cell tumor. S-100 protein exhibited a granular or a

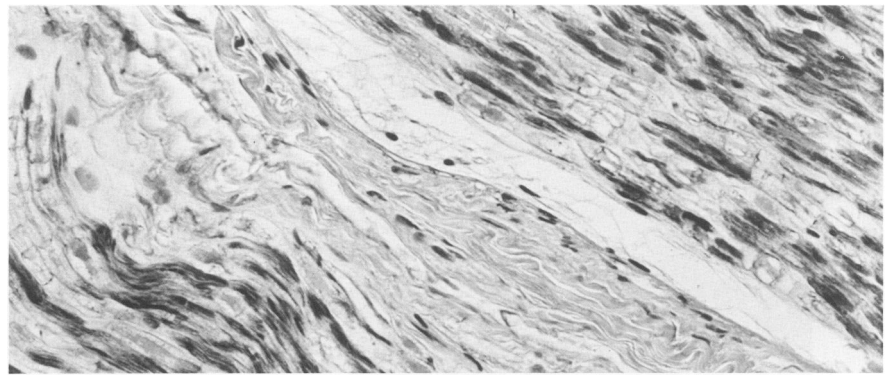


Figure 1—Longitudinal section of nerve bundles. Only Schwann cells show positive immunoperoxidase staining for S-100 protein. (Immunoperoxidase staining for S-100 protein with hematoxylin, ×300)

diffuse positive staining pattern but was negative in the interstitial cells in the periphery of the granular cells (Figures 3 and 10). The positive findings were observed in the cytoplasm and the nuclei in the granular cells, often with more intense reactions in the nuclei (Figure 10).

On the other hand, the positive findings for both P2 protein and P0 protein were similar, showing intracytoplasmic positive granules of various sizes and various staining intensities in the granular cells (Figures 4, 5, 6, and 11). The tendency observed was similar to that for PAS staining. In order to ascertain the correspondence of PAS-positive characteristic granules and the localization of P2 protein and P0 protein, PAS staining and staining by the PAP technique for P2 and P0 proteins were performed on serial sections. Cases that concurrently showed intensely PAS-positive cells and weakly PAS-positive cells were deliberately selected for a definitive demonstration. As a result, P2 protein and P0 protein were demonstrated in the PAS-positive granules (Figures 12 and 13). Positive findings for both P2 protein and P0 protein were also found in the interstitial cells filled with angular bodies around the granular cells (Figures 6 and 11).

The above reactions were invariably negative when normal rabbit serum as well as antiserums absorbed with the respective specific proteins were used. In the control cases, all cases of schwannoma and neurofibroma were S-100-protein-positive, but in the remaining cases the findings were totally negative. Positive findings for P2 protein and P0 protein were detected only in the tumor tissues of all cases of schwannoma and neurofibroma. Although definite positive findings were observed for S-100 protein, P2 protein, and P0 protein in the peripheral nerves, they were negative in the striated and smooth muscles, which happened to be mixed in the surgical specimens. The localization of myoglobin and myosin were demonstrated only in the rhabdomyosarcoma (15 cases) in the control cases, but not in the others. Positive findings were observed in the striated muscles, but not in the granular cells in the granular cell tumor (Figure 14).

Discussion

Despite the fact that granular cell tumor is known widely as a definite pathologic entity, many theories have been proposed as to its histogenesis. Representative theories have postulated its origin from the striated muscle[1,4] or from the peripheral nerve.[3,5,6,10,13] Ever since the first report by Abrikossoff,[1] many have regarded the striated muscle cell to be the cell of

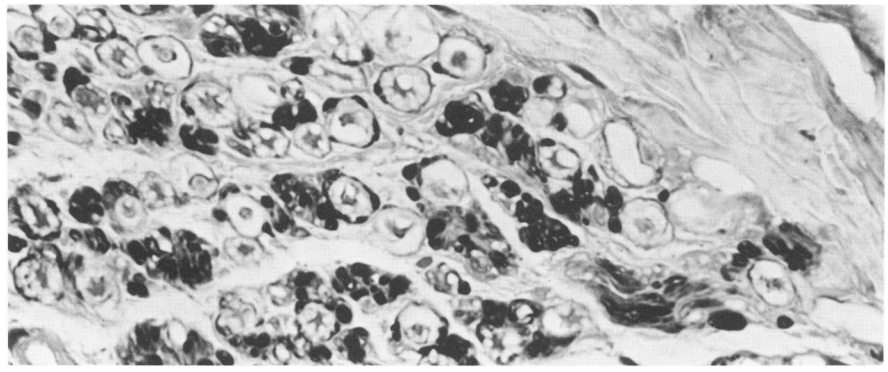


Figure 2—Cross-section of a nerve bundle. Axons are encircled by Schwann cells with positive immunoperoxidase staining for S-100 protein. (Immunoperoxidase staining for S-100 protein with hematoxylin, ×600)

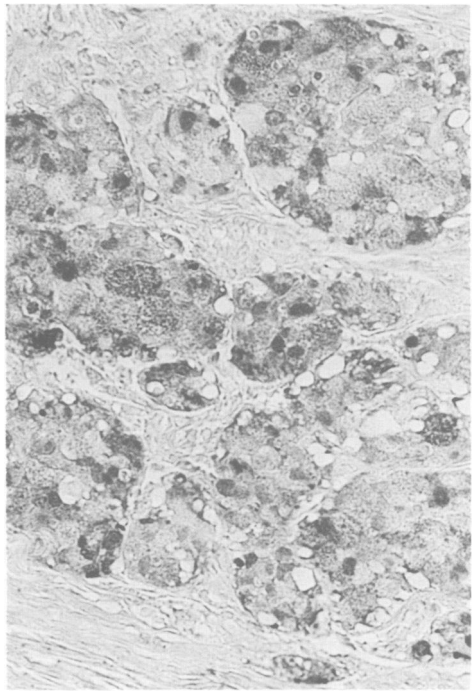


Figure 3 – Strands or sheets of the granular cells stained positively with anti-S-100 protein antiserum. (Case 4, immunoperoxidase staining without counterstain, ×320)

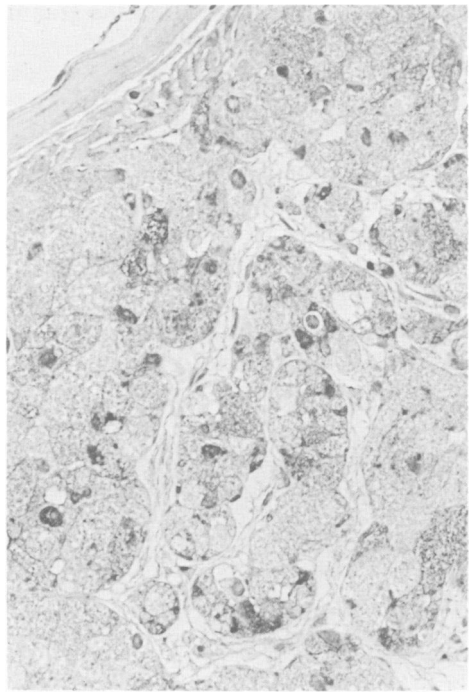


Figure 4 – The granular cells stained positively with anti-P2 protein antiserum. (Case 8, immunoperoxidase staining without counterstain, ×320)

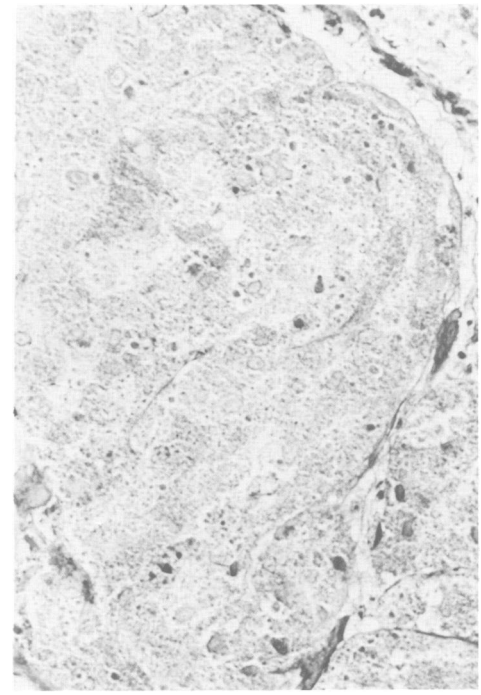


Figure 5 – The granular cells stained positively with anti-P0 protein antiserum. (Case 5, immunoperoxidase without counterstain, ×320)

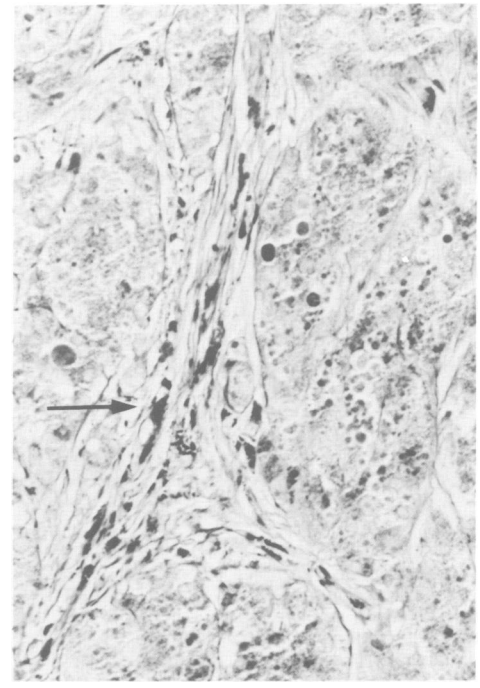
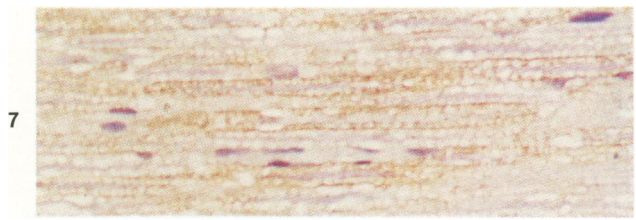


Figure 6 – The interstitial cells (*arrow*) filled with coarse granules, ie, angular bodies, and granular cells show positive immunoperoxidase staining for P0 protein. (Case 12, immunoperoxidase without counterstain, ×320)



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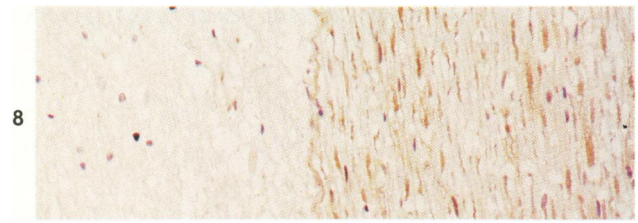

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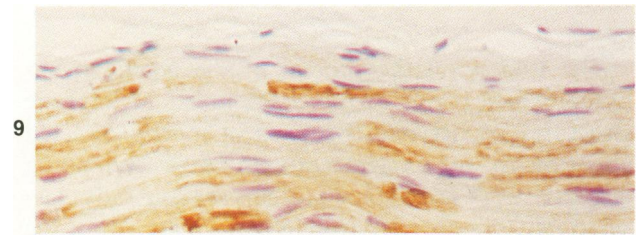

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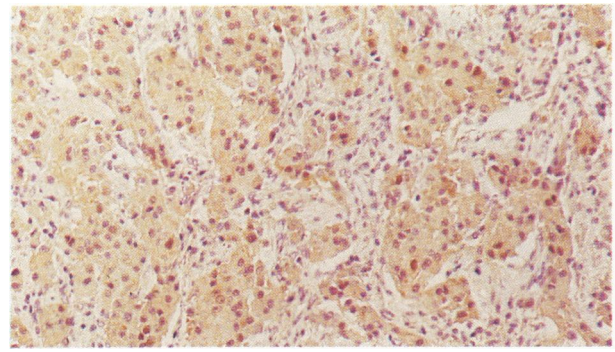

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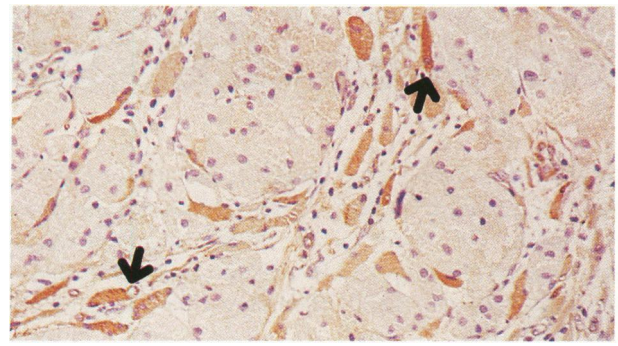

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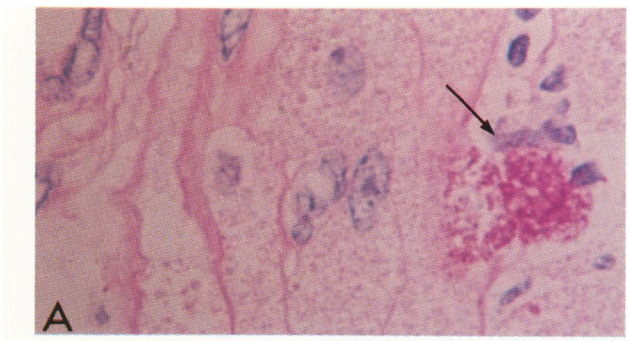

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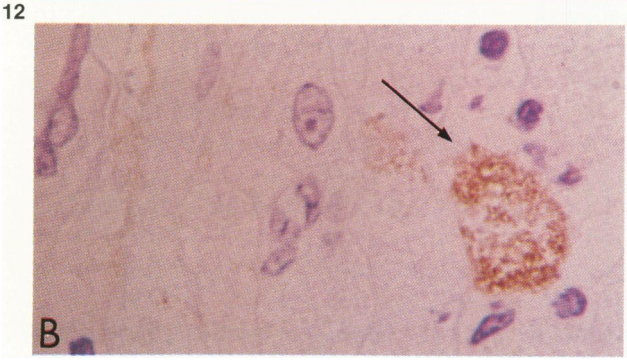

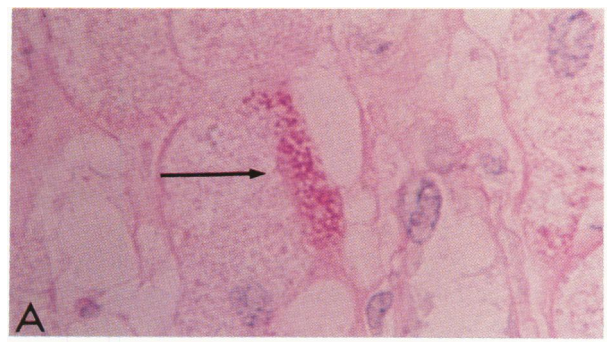

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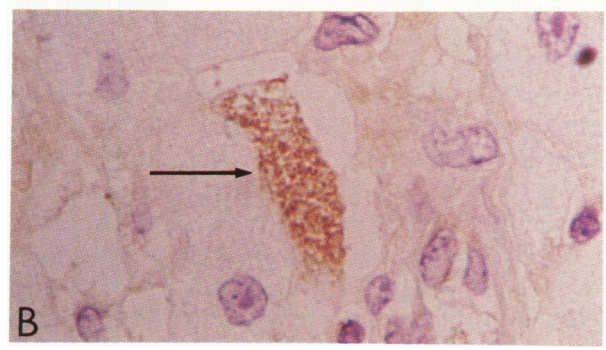

Figure 7 — Longitudinal section of a nerve bundle. Positive immunoperoxidase staining for P2 protein corresponds with the sites of myelin. (Immunoperoxidase with hematoxylin, × 400) **Figure 8** — Transverse section through the sacral region of the rat spinal cord. **Left** — Central nervous tissue (spinal cord). **Right** — Peripheral nervous tissue. Only peripheral nervous tissue shows positive immunoperoxidase staining for P2 protein. (Immunoperoxidase with hematoxylin, × 160) **Figure 9** — Longitudinal section of a nerve bundle. Positive immunoperoxidase staining for P0 protein corresponds with the sites of myelin. (Immunoperoxidase with hematoxylin, × 400) **Figure 10** — Granular cells showing reaction products in their nuclei and cytoplasm. (Immunoperoxidase staining for S-100 protein with hematoxylin, × 160) **Figure 11** — Granular cells and interstitial cells (*arrow*) filled with granules, ie, angular bodies, show positive immunoperoxidase staining for P2 protein. (Case 9, immunoperoxidase with hematoxylin, × 160) **Figure 12** — Serial sections (Case 1). *Arrows* indicate the same cell. **A** — PAS reaction with diastase pretreatment. **B** — Immunoperoxidase staining for P2 protein with hematoxylin. (× 600) **Figure 13** — Serial section (Case 17). *Arrows* indicate the same cell. **A** — PAS reaction with diastase pretreatment. **B** — Immunoperoxidase staining for P0 protein with hematoxylin. (× 600)

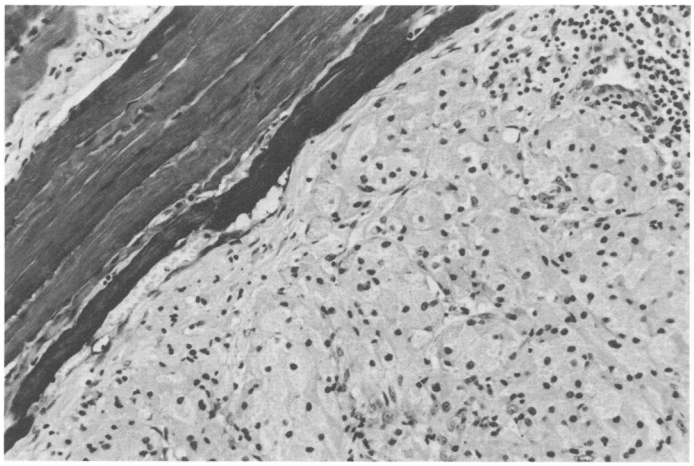

Figure 14 – Case 16. Striated muscle fibers show positive immunoperoxidase staining for myoglobin. The granular cells show negative staining. (Immunoperoxidase with hematoxylin, × 150)

origin. There have been, however, many reports[3,5,7,9] of its occurrence in sites where striated muscle does not exist. The result of the immunohistochemical study using anti-myoglobin antiserum and anti-myosin antiserum also disqualified the striated muscle cell origin because it is well known that immunohistochemical demonstration of myoglobin and myosin is a positive proof of cellular differentiation into striated muscle cells.[30-32]

On the other hand, many reports today advocate the neurogenic theory. The close relationship that has been observed between the lesion and the peripheral nerves and the results of histochemical and electron-microscopic observations have provided support for this theory. Some[5,13] consider the endoneural fibroblast or perineural fibroblast as its origin; others regard the Schwann cell[3,6,10] as its origin. Recently, some have further advocated the Schwann cell origin on the grounds of immunohistochemical S-100 protein localization in the granular cells.[16-18] The localization of S-100 protein was detected in all cases in the present study. It was not identified in the muscular tissue or myogenic tumor, while in the peripheral nerve it was detected only in the Schwann cells but not in perineural cells or endoneural fibroblasts.

Although many reports have addressed the question of the histogenesis of the lesion, not many have referred to the true nature of intracellular granules and the angular bodies. Pearse[13] reported that granules contained lipid, protein, and the glycol groups; whereas Bangle,[8] in a histochemical study, and Thorén,[19] in a biochemical study, reported that the granular component was similar to myelin. Moreover, based on their electron-microscopic and histochemical studies, Fisher et al[3] indicated that the granules were either myelin or its metabolic products.

The studies on the protein of the myelin membrane, which is one of the characteristic membrane structures of nervous tissue, have mostly directed attention to an antigenic protein of demyelinating diseases.[24,33-35] Membrane proteins are generally classified into the integral protein embedded within the membrane and the peripheral protein binding relatively weakly with the membrane. In the peripheral nerves P0 protein[29,36] comprises the major integral protein; whereas P2 protein[26,27,36] comprises the major part of the peripheral protein. With a molecular weight of 28,000, P0 protein is known to be a glycoprotein with a single carbohydrate chain containing glucosamine, mannose, fucose, and galactose and binds to the protein site by an asparagin-N-glycoside bond.[28,29] On the other hand, P2 protein has a molecular weight of 14,000 and is extractable by saline as well as by acidic solutions.[27] These two proteins have not been given any attention in the field of tumor pathology.

P2 protein and P0 protein were identified in the cytoplasm of every case of granular cell tumor in the present study. Furthermore, the correspondence of characteristic PAS-positive granules to the localization of P2 and P0 proteins was demonstrated by the use of serial sections. The results strongly support the theory that considers the granules as myelin or its metabolic products based on electron-microscopic, biochemical, and histochemical studies.[3,8,19] Moreover, it provides a solid basis in support of the neurogenic–Schwann cell theory.

Very much like the granules in the granular cells, PAS-positive diastase-resistant coarse granules filled the interstitial cells around the granular cells. Bangle[8] defined these as angular bodies and assumed that cells containing them were phagocytes. The present

study has provided grounds for the theory that these angular bodies possess P2 protein as well as P0 protein and that, much like the granules in the granular cells, they are either myelin protein or its metabolic products. The fact that S-100 protein is detected in the granular cells but not in the interstitial cells with angular bodies suggests that these interstitial cells are quite different from the granular cells in terms of their nature. Moreover, S-100 protein is known to be absent in phagocytes,[37] and thus it may be worthwhile to mention that it is not inconsistent to regard these cells containing angular bodies as phagocytes.

Finally, concerning the biologic significance of the results of this study, the fact that granular cells contain myelin-specific proteins in the cytoplasm clearly indicates the following: 1) there is endocytotic incorporation of myelin into the cytoplasm, or 2) granular cells *per se* produce myelin proteins. Obviously, there are some[3] who would support the former, namely, that the granular cell tumor is not a true neoplasm but a histiocytic reaction of Schwann cells. However, if the latter holds true, this phenomenon would be extremely interesting in relation to myelinogenesis. Although the basic intercellular interactions that initiate and sustain myelination remain unknown, much evidence has shown that the axon, in some unknown way, stimulates the Schwann cells to form myelin.[38] It may be said that the production of P0 and P2 protein by neoplastic cells in the absence of axons lessens the role of the axon in the synthesis of the components of myelin.

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