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The Expression of PNA-lectin Binding Sites and S-100 Protein in Histiocytic Lesions of the Skin

A Comparative Immunohistochemical Study

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In this work the potential usefulness of antibodies to S-100 protein and the lectin Peanut Agglutinin (PNA) in the differential diagnosis of histocytic lesions of the skin was assessed. Out of 54 deparaffinized specimens studied through an avidin-biotin-alkaline phosphatase technique, 35 proved to comprise S-100 (+) and 12 PNA (+) cells. The results show that antibodies to S-100 protein are useful in distinguishing the "X" (Langerhans'cell) from the "non-X" types of histiocytosis, and cast some shadow on the usefulness of the lectin PNA as a histiocytic marker. (Received May 5, 1987.)

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Cells belonging to the system of mononuclear phagocytes may give rise to a wide variety of benign or malignant, reactive or neoplastic proliferations (1, 2). The precise diagnosis and classification of these lesions relies mainly on morphological criteria, obtained through light and electron microscopic examination, but often presents difficulties. Immunohistochemical techniques, using monoclonal or polyclonal antibodies represent a useful tool in diagnostic pathology, since in many instances they allow the diagnosis of morphologically poorly-differentiated lesions. In this respect, the use of lectins may also be helpful in defining the origin of a cell, by virtue of recognition of cell-surface carbohydrates that have a cell-specific distribution. The lectin Peanut Agglutinin (PNA) has been claimed to be a useful marker for tissue histiocytes (3) yielding sometimes disease-specific labelling patterns (4, 5). This fact prompted us to test the distribution of PNA-binding sites in a group of cutaneous lesions characterized by a predominant histiocytic infiltrate. The aim was to assess the potential usefulness of the lectin PNA in diagnostic pathology, by applying standardized immunohistochemical techniques and to compare the results with those obtained on the same lesions with antibodies to S-100 protein, another potential histiocytic marker.

MATERIAL AND METHODS

1. *Specimens studied*

These included a total of 54 lesions (Table I) that were retrieved from the files of the Laboratory of Pathology, Clinic of Dermatology, Hop. Ed. Herriot (Drs C. Hermier, B. Chouvet). They had been collected over the past 5 years, fixed in formalin and embedded in paraffin. The diagnosis had been established in all cases through standard histological examination of haematoxylin-eosin-stained slides.

2. *Immunohistochemical labelling*

3 μ-thick sections were cut from each specimen, deparaffinized in xylene-toluene and rehydrated through a graded series of alcohol.

An avidin-biotin-alkaline phosphatase reaction was carried out to reveal the presence of S-100 protein, according to the following procedure: (*a*) incubation of slides with normal goat serum (diluted at 2% in Tris-buffered saline-TBS) (10 min), (*b*) incubation with polyclonal antibody to S-100 protein

(Dako, Denmark) (diluted 1:200 in TBS) (45 min at 37°C or overnight at 4°C) (*c*) wash in TBS (10 min), (*d*) incubation with biotinylated antibody to rabbit immunoglobulins (Vector Lab., Burlingame) (diluted 1:200 in TBS) (45 min at 37°C), (*d*) wash as in (*c*), (*e*) incubation with avidin-biotin-alkaline phosphatase preformed complex (Vector Lab., Burlingame) (0.5 μg of avidin and 0.001 U of biotinylated alkaline phosphatase in 5 ml of TBS) (45 min at 37°C). After a final wash in TBS, the reaction was revealed by (*f*) incubating the slides (10–15 min at room temperature) with 10 mg of fast-red TR salt, 2.4 mg levamisole and 2 mg naphthol-AS MX phosphate (Sigma, St Louis) in 10 ml TBS. Slides were then washed in tap water, counterstained with Meyer's haematoxylin and mounted with a medium containing 3 g of gelatin in 25 ml of glycerin and 75 ml of phosphate-buffered saline.

PNA-binding sites were revealed by incubating the deparaffinized and rehydrated slides with a solution of biotinylated PNA lectin (E.Y., San Mateo) (100 μg/ml of TBS) (45 min at 37°C). Thereafter, the steps (*d*) through (*f*) were carried out as above.

Controls were obtained by omitting the first-layer reactants (antibody to S-100 protein and biotinylated PNA); they proved consistently negative.

RESULTS

The results are summarized in Table I.

On normal skin adjacent to the lesions, the antibody to S-100 protein consistently revealed dendritic intraepithelial cells (melanocytes and Langerhans' cells), Schwann cells in dermal nerves and a subset of cells of the eccrine secretory coil. PNA-lectin revealed in most—but not all—cases a generally weak intercellular labelling of the upper epidermal layers, a diffuse labelling of collagen fibers in the mid- and lower dermis, and a labelling of a subset of cells of the eccrine secretory coil. Other cutaneous structures that were occasionally labelled comprised Schwann cells of cutaneous nerves (2 cases) and the basement membrane of dermal vessels (1 case).

With respect to the histiocytic lesions, the two markers generally gave different staining patterns: S-100 protein was consistently detected in cases of histiocytosis X, where 70–100% of cells were positive. In reticulohistiocytoma (comprising both solitary forms and cases of multicentric reticulohistiocytosis), S-100 (+) cells were noted in 6 out of the 7 cases studied (Fig. 1); in most cases the percent of labelled cells was low (5–10%), but in one case it rose up to 70%. S-100 (+) giant or epithelioïd cells were rarely observed in cases of sarcoïdosis (Fig. 2), granuloma annulare, actinic granuloma, atypical fibroxanthoma and histiocytofibroma, where they constituted less than 5% of the total cellular

Table I. *S-100 protein and PNA-binding sites in the lesions studied (the number in parenthesis indicates the percentage of positive cells)*

Lesions	Total	S-100 (+)	PNA (+)
Histiocytosis X	9	9/9 (60–100%)	3/9 (20%)
Hashimoto-Pritzker disease	1	1/1 (90%)	0/1
Reticulohistiocytoma	7	6/7 (10%)	3/7 (25%)
Reticulosarcoma	1	0/1	0/1
Histiocytofibroma	8	5/7 (1%)	0/8
Sarcoïdosis	4	3/4 (1%)	1/4
Granuloma annulare	8	4/8 (5%)	1/7 (70%)
Actinic granuloma	5	5/5 (5%)	2/5 (20%)
Juvenile xanthogranuloma	8	0/8	2/8 (90–100%)
Atypical fibroxanthoma	3	2/3 (1%)	0/3
Total	54	35/53	12/53

ca. 70% in one case.

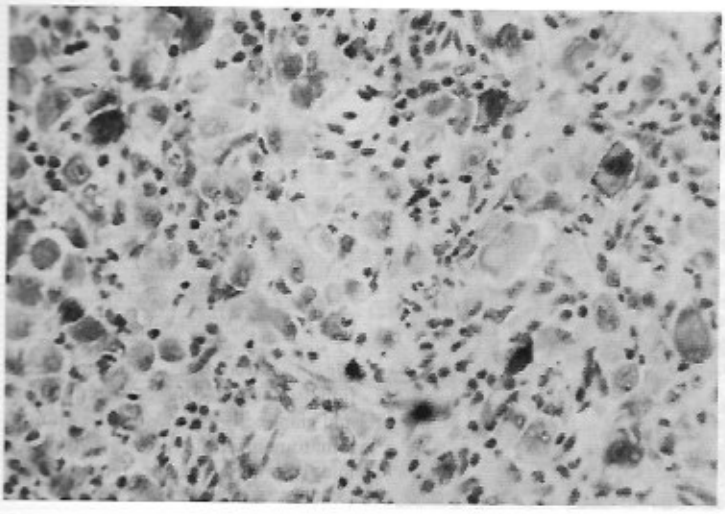

Fig. 1. Multicentric reticulohistiocytosis. Scattered histiocytic cells show a cytoplasmic staining for S-100 protein (×200)

population. The cases of juvenile xanthogranuloma and reticulo-sarcoma remained completely negative.

PNA-lectin binding sites had generally a more restricted distribution among the lesions studied. A strong, cytoplasmic reactivity was obtained on all epithelioïd or giant cells in two cases of juvenile xanthogranuloma. PNA (+) cells were also found in few numbers in occasional cases of histiocytosis X, reticulohistiocytoma, sarcoïdosis (Fig. 3), granuloma annulare (Fig. 4) and actinic granuloma, while the cases of reticulosarcoma, histiocytofibroma and atypical fibroxanthoma were always negative.

DISCUSSION

In the present study the potential usefulness of antibodies to S-100 protein and the lectin Peanut Agglutinin (PNA) in the diagnosis of histiocytic lesions of the skin was compara-

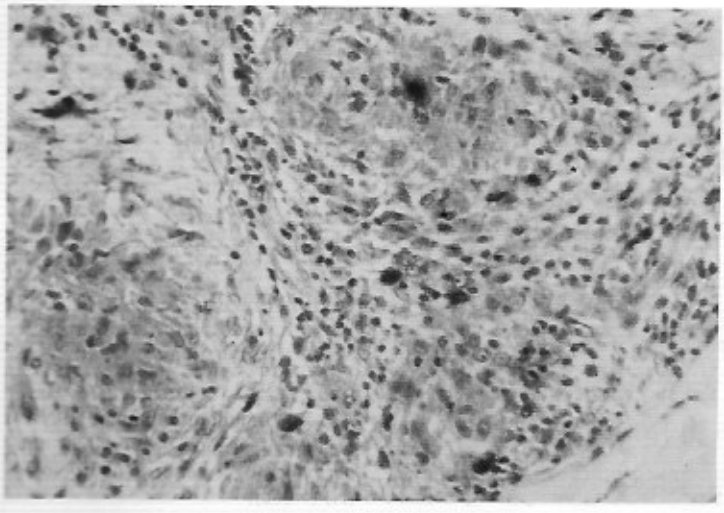

Fig. 2. Sarcoïdosis. Rare S-100 (+) mononuclear cells are observed amidst the granulomatous infiltrate (×200)

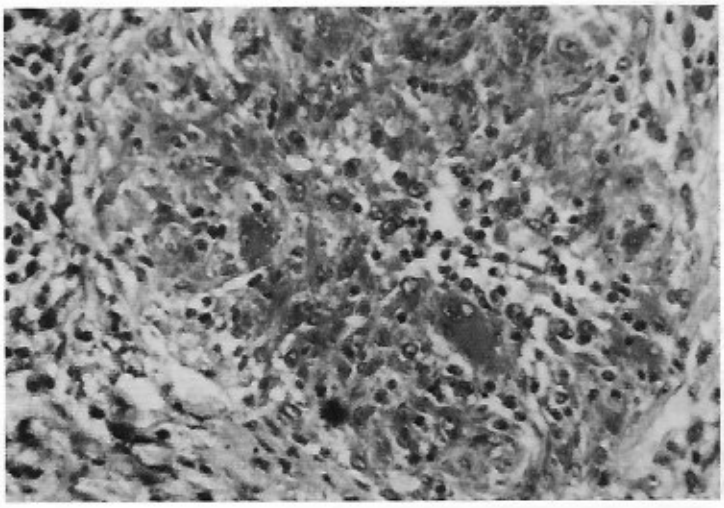

Fig. 3. Sarcoïdosis (adjacent section to that of Fig. 2). Epithelioïd and giant cells composing the granulomatous infiltrate show a cytoplasmic staining with the lectin PNA (×200)

tively evaluated. This investigation was prompted for several reasons: the histiocytic lineage of a cell is often difficult to ascertain by routine histology in tissue sections. Furthermore, histiocytic lesions of the skin encompass a wide clinico-pathological spectrum of benign and malignant entities with overlapping features, that are often difficult to distinguish from each other. Hence, the contribution of markers recognizing the histiocytic origin of a cell and, when possible, its level of differentiation or maturation, is particularly welcome. Immunohistochemical markers that are usually considered in this respect and are applicable in routinely-processed tissue specimens include degradative, hydrolytic enzymes such as alpha-1-antitrypsin, alpha-1-antichymotrypsin and lysozyme (muramidase) (6, 7, 8). The discovery of the presence of S-100 protein in Langerhans' cells (LC) (9) prompted the study of various histiocytic disorders with antibodies directed against that protein. On the other hand, the lectin PNA, derived frome the plant Arachis hypogea and binding specifically to beta-D-galactose (1–3)-N-acetyl-D-galactosamine moieties, has

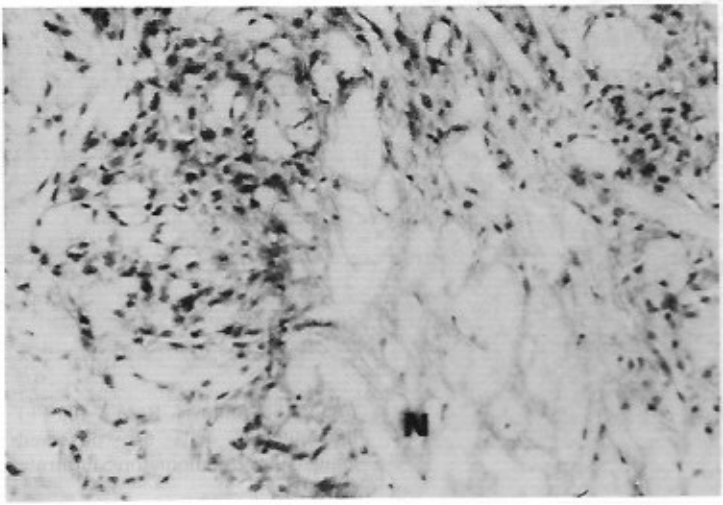

Fig. 4. Granuloma annulare. PNA-positive cells are observed at the periphery of a zone of necrobiosis (N) (×200).

originally been used to study epidermal differentiation. At the epidermal level it recognizes the surface of the keratinocytes of the upper epidermal layers (10). PNA lectin has been reported to be a marker of malignancy of breast (11, 12) and sweat-gland (13) tumours, since in benign forms the labelling it imparts is membranous, whereas in malignant counterparts it becomes membranous and cytoplasmic. On premalignant and malignant epidermal proliferations (solar keratosis, Bowen's disease, squamous-cell carcinoma), PNA-binding sites have been found to be decreased as compared to normal epidermis (14, 15).

Following the work of London et al. (16), it was reported that PNA represented a useful marker of tissue histiocytes (3), helpful in differentiating benign from malignant histiocytes (17) and in establishing the diagnosis of histiocytosis X (5) and Hodgin's disease (4), by virtue of a particular labelling pattern.

The results we obtained in our study with the antibody to S-100 protein are in keeping with data available in literature. As in previous studies, we found low percents of S-100 (+) cells in cases of granuloma annulare (18), atypical fibroxanthoma (19), sarcoïdosis (18, 20) and reticulohistiocytoma (21). High numbers of S-100 cells were found in histiocytosis X, as previously reported (22, 23, 24) and in Hashimoto-Pritzker disease (25). Thus, the consistent finding of the majority of histiocytosis X cells being S-100 (+) further underlines the usefulness of the corresponding antibody in distinguishing the "X" (Langerhans' type) from the "non-X" forms of histiocytosis (26). This marker, even though less specific for LC than the CD 1 (e.g. OKT 6) molecules, is invaluable in diagnostic pathology, since it is easily revealed in routinely-processed specimens. The strong positivity obtained in the case of Hashimoto-Pritzker disease (congenital self-healing histiocytosis) is in keeping with additional results obtained with a panel of monoclonal antibodies, showing the immunohistochemical identity between histiocytosis X and Hashimoto-Pritzker disease (25). In the remaining histiocytic disorders, the percent of S-100 (+) cells was low, never exceeding 10% of the cellular infiltrate (with the exception of one case of reticulohistiocytoma). Previous ultrastructural and immunohistochemical reports have shown the absence of LC from histiocytofibromas, granuloma annulare and sarcoïdosis (20, 27, 28). The nature of the S-100 (+) cells we occasionally observed cannot be defined with certainty. Although there is some evidence that in granuloma annulare some of them are LC (18), these cells could also represent melanocytes (9), T-lymphocytes—a subset of which has been shown to express S-100 protein (29), interdigitating reticulum cells (20, 30) or T-zone histiocytes (18).

As far as the lectin PNA is concerned, our results only partly agree with those already reported. Labelling of histiocytes was always cytoplasmic and diffuse, with the exception of one case of histiocytosis X (where it was membranous). On the whole, much fewer histiocytic lesions were PNA (+) (12 out of 53) than S-100 (+) (35 out of 53). The two markers apparently do not recognize the same cells, since in the two cases of juvenile xanthogranuloma that comprised strongly PNA (+) cells, the latter were completely S-100 (−). Thus, under the conditions used in this study, under which a wide panel of monoclonal and polyclonal antibodies give clear-cut results, PNA lectin did not prove to be a reliable marker of tissue histiocytes. Pretreatment of the sections with neuraminidase has been reported to reveal PNA-binding sites "masked" by sialic acid (15), but this procedure has not been applied by all investigators for identifying histiocytes in situ (3).

In conclusion, we believe that S-100 protein is a useful marker for distinguishing the "X" (LC) from the "non-X" types of histiocytosis. Under the conditions used in this study, PNA-lectin cannot be considered as a reliable marker of tissue histiocytes and therefore it should not be routinely used in this respect. In any event, our results point out to the relative heterogeneity of tissue histiocytes. This becomes obvious when one

considers the varying staining patterns observed not only among different entities but also among different cases of the same entity. More generally, this point should be considered when immunohistochemical markers are used for a diagnostic purpose.

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