Virchows Arch [Pathol Anat] (1985) 406:27–32

Virchows Archiv A

S-100 protein immunostaining in cells of dendritic morphology within reactive germinal centers by ABC immunoperoxidase method *

Antonino Carbone[1], Riccardo Manconi[1], Alessandro Poletti[1], Rachele Volpe[1], and Leonardo Santi[2]

[1] Division of Pathology, Centro di Riferimento Oncologico, I-33081 Aviano
[2] Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

Summary. The "dendritic" and the "interdigitating" (IRC) reticulum cells are commonly reported to have different topographical distribution in the human lymphoid tissue and some peculiar cytochemical and immunohistochemical features. These include the detection of S-100 protein by PAP (peroxidase anti-peroxidase) method only in IRC located in the thymus-dependent areas. In 15 reactive lymphoid tissue specimens (11 lymph nodes, 3 tonsils, and 1 adenoid) the presence of S-100 protein was tested by ABC (avidin-biotin complex) immunoperoxidase method. IRC were constantly positive. Other positive cells were located within the follicular germinal centers; these immunostained cells appeared as a striking network composed of dendritic-shaped processes displaying a finely granular positivity for S-100 protein. It is suggested that by using this very sensitive technique, S-100 protein can also be detected in intrafollicular cells of dendritic morphology.

Key words: S-100 protein – Lymphoid tissue – Avidin biotin peroxidase – Dendritic cells – Interdigitating cells

Introduction

S-100 protein, first detected in nervous tissue (Moore 1965), can be also found in other normal cells and tissues and in their neoplastic counterparts (Kahn et al. 1983). It has been demonstrated in many neoplasms including melanomas, schwannomas, thymomas, and carcinoids by peroxidase anti-peroxidase (PAP) methods (Nakajima et al. 1982a; Nakajima et al. 1982b; Kahn et al. 1983; Lauriola et al. 1984) and by this latter technique, S-100 protein can be also demonstrated in the Langerhans' cells – including those

* This study was in part supported by Grant n.84.00525.44 from "Consiglio Nazionale delle Ricerche" Progetto Finalizzato "Oncologia" Roma

Offprint requests to: A. Carbone at the above address

of histiocytosis X- (Nakajima et al. 1982b; Ide et al. 1984) and in the so called "interdigitating" reticulum cells (IRC) of the lymph nodes (Takahashi et al. 1981; Kahn et al. 1983).

IRC, which are similar to the Langerhans' cells, are particularly abundant in dermatopathic lymph nodes; IRC are also found in the thymus whereas in normal lymph nodes and spleen they are typically located in the thymus-dependent areas (Müller-Hermelink and Lennert 1978; Klug 1980).

In contrast, the so called "dendritic" reticulum cells (DRC) of the lymph node are specific to the B-cell region and, accordingly, are found in large number in the germinal centers (Müller-Hermelink and Lennert 1978; Heinen et al. 1984); these cells have been reported as negative for the presence of S-100 protein by PAP method (Takahashi et al. 1981).

Surprisingly, during a study carried out in our laboratory to investigate the relationships between the lymphoid and the stromal components in the reactive lymph nodes taken from intravenous (i.v.) drug abusers, we found S-100 protein immunoreactivity in cells of dendritic morphology within germinal centers by avidin-biotin complex (ABC) immunoperoxidase method. This result was confirmed in an additional series of reactive lymphoid tissues.

Materials and methods

Human lymphoid tissue for study consisted of 11 reactive lymph nodes (6 from i.v. drug abusers with generalized lymphadenopathy and 5 from other patients with non-specific reactive lymph node hyperplasia), of 3 reactive palatine tonsils and of 1 adenoid. All the tissues were obtained at surgery.

Eleven biopsy specimens were fixed in Bouin's solution and four in B-5 fixative. After paraffin embedding, sections 2–4 μm thick were cut and tested by ABC method according to Hsu et al. (1981) for light microscopy, with some modifications.

ABC method. Deparaffinized sections were soaked in phosphate-buffered saline (PBS) containing 3% hydrogen peroxide for 5 min at room temperature in order to eliminate endogenous peroxidase activity. After washing and incubation with normal goat serum (1:200, 30 min, ABC kit PK-4001 Vector Lab.), each section was incubated at 4° C with the primary antibody (1:100, 12 h, Rabbit anti-cow S-100 protein DAKO, lot n. 113) followed by biotin-labelled secondary antibody (1:200, 30 min, ABC kit PK-4001 Vector Lab.). After washing, ABC complex (ABC kit PK-4001 Vector Lab.) was applied for 45 min. Brown staining was produced by 5 min treatment of diaminobenzidine (DAB) (50 mg of DAB in 100 ml of PBS, pH 7.4, containing 0.01% hydrogen peroxide). Counterstaining was performed with Mayer's haematoxylin.

Controls. Negative controls were performed by treating all sections with immunoglobulin fraction of non-immune rabbit serum (1:100 DAKO) as substitute for the primary antiserum. None of these control sections was immunostained.

Positive control tissues included 1 melanoma, 1 normal skin, 1 neurogenic sarcoma, 1 salivary gland, 5 normal thymuses, and 1 neoplastic thymic tissue. All these tissues showed a pronounced S-100 immunoreactivity when tested by the same ABC procedure.

Additional controls were performed by incubating each tissue section with three different dilutions (1:50, 1:100, 1:200) of the primary antiserum. All the tissues showed a marked variation of the intensity of the immune staining which was proportional to the dilution employed.

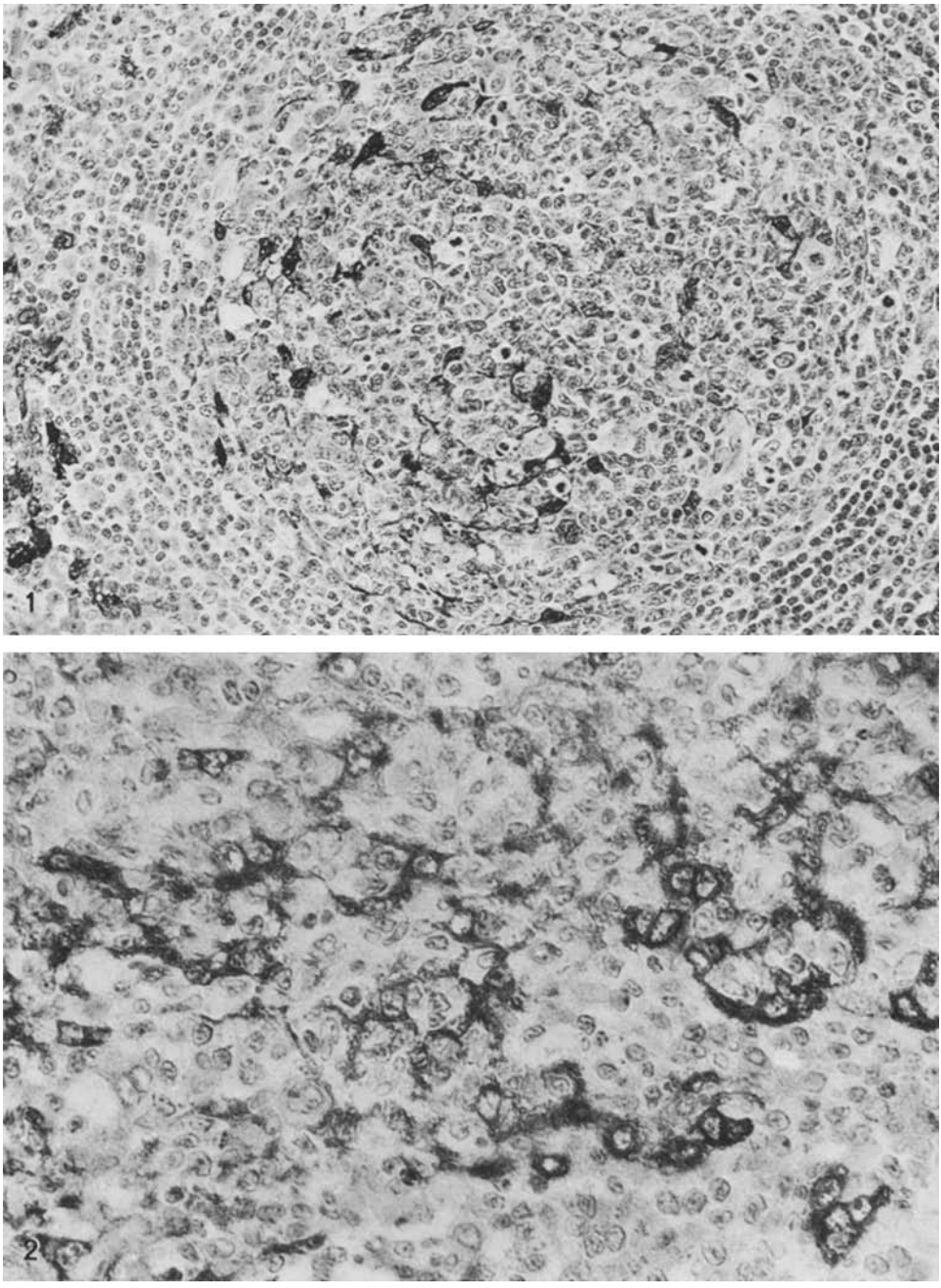

Fig. 1. *Lymph node:* In the extrafollicular area some cells to be referred to interdigitating reticulum cells show a homogeneously diffused positivity for S-100 protein of variable intensity (*bottom left*). Intrafollicular cells positively immunostained for S-100 protein show dendritic morphology. Some scattered macrophages appear negative. (ABC counterstained with Haematoxylin $\times 250$)

Fig. 2. *Tonsil:* In the central portion of a germinal center, cells positively immunostained for S-100 protein appear as a well delineated network which is made up by dendritic processes stained with a finely granular pattern. Some positive cells appear binucleated (ABC counterstained with Haematoxylin $\times 400$)

Results

All the cases showed a strong positivity in the nervous structures present in the lymphoid tissue and this finding was considered as an internal positive control of the specificity of the primary antibody.

Two different patterns of cellular positivity for S-100 protein were found. These differences concerned the topographical distribution of the positive cells, their cytological features, and the intensity and quality of the staining.

Cells positive for S-100 protein, which morphologically resembled IRC, were found predominantly in the interfollicular and paracortical areas. These cells, sometimes arranged in clusters or aggregates, possessed a large cytoplasm containing an irregularly shaped nucleus. They generally showed a very strong and homogeneously diffused positivity in their cytoplasm, which in several instances masked the nucleus (Fig. 1).

Other cells positive for S-100 protein were located within the follicular germinal centers. This finding, although present in all the cases, showed a wide morphological variability ranging from few follicles with a small number of S-100 protein positive cells to a great number of follicular centers which contained a variable amount of positively stained cells. These immunostained cells were often binucleated and appeared as a striking network composed of dendritic-shaped processes displaying a finely granular positivity for S-100 whose degree ranged from intense to moderate (Fig. 1 and 2). Their nuclei were clear, oval in shape, had a regular contour and contained an inconspicuous nucleolus (Fig. 2).

In all the cases the histiocytic reticulum cells (macrophages) and the lymphoid cells of the germinal centers were devoid of the immunoreaction products indicating the presence of the S-100 protein (Fig. 1). Few S-100 protein positive cells were present in the sinuses.

Discussion

Not only do DRC and IRC differ in their topographical distribution within the B and T areas of the lymph nodes but they are also reported to have some peculiar cytochemical and immunohistochemical features.

By enzyme histochemistry, IRC have been reported to be strongly positive for the presence of ATPase which is not demonstrable in DRC (Beckstead 1983); conversely, these latter cells have been found to be strikingly positive for the presence of 5′-Nucleotidase, whose activity is not demonstrable in IRC (Beckstead 1983) or in the starry-sky cells (histiocytic reticulum cells) of the germinal centers (Müller-Hermelink and Lennert 1978).

By means of immunohistochemistry – particularly with monoclonal antibodies – recent studies have demonstrated that it is possible to differentiate IRC from DRC selectively on frozen tissue sections of lymph nodes and tonsils (Gerdes et al. 1983; Parwaresch et al. 1983; van der Valk et al. 1984). In these sections, DRC have been easily identified by their dendritic, web-like, cytoplasm staining (Parwaresch et al. 1983; van der Valk et al. 1984). Unlike the DRC and the histiocytes (macrophages), IRC have been reported

to be positive for the presence of S-100 protein in paraffin embedded specimens immunostained by PAP method (Takahashi et al. 1981; Kahn et al. 1983).

In the present study we found S-100 protein immunoreactivity by ABC method both in cells of dendritic morphology placed within the germinal centers and in cells of the paracortical and interfollicular areas in paraffin embedded lymphoid tissues; to our knowledge, ABC method has not been employed to detect S-100 protein in embedded tissues.

It has been reported that ABC technic provides a superior sensitivity when compared with the PAP method (Hsu et al. 1981). We are tempted to suggest that the use of a very sensitive immunostaining technic like ABC revealed the presence of S-100 protein unexpectedly in a hitherto undescribed location. The present study, not only confirmed the presence of S-100 protein in the IRC but also detected S-100 protein immunoreactivity in intrafollicular cells of dendritic morphology.

Recent studies on semi-thin Epon sections (1 μm) indicated that DRC can be recognized by light microscopy with great accuracy also due to their characteristic double clear nucleus (Peters et al. 1984). In our cases this feature was remarkable in the intrafollicular cells of dendritic morphology positively immunostained for S-100 protein (Fig. 2). Thus, on purely morphological grounds and by also excluding other follicular center cell types, we would suggest the hypothesis that these intrafollicular cells may correspond to DRC.

Further studies are clearly necessary to compare the cytochemical properties and the ultrastructural features of these intrafollicular cells of dendritic morphology which express S-100 protein immunoreactivity in order to confirm this hypothesis.

References

Beckstead JH (1983) The evaluation of human lymph nodes, using plastic sections and enzyme histochemistry. Am J Clin Pathol 80:131–139

Gerdes J, Stein H, Mason DY, Ziegler A (1983) Human dendritic reticulum cells of lymphoid follicles: their antigenic profile and their identification as multinucleated giant cells. Virchows Arch [Cell Pathol] 42:161–172

Heinen E, Lilet-Leclercq C, Mason DY, Stein H, Boniver J, Radoux D, Kinet-Denoël C, Simar LJ (1984) Isolation of follicular dendritic cells from human tonsils and adenoids II. Immunocytochemical characterization. Eur J Immunol 14:267–273

Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Ide F, Iwase T, Saito I, Umemura S, Nakajima T (1984) Immunohistochemical and ultrastructural analysis of the proliferating cells in histiocytosis X. Cancer 53:917–921

Kahn HJ, Marks A, Thom H, Baumal R (1983) Role of antibody to S100 protein in diagnostic pathology. Am J Clin Pathol 79:341–347

Klug H (1980) Ultrastructural differences of interdigitating cells in human lymph nodes. Virchows Arch (Cell Pathol) 34:291–298

Lauriola L, Michetti F, Stolfi VM, Tallini G, Cocchia D (1984) Detection by S-100 immunolabelling of interdigitating reticulum cells in human thymomas. Virchows Arch [Cell Pathol] 45:187–195

Moore BW (1965) A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 19:739–744

Müller-Hermelink HK, Lennert K (1978) The cytologic, histologic, and functional bases for a modern classification of lymphomas. In: Lubarsch O, Henke F, Rossle R, Uehlinger E (eds) Handbuch der Speziellen Pathologischen Anatomie und Histologie Bandteil B. Malignant Lymphomas other than Hodgkin's disease. Springer, Berlin Heidelberg New York, pp 59–68

Nakajima T, Watanabe S, Sato Y, Kameya T, Shimosato Y, Ishihara K (1982a) Immunohistochemical demonstration of S-100 protein in malignant melanoma and pigmented nevus, and its diagnostic application. Cancer 50:912–918

Nakajima T, Kameya T, Watanabe S, Hirota T, Sato Y, Shimosato Y (1982b) An immunoperoxidase study of S-100 protein distribution in normal and neoplastic tissues. Am J Surg Pathol 6:715–727

Parwaresch MR, Radzun HJ, Hansmann M-L, Peters K-P (1983) Monoclonal antibody Ki-M4 specifically recognizes human dendritic reticulum cells (follicular dendritic cells) and their possible precursor in blood. Blood 62:585–590

Peters JPJ, Rademakers LHPM, Roelofs JMM, de Jong D, van Unnik JAM (1984) Distribution of dendritic reticulum cells in follicular lymphoma and reactive hyperplasia. Light microscopic identification and general morphology. Virchows Arch [Cell Pathol] 46:215–228

Takahashi K, Yamaguchi H, Ishizeki J, Nakajima T, Nakazato Y (1981) Immunohistochemical and immunoelectron microscopic localization of S-100 protein in the interdigitating reticulum cells of the human lymph node. Virchows Arch [Cell Pathol] 37:125–135

van der Valk P, van der Loo EM, Jansen J, Daha MR, Meijer CJLM (1984) Analysis of lymphoid and dendritic cells in human lymph node, tonsil and spleen. A study using monoclonal and heterologous antibodies. Virchows Arch [Cell Pathol] 45:169–185

Accepted January 16, 1985