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Immunocytochemical study of human lymphoid tissues with monoclonal antibodies against S-100 protein subunits

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Summary. The present study concerns the immunocytochemical localization of S-100 protein α and β subunits in the cells of human lymphoreticular tissue and their related tumours. The α subunit is mainly localized in dendritic cells, most likely the dendritic reticulum cells (DRCs) located within the germinal centers, while the β subunit is mainly localized in the interdigitating reticulum cells (IRCs) in the paracortical area and in Histiocytosis "X" cells. No immunoreactivity for either subunit was found in the majority of normal lymphocytes, macrophages, malignant lymphoma cells, or xanthoma cells.

The DRCs and IRCs are generally considered to show different distribution in the lymphoid tissues and demonstrate some difference in their immunocytochemical and enzyme-histochemical features. It is suggested that S-100 subunits can be used as useful markers for these two types of dendritic cells and investigation of these subunits may provide more information for the study of human lymphoreticular system.

Key words: S-100 protein (its subunits) – Lymphoreticular system – Dendritic reticulum cell – Interdigitating reticulum cell.

Introduction

S-100 protein (S-100) is composed of heterogenous fractions of low-molecular weight (M.W. approximately 20,000) acidic calcium binding proteins. S-100 was first detected in the central nervous tissue (Moore 1965) and was named according to its partial solubility in saturated ammonium sulfate at pH 7. Two S-100 subunits, termed α and β, have been purified from the S-100 fraction of bovine brain and their amino acid sequences have been elucidated (Isobe et al. 1978; Isobe et al. 1981). Isobe and his co-workers demonstrated that S-100 isolated from the brain of mammalian species (including human) is not a single protein, but a mixture of at least three proteins, S-100ao, S-100a and S-100b, with a subunit composition of $\alpha\alpha$ (S-100ao), $\alpha\beta$ (S-100a), and $\beta\beta$ (S-100b). They also showed that brain S-100 is predominantly composed of β subunit containing proteins, S-100b and S-100a. S-100 appears to be a remarkably conserved protein as demonstrated by the conservative immunological response among a wide variety of vertebrates (Moore 1972).

Until recently, S-100 had been considered to be a neuroectodermal specific protein, primarily localized to glial cells and to Schwann cells. However, S-100 has also been demonstrated in non-nervous tissues, such as adipose tissue, cartilage, myoepithelial cells, and their related tumours (Nakajima et al. 1982; Kahn et al. 1983). Studies have revealed that S-100 is more widely distributed than originally thought. Immunocytochemical demonstration of S-100 has widely expanded its clinical applications.

In the lymphoreticular system, S-100 has been demonstrated in Langerhans cells (LCs) in the skin (Cocchia et al. 1981), interdigitating reticulum cells (IRCs) in the lymph nodes and in Histiocytosis "X" cells (Takahashi et al. 1981; Nakajima et al. 1982; Ide et al. 1984). IRCs, which are supposed to have similar functional and morphological characterization as LCs are located in the thymus-dependent areas of lymph nodes, spleen, and other lymphoid organs (Lennert 1978). Recently, in addition to the above-mentioned cells, S-100 has also been detected by the avidin-biotin immunoperoxidase technique in the intrafollicular cells of den-

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dritic appearance, which are probably the dendritic reticulum cells (DRCs) (Carbone et al. 1985). Although S-100 has been detected immunocytochemically in these two types of cells, the details of distribution of its subunits have not been elucidated.

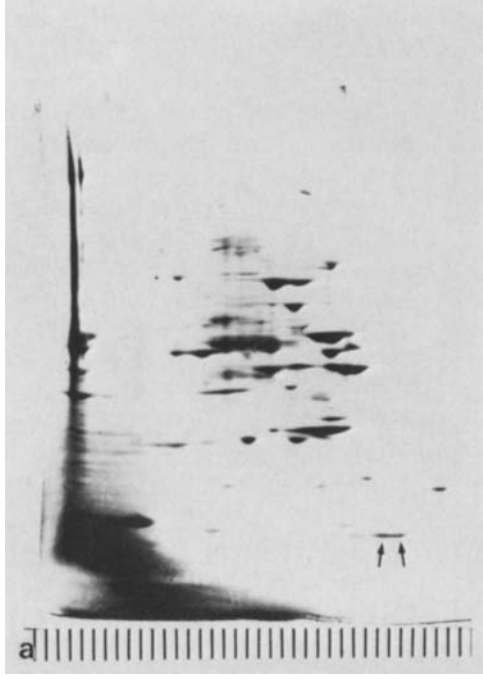
This immunocytochemical study of S-100 subunits in human lymphoreticular tissue, using monoclonal antibodies against S-100 subunits, revealed that α subunit was demonstrated in the dendritic cells in the germinal centers and the β subunit was mainly demonstrated in the IRCs, Histiocytosis "X" cells, and occasionally in the dendritic cells in the germinal centers.

Materials and methods

Materials. The lymphoid tissue examined consisted of 36 cases of non-specific lymphadenitis, 6 cases of dermatopathic lymphadenopathy, 11 cases of tonsils, 3 cases of Letterer-Siwe disease, 4 cases of eosinophilic granuloma, 21 cases of malignant lymphoma (including 8 mycosis fungoides), and 4 cases of xanthoma. All tissues were fixed in 10% formalin and embedded in paraffin for routine surgical pathology. Three to four micron thick sections were cut from each block of 85 cases and used for light microscopic and immunocytochemical studies for S-100 subunits.

The monoclonal antibody against the β subunit was produced by the hybridoma technique in our laboratory (Tanaka et al. 1986). The monoclonal antibody against the α subunit was a product of JIMRO (Takasaki, Japan). Each subclass of immunoglobulin was IgGl. Specificity of each antibody was tested by immunoblotting and dot-immunobinding assay.

Immunoblotting was performed as described by Manabe et al. (Manabe et al. 1983; Manabe et al. 1985). After two dimensional electrophoresis, proteins were transferred electrophoretically from two dimensional gels to nitrocellulose membranes and the blots were stained with monoclonal antibody peroxidase staining. As shown in Fig. 1, the monoclonal antibody against the α subunit reacts only to S-100a ($\alpha\beta$) (Fig. 1C) and the monoclonal antibody against β subunit reacts to both S-100a ($\alpha\beta$) and S-100b ($\beta\beta$) (Fig. 1B). Neither antibody reacts



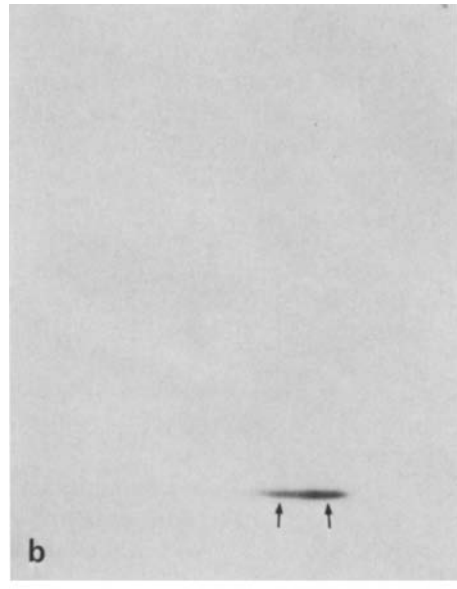


Fig. 1. a Coomasie blue stained gel of polypeptide map of bovine brain soluble proteins. *Arrows* indicate location of S-100 (*left*; S-100b *right*; S-100a). **b** immunostained-blot of the antibody against β subunit used in this study, which reacts to both S-100a ($\alpha\beta$, *right arrow*) and S-100b ($\beta\beta$, *left arrow*). **c** immunostained-blot of the antibody against α subunit used in this study, which does not react to S-100b ($\beta\beta$, *left arrow*) but reacts to S-100a ($\alpha\beta$). S-100ao ($\alpha\alpha$) can not be identified in this polypeptide map because only a small amount of S-100ao is contained in bovine brain

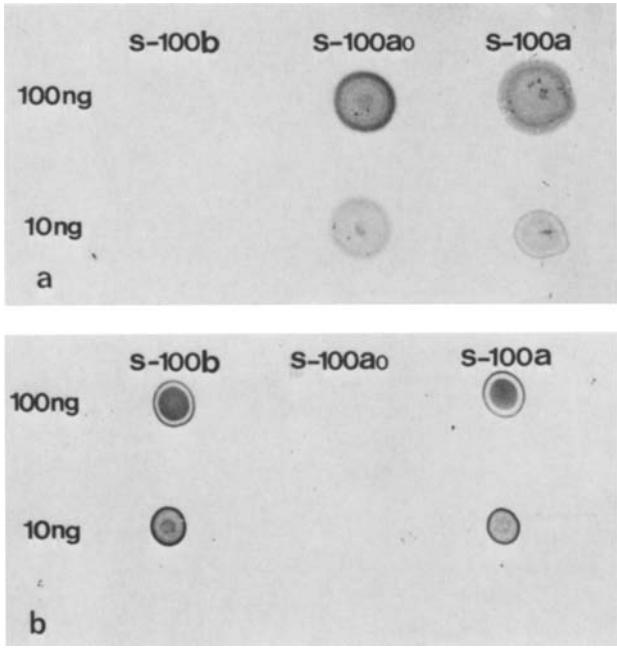


Fig. 2a, b. Dot-immunobinding assay tests of the antibodies used for this study. **a** Anti α subunit antibody which reacts to S-100ao (αα) and S-100a (αβ). **b** Anti β subunit antibody which reacts to S-100b (ββ) and S-100a (αβ).

with any other protein of the extract from bovine brain. S-100ao (αα) cannot be identified in this polypeptide map because only a small amount of S-100ao is contained in the brain.

1μl of each protein solution, which contained 100 ng to 10 ng protein, was dropped on a nitrocellulose sheet and was treated for immunochemical staining according to the method of Manabe et al. (Manabe et al. 1983). Fig. 2 demonstrates that the monoclonal antibody against α subunit reacts to S-100ao (αα) and S-100a (αβ) containing α subunit and does not react to S-100b (ββ) (Fig. 2A), and, in contrast the monoclonal antibody against β subunit reacts to S-100a (αβ) and S-100b (ββ) containing β subunit and does not react to S-100ao (αα) (Fig. 2B).

For immunocytochemistry, the sections were deparaffinized with xylene and soaked in absolute methanol solution containing 1.0% H_2O_2 for 30 min at room temperature, to eliminate endogenous peroxidase activity. After washing and incubation with 10% normal goat serum (DAKO) in the phosphate buffer saline pH 7.6 for 10 min at room temperature, each section was incubated with monoclonal antibodies against S-100 subunits (1:200) at 4 °C for 12 h. After washing, peroxidase labelled anti mouse immunoglobulin (TAGO, 1:40) was applied at 37 °C for 60 min. Brown staining was produced by 2 min treatment of 0.05 M-ammonium acetate citric buffer pH 5.7 containing 20 mg/100 ml of 3-3′-diaminobenzidine tetrachloride (WAKO, Japan) and 0.01% H_2O_2. Counterstaining was performed with Mayers' haematoxylin or methyl green.

Negative controls were performed by treating each section with the culture supernatant of hybridomas which secreted no immunoglobulin, as substitute for the primary antibodies.

Results

In non-neoplastic lymphoid tissues, cells positive for the α subunit stain were found only within the follicular germinal centers (Fig. 3, Fig. 5A). These cells appeared as a network with dendritic-shaped processes and oval or irregular-shaped nuclei displaying frequent binucleation.

This histological finding was observed in all cases of lymphadenitis, dermatopathic lymphadenopathy, and in the tonsils, but the morphological pattern of distribution was widely variable. It ranged from a few follicles with a small number of positive cells to many germinal centers, which contained a varying number of positively stained cells. The morphological appearance of these cells closely resembled DRCs.

In all cases, macrophages (including tingible body macrophages) and lymphoid cells in the germinal centers and other regions were devoid of immunostaining. In the paracortical area no cells, including the dendritic cells which are stained by anti β subunit antibody, showed positive immunoreactivity. The tumour cells of malignant lymphoma, xanthoma, and Histiocytosis "X" (Letterer-Siwe disease, eosinophilic granuloma) showed no immunostaining (Fig. 6A). The nerve fibers in the stroma showed little or no positivity.

In non-neoplastic lymphoid tissues, cells positive for the β subunit were located mainly in the paracortical areas (Fig. 4, Fig. 5B). These immunostained cells possessed an irregularly shaped nucleus and dendritic shaped processes, and resembled IRCs. However, intrafollicular cells were occasionally also positive for the β subunit (9/36 lymphadenitis, 2/6 dermatopathic lymphadenopathy, 6/11 tonsils). These intrafollicular cells which were positive for β subunit demonstrated similar morphological features both in cellular appearance and distribution pattern as observed in α subunit positive intrafollicular cells (Fig. 7). Although no distinct differences between the cases which contain β staining positive intrafollicular dendritic cells and the others which show no β staining positive cells in the germinal center was evident, the majority of the former group showed moderate to marked follicular hyperplasia. Histiocytosis "X" cells showed positive staining for β subunit in all cases (Fig. 6B). Although the majority of lymphoid cells and macrophages were devoid of immunostaining, a few small lymphoid cells occasionally showed positive staining, but they could not be classified accurately. Malignant lymphoma cells and xanthoma cells were also devoid of immunostaining. Scattered non-neoplastic cells with dendritic morphology were positively stained in some cases of malignant lymphoma. The nerve fibers in the lymphoid tissues showed strong positivity.

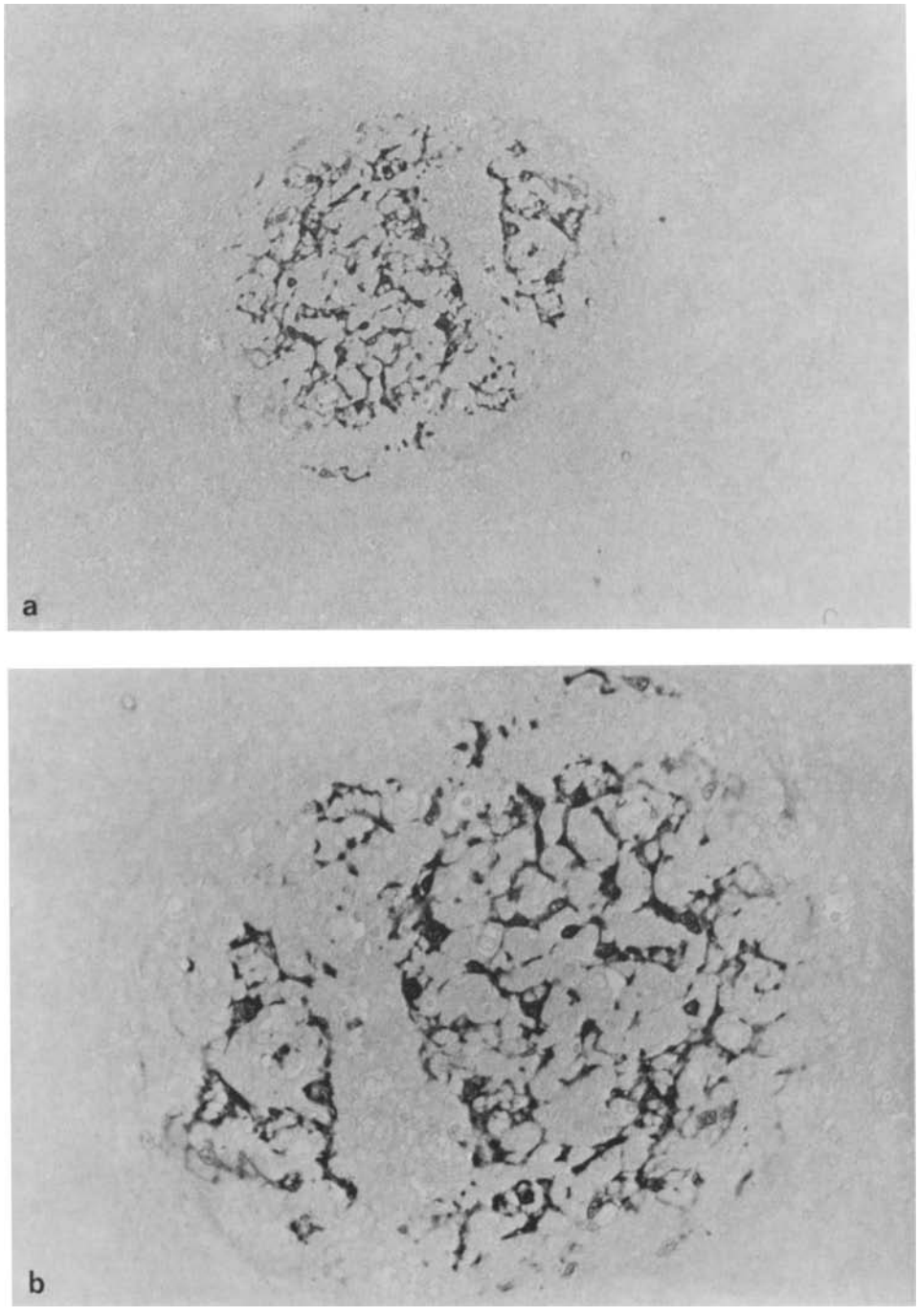


Fig. 3a, b. Immunoperoxidase staining of tonsil with follicular hyperplasia. α staining showing that IRCs are negative but dendritic cells in the germinal center are strongly positive. (**a**; ×120, **b**; ×280, methyl green counterstain)

Discussion

Not only do DRCs and IRCs differ in their distribution within the lymphoreticular system but they also have some different immunocytochemical features (Rausch et al. 1977; Murphy et al. 1981; Beckstead 1983; Naiem et al. 1983). Although the function of these dendritic cells has not been confirmed, they are considered to play an important role in immune response, especially antigen presentation (Kalus et al. 1980; Silberberg-Sinaken et al. 1980).

DRCs, mainly located in the germinal centers (B-cell area), shows 5′-nucleotidase activity on their cell membrane (Beckstead 1983). By means of immunocytochemistry, complement and immunoglobulins have been demonstrated in DRCs and some monoclonal antibodies were reported to react

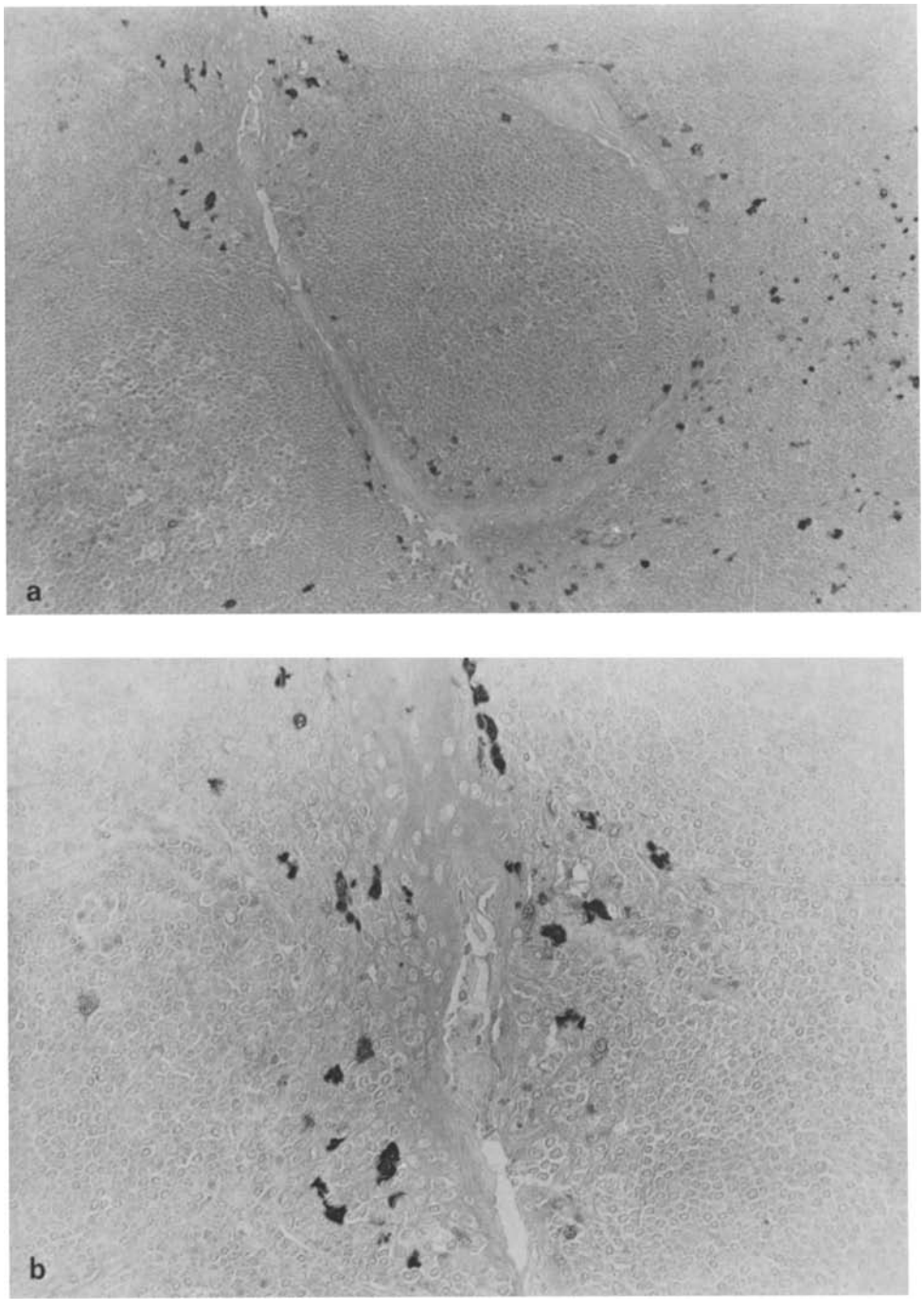


Fig. 4a, b. Immunoperoxidase staining of tonsil with follicular hyperplasia. β staining showing that IRCs are intensely positive but dendritic cells in the germinal center are negative. (**a**; ×120, **b**; ×280, methyl green counterstain)

with DRCs (Naiem et al. 1983). These markers can ordinarily be applied only to frozen sections.

IRCs, located in the paracortical area (T-cell area), have been reported to show ATPase activity (Rausch et al. 1977). Immunocytochemical studies revealed that OKT6 and other monoclonal antibodies are able to detect IRCs in frozen sections (Murphy et al. 1981; Van der Valk et al. 1984).

Many papers concerning the topographical distribution of S-100 in the human lymphoreticular system have been published and S-100 immunoreactivity has been usually described only in IRCs. Recently Carbone et al. reported that S-100 was present in the intrafollicular cells of dendritic morphology in formalin-fixed and paraffin-embedded tonsils and lymph nodes by the ABC immunoperoxidase method (Carbone et al. 1985). They used polyclonal antibody against "whole" S-100

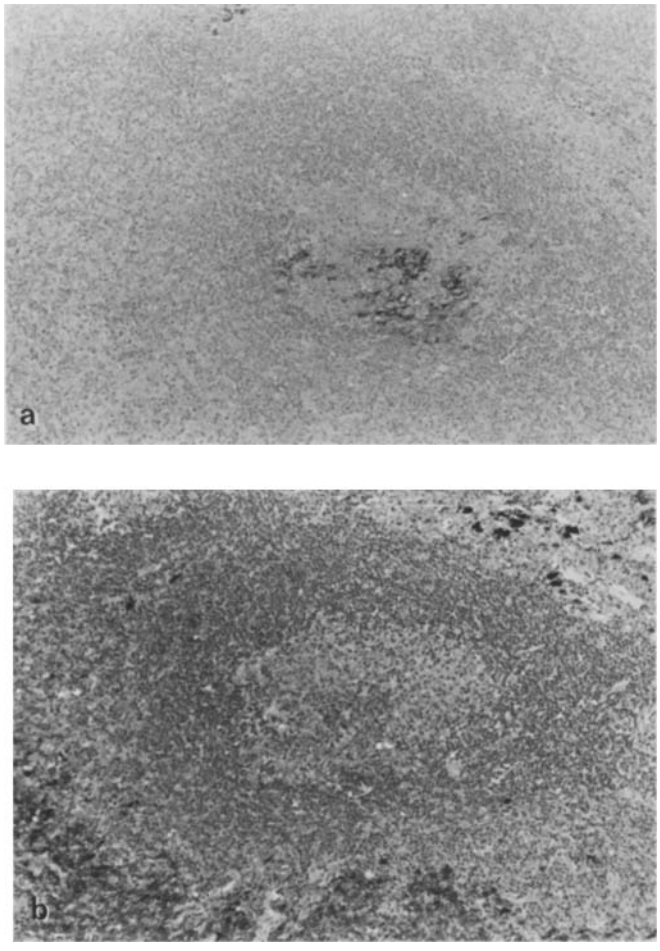


Fig. 5a, b. Immunoperoxidase staining of lymph node of dermatopathic lymphadenopathy. **a** α staining showing that proliferating histiocytic cells and IRCs in the paracortical area are completely negative and that dendritic cells in the germinal center are strongly positive. **b** β staining showing that proliferating histiocytic cells and IRCs in the paracortical area are strongly positive and that dendritic cells in the germinal center are completely negative. (× 120, haematoxylin counterstain)

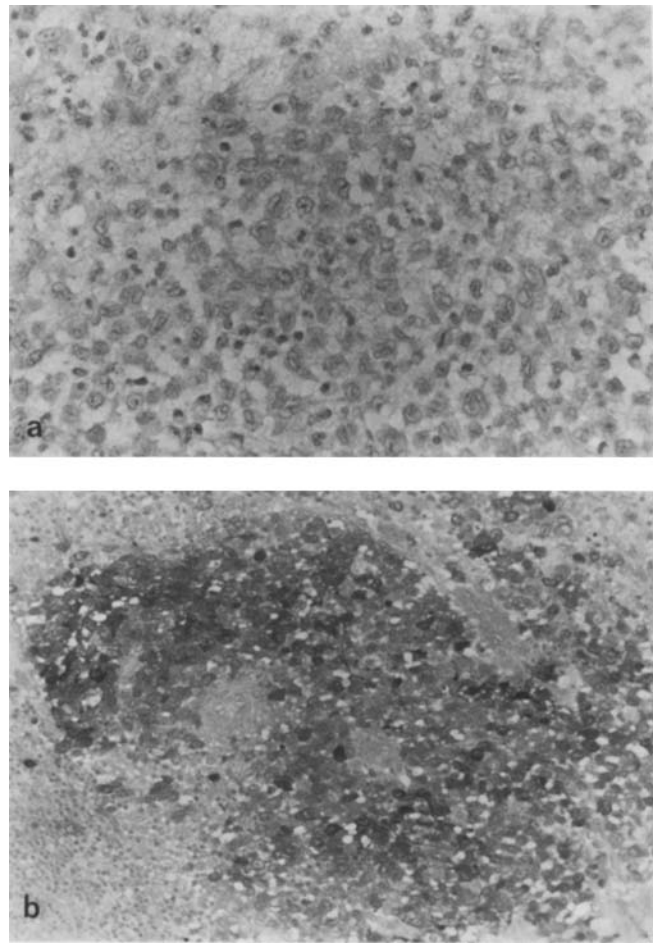


Fig. 6a, b. Immunoperoxidase staining of eosinophilic granuloma of the bone. **a** α staining showing completely negative reaction. **b** β staining showing positive reaction of proliferating histiocytic cells. (× 280, haematoxylin counterstain)

(DAKO) as the primary antibody so that the distribution of its subunits (α, β) was not determined. Our preliminary examinations, using the same antibody against whole S-100 showed very similar findings to those observed by Carbone et al..

To determine the precise localization of S-100 subunits, I conducted the present study and found that α subunit was present in intrafollicular dendritic cells, which are most likely to be DRCs. The β subunit was mainly detected in IRCs and Histiocytosis "X" cells, using a routine indirect immunoperoxidase technique. The β subunit was occasionally demonstrated in the dendritic cells within the follicles. The staining intensity of the β positive dendritic cells in the follicles is similar to that of IRCs which are observed in the paracortical area.

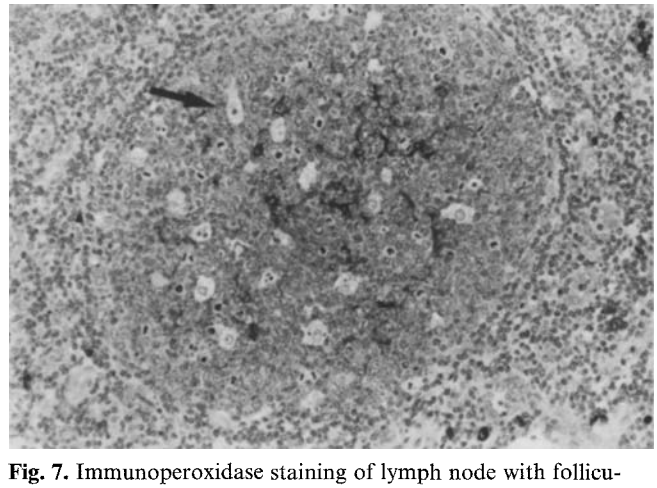


Fig. 7. Immunoperoxidase staining of lymph node with follicular hyperplasia. β staining showing that dendritic cells in the germinal center, as well as IRCs, are occasionally positive while tingible body macrophages ("starry-sky" cells, *arrow*) are completely negative. (× 120, haematoxylin counterstain)

However, these cells were morphologically similar to the intrafollicular cells stained positively for α subunit. Althoug it is difficult to explain this finding clearly, I consider that these cells are more likely to be DRCs. It is difficult to judge whether these β positive dendritic cells in follicles are an unusual type of DRCs, or cells which may contain not only the β subunit but also the α subunit. Nonspecific trapping of S-100 on the membranes of these dendritic cells in the specimens can be ruled out since these cells demonstrated distinct positivity for S-100 not only in the cytoplasm but also in the nuclei.

It is suggested that commercially available polyclonal antibody against "whole" S-100 may contain two components – antibodies against α and β subunits. It is thought that the β subunit is usually dominant to α in the bovine brain S-100 fraction used for immunization and the antibody against β subunit is also much more reactive than the antibody against the α subunit. This may be the reason that S-100 had not been detected in the intrafollicular dendritic cells until Carbone et al. demonstrated S-100-positive dendritic cells within the germinal centers with the sensitive ABC method. No immunoreactivity of the α subunit was detected in the cells within the region other than in the germinal centers. Histiocytosis "X" cells were completely negative for α subunit. These results appear to support a derivation of histiocytosis "X" cells from IRCs and their related cells.

α and β subunits were not demonstrated in the macrophages (including tingible body macrophages), various types of malignant lymphoma cells, and xanthoma cells. This finding suggests that S-100 is a rather specific protein mainly present in "dendritic cells" in the human lymphoreticular system. The small number of lymphoid cells positively stained for the β subunit may correspond to the S-100 positive T lymphocytes reported in recent years (Takahashi et al. 1985). However, as yet, no conclusive evidence has been obtained.

The results of this study are in agreement with the data of Carbone et al. Recently, Kato et al., (Department of Biochemistry, Aichi Prefectural Colony) performed immunocytochemical studies with purified antibody against human S-100 α subunit and demonstrated that intrafollicular dendritic cells were positive for the α subunit (personal communication). However, Takahashi et al. reported that tingible body macrophages and some of sinus macrophages were positive for α subunit by PAP method with non-commercial antisera against S-100 (Takahashi et al. 1984). The observations in the present study differ from their findings and the reasons for this remain unclear. The results of this study, with regard to reaction to anti β subunit, are generally in agreement with the data of Takahashi et al.

It is interesting that S-100 α subunit was detected only in dendritic cells in the germinal center (B-cell region). These are most likely DRCs. The S-100 β subunit was mainly detected in IRCs in the thymus-dependent area (T-cell region). Application of S-100 subunits may provide useful information in the study of so called "dendritic cells" which are considered to play a significant role in antigen presentation. The author speculates on the existance of α subunit positive neoplasm which originates from DRCs and has conducted series of studies on available material. However, such a tumour has not been found in this study. Further examination may reveal a significant functional role for S-100 in lymphoreticular tissue, especially in the antigen presenting system.

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