Dendritic Cells in the Rat Pituitary Gland Evaluated by the Use of Monoclonal Antibodies and Electron Microscopy*

Tetsuji SATO and Kouji INOUE

Department of Anatomy (Division II), School of Dental Medicine, Tsurumi University, Yokohama, Japan

Received January 27, 2000; revised April 13, 2000

Summary. A detailed analysis of the difference in the localization and the immunoreactivity for various surface markers among folliculo-stellate cells, macrophages, and dendritic cells was performed using immunohistochemistry and electron microscopy of the rat pituitary gland. The folliculo-stellate cells were selectively labeled by an antiserum against S100 protein. The majority of dendritic cells were immunoreactive for the MHC class II (Ia) antigen (OX6) and/or the dendritic cell antibodies (OX62). The main population of macrophages was positive for the macrophage antibodies (ED1, ED2, and/or OX42). The cellular density of adenohipophyseal macrophages was significantly lower than that of folliculo-stellate cells and of dendritic cells. All the neurohypophyseal microglial cells were labeled with OX42, while the mAb OX6 labeled a small population of cells different from the cells identified by OX42 in the neurohypophysis. Double-immunoperoxidase staining for ED1 and OX6 revealed that positively stained cells could be classified into ED1"OX6", ED1"OX6", and ED1"OX6" cells. Double staining with OX62 and OX6 mAbs showed that about 60% of the OX62 cells were also immunolabeled with OX62 in the anterior lobe; OX62 detects a subpopulation of dendritic cells but does not recognize macrophage populations. Furthermore, double staining for S100 and OX6 resulted in no S100+ OX6+ cells. At the electron-microscopic level, reaction products for OX6 were confirmed in the cell membrane and labeled cells were distinguished from macrophages and folliculo-stellate cells by distinctive short, broad cytoplasmic processes and the rare presence of cytoplasmic organelles. Such cytological characteristics of the OX6-positive cells in the pituitary gland are similar to dendritic cells. Our results suggest that resident dendritic cells and folliculo-stellate cells are two different main components of interstitial cells in the pituitary gland.

Morphological studies have identified six different types of granulated cells and one type of non-granulated cell in the human anterior pituitary gland. Each type of granulated cell can easily be differentiated from other granulated cells by immunohistochemical staining for the corresponding hormone. Non-granulated cells in the rat anterior pituitary have been found by immunohistochemical techniques to contain S100 protein in the nuclei and cytoplasm (NAKAJIMA et al., 1980). These S100-immunoreactive cells have exhibited the same morphological features as the folliculo-stellate cells reported by many investigators (VILA-POCRE, 1972; GIROD et al., 1975). Over the past years, increasing evidence has indicated that folliculo-stellate cells are important regulators of the pituitary endocrine function (ALLAERTS et al., 1990; INOUE et al., 1999). S100 protein is also expressed in the Langerhans cells in the skin (COCCHIA et al., 1981) and the interdigitating cell in the lymph nodes (TAKAHASHI et al., 1981). According to ALLAERTS et al. (1991, 1996), dendritic cells and folliculo-stellate cells have many morphological and other features in common. The presence of major histocompatibility complex (MHC) class II (Ia) determinants in both mouse and rat pituitary stellate cells, and the weakness or absence of any staining ability for specific macrophage markers in these cells identifies these as folliculo-stellate cells along with lymphoid dendritic cells (ALLAERTS et al., 1991).

Dendritic cells and macrophages constitute two groups of nonlymphoid mononuclear cells involved in immune responses (ROSENTHAL, 1980; STEINMAN and NUSSENZWEIG, 1980; Tew et al., 1982; VAN VOORHIS et al., 1983). Dendritic cells exhibit several features that distinguish them from macrophages, including

---

*This work was supported in part by a fellowship from the Alexander von Humboldt Foundation to T. S., invited to the Institute of Anatomy and Cell Biology in Giessen, Germany.
the morphology of their processes, a more potent accessory cell function, minimal phagocytic capacity, and lower levels of lysosomal enzymes (Tew et al., 1982; Van Voorhis et al., 1983). Ultrastructurally, dendritic cells typically contain relatively few cytoplasmic organelles associated with phagocytic function, while macrophages are rich in lysosomes and endocytic vacuoles (Tew et al., 1982; Van Voorhis et al., 1983; Sato et al., 1998). The progenitors for dendritic cells are present in bone marrow: a small CD34+ subset of hematopoietic progenitors gives rise to all blood cells and dendritic cells (Banchereau and Steinman, 1998). Granulocyte-macrophage colony stimulating factor (GM-CSF) and Interleukin-3 (IL-3), products of activated T cells and other cells, enhance the differentiation of dendritic cells, whereas the macrophage-colony stimulating factor (M-CSF) favors the differentiation of the precursors into macrophages (Inaba et al., 1992; Saunders et al., 1996). Cells that express the marker CD34 contain progenitors for two discrete populations of dendritic cells: the epidermal Langerhans cells, and dermal or interstitial type of dendritic cells (Caux et al., 1996; Strunk et al., 1997). The Langerhans cell progenitor expresses CLA (a ligand for E-selectin and a skin-homing molecule) and lacks CD14 (a marker that is abundant on monocytes) and cannot form macrophages. In contrast, the dermal dendritic cell progenitors lack CLA, give rise to CD14-positive cells that resemble monocytes, and can form either macrophages in response to M-CSF or dendritic cells in response to GM-CSF and TNF-α (Caux et al., 1996; Szabolcs et al., 1996). These findings agree with the report that op/op mice lacking functional M-CSF activity (Yoshida et al., 1990; Wiktork-Jedrzejczak et al., 1990; Naito et al., 1991) show no statistically significant differences in the number of dendritic cells compared with normal litter mates (Takahashi et al., 1995).

An immunohistochemical demonstration of MHC class II antigen or la determinant on the cell surface characterizes cells belonging to the antigen-presenting cell family; under normal conditions, this family comprises macrophages, dendritic cells, and B lymphocytes. These immunocompetent cells can process and present antigens to CD4 T lymphocytes. CD4 helper T cells recognize foreign antigens only in connection with MHC class II molecules (Benacerraf, 1981). However, because of the lack of specific markers which are exclusively reactive with dendritic cells, it is difficult to classify them histochemically. The present study offers a detailed analysis of differences in the localization and immunoreactivity for various surface markers among folliculo-stellate cells, macrophages, and dendritic cells using immunohistochemistry and electron microscopy of the pituitary gland of normal Wistar rats.

MATERIALS AND METHODS

Inbred Wistar rats (n = 18) of both sexes weighing 200–220 g at 7 weeks of age, and kept in a specific pathogen-free environment, were used for the experiments. All procedures followed the guidelines of Tsurumi University for Animal Research. Under ether narcosis, animals were perfusion-fixed via the left ventricle with periodate-lysine-paraformaldehyde (PLP) solution preceded by phosphate-buffered saline (PBS) for light- and electron-microscopic immunocytochemistry. The tissue blocks containing the pituitary gland were immersed in the same fixative for 4–6 h at 4°C. For light-microscopic immunohistochemistry, the tissue was thoroughly rinsed in 0.1 M phosphate buffer (PB; pH 7.4) including 15% sucrose, embedded in Tissue-Tek O.C.T. compound (OCT; Miles, Elkhart, Ind., USA) after being infiltrated overnight at 4°C in a mixed solution (1:1) of PB containing 15% sucrose and OCT, and frozen in liquid nitrogen. Cryostat sections (6 μm in thickness) were cut coronally or sagittally through the brain and pituitary, collected in six parallel serial sets on slides coated with gelatin-chrome alum, and stored at −20°C.

Immunohistochemistry

The distribution of folliculo-stellate cells and various immunocompetent cells was demonstrated immunohistochemically with the streptavidin-biotin-peroxidase complex (SAB) method and the peroxidase anti-peroxidase (PAP) method. The latter was used only for double-staining, as described below. Specific populations of cells were immunostained with the polyclonal rabbit anti-human S100 protein antibody that selectively stained the folliculo-stellate cells (Nakajima et al., 1980) and monoclonal antibodies (mAbs) that recognized a cytoplasmic antigen in monocytes and in monocyto-derived macrophages (EDI) (Dickstra et al., 1985; Damaudeau et al., 1994), membrane antigens of tissue macrophages (ED2) (Dickstra et al., 1985; Barbe et al., 1990), the complement receptor type 3 (CR3) on macrophages and microglia (MRC OX42) (Robinson et al., 1986; Ling et al., 1990), MHC class II (Ia) antigen (MRC OX6) (McMaster and Williams, 1979), and the plasmalemmal antigen of dendritic cells/γ, δT-cells (MRC OX62) (Brenan and Puklavec, 1992). Anti-S100 were purchased from
Chemicon (Temecula, CA, USA), ED1, ED2, and MRC OX62 from Serotec (Blacktown Bicester, UK), MRC OX42 from Pharmingen (San Diego, CA, USA) and MRC OX6 from Cedarlane (Hornby, Ontario, Canada). Sections to be stained with antisera or mAbs were blocked with 20% normal goat serum in PBS for 30 min. Primary antibodies were then used at an appropriate dilution of each one (S100, 1:200–1:800; ED1 and ED2, 1:400–1:1000; OX42, 1:25–1:100; OX6, 1:1000–1:3000; OX62, 1:100–1:400) in 1% bovine serum albumin (BSA; Sigma, St. Louis, Mo., USA) in PBS with 0.03% Triton X-100 (Sigma) overnight at room temperature. The sections were incubated in biotinylated goat anti-mouse Ig (1:600; Dako) or anti-rabbit Ig (1:800; Dako), diluted in 1% BSA in PBS, and then incubated (30 min, room temperature) with peroxidase-conjugated streptavidin (Dako), diluted 1:300. Sections were washed in PBS without Triton X-100 (3×20 min) between each step. Endogenous peroxidase was blocked with hydrogen peroxide (0.3%) in 100% methanol. The immunoreaction was visualized using 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0.01% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.3) for 5–10 min. Some immunostained sections were counterstained with hematoxylin staining. The immunoreaction for S100, OX42, and OX62 was enhanced by 2.5% ammonium-nickelsulfat.

**Double immunohistochemical staining**

A double-immunolabeling was performed by applying the SAB and PAP methods sequentially according to the method by Hsu and Soban (1982), with some modifications. In the first staining sequence, DAB-H2O2 was used as the chromogenic substrate and yielded a brown color. After the first sequence was completed, the sections were washed with Tris-HCl buffer and then PBS, before proceeding to the second staining sequence. The sections were again incubated with mAb at 37°C for 2 h. The peroxidase activity was made visible by incubation in True Blue® peroxidase substrate (Kirkegaard & Perry Lab, MD, USA) for 10 min (for details see SATO et al. 1999). The reaction products were colored blue. The sections were dehydrated only in a graded ethanol series and mounted in Permount (Fisher Sci., Fair Lawn, New Jersey, USA). When the sections were double-stained for S100/OX6, the reaction products of S100 were colored dark blue by DAB-H2O2-Ni. In the second sequence, the immunoreactivity for OX6 yielded a brown color for DAB-H2O2. The localization of the respective antigens on the double-stained sections was essentially the same as that demonstrated by single-staining.

For double-staining, the following five slides were simultaneously stained so that the specificity and reliability of the staining could be checked: 1) SAB (mAb A)-PAP (mAb B), 2) SAB (B)-PAP (A), 3) SAB (A or B)-PAP (omission of the primary antibody), 4) SAB (omission of the primary antibody)-PAP (A or B), and 5) SAB (omission of the primary antibody)-PAP (omission of the primary antibody).

**Cell enumeration**

Quantitative analysis was performed in one to five representative sections of each specimen (n=6). The areas of the anterior, intermediate, and posterior lobes were measured by use of an Image Processor and Analyzer (Luzex III; Nikon, Tokyo, Japan) and cellular density was expressed as the cell number per square millimeter. For assessment of labeled cells, only cells revealing a distinct nucleus were counted. The unpaired two-sided Student’s t test was used to evaluate statistical significance. A value of P<0.05 was considered significant.

**Electron-microscopic immunocytochemistry for OX6**

Pituitary glands were fixed and processed according to the protocols used for light microscopy. Vibratome sections, 30–50 μm-thick, from the tissue blocks were collected directly into cold PBS for further processing as free-floating sections for immunocytochemistry. The same protocols were used for staining except that none of the antibody solutions or washing buffers contained Triton X-100. After visualizing immunoreactive materials with the SAB-method, the sections were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1 h at 4°C, dehydrated in a graded series of ethanol, and embedded in Polybed 812 epoxy resin. Ultrathin sections were collected on 150-mesh fine-bar copper grids, counterstained only with 0.4% lead citrate, and examined in a JEOL 100 CX electron microscope operated at 100 kV.

**RESULTS**

The detailed distribution and heterogeneity of folliculo-stellate cells, dendritic cells, and macrophages were characterized in the normal pituitary gland of rats. These cells were clearly distinguished from granulated cells and pituicytes, respectively, in the anterior and posterior pituitary, by their morphology and location. The folliculo-stellate cells were labeled exclusively for the anti-sera against S100 protein. The majority of dendritic cells were immunoreactive for the MHC class II (Ia) antigen (MRC OX6) and/or for the dendritic cell antibodies (MRC OX62); the
Fig. 1. Legend on the opposite page.
main population of macrophages was reactive to the macrophage antibodies (ED1 and/or MRC OX42). The immunoreactive product of the S100 protein was mainly localized in the cell nucleus and perinuclear cytoplasm, although a few cytoplasmic processes could be identified. ED2, OX42, OX6, and OX62 caused membrane staining and ED1 gave a patchy granular pattern within the cytoplasm. A quantitative comparison of the densities of S100+, ED1+, ED2+, OX42+, OX6+, and OX62+ cells is given in Table 1. Immunohistochemical localization of macrophages revealed ED1- or ED2- positive cells with either a flat spindle-shaped or dendritic appearance. OX6- and OX62-positive cells appeared oval or dendritic, characterized by the extension of dendritic processes.

### Table 1

Numbers of S100+, ED1+, ED2+, OX42+, OX6+, and OX62+ cells in the rat pituitary gland. Each value is the mean ± SD of 8-20 samples. Values with the same superscript (a-i) in a column were significantly different. Values with the same superscript (1-3) were statistically significant compared with the number of corresponding cells in other zones. ND: not detectable.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Number of cells per square millimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior lobe</td>
</tr>
<tr>
<td>S100+</td>
<td>93.5 ± 38.9abc</td>
</tr>
<tr>
<td>ED1+</td>
<td>22.2 ± 15.4abcde</td>
</tr>
<tr>
<td>ED2+</td>
<td>7.7 ± 2.0abcdef</td>
</tr>
<tr>
<td>OX42+</td>
<td>8.2 ± 3.3abcdef</td>
</tr>
<tr>
<td>OX6+</td>
<td>98.9 ± 23.6abcdef</td>
</tr>
<tr>
<td>OX62+</td>
<td>28.3 ± 6.2abcdef</td>
</tr>
</tbody>
</table>

### Anterior pituitary

Cells immunostained for ED1 (Fig. 1a), ED2 (Fig. 1b), or OX42 mAbs (data not shown in Figures) were scattered throughout the anterior pituitary gland and frequently located in the connective tissue close to the blood vessels. Overall, adenohypophysal macrophages (ED1+, ED2+, or OX42+) were significantly smaller than neurohypophysal OX42+ microglia (compare Fig. 1a, b, and c) and the numerical density of adenohypophysal macrophages was comparatively lower than that of neurohypophysal microglia (Table 1). The cellular density of adenohypophysal macrophages was significantly lower than that of S100+ folliculo-stellate cells and OX6+ dendritic cells (Table 1). Immunoperoxidase staining of cryosections of the rat anterior pituitary with the S100 antiserum revealed stellate-shaped positive cells scattered throughout the entire gland, matching the...
description of S100+ folliculo-stellate cells in the rat (Cocchia and Miani, 1980; Nakajima et al., 1980). S100+ cells were found scattered among granulated endocrine cells, and the long slender cytoplasmic processes of these cells extending between granular cells were also immunopositive (Fig. 1d). The localization of S100+ cells was mainly restricted to the epithelial parenchyma cords within the anterior lobe (Fig. 1d), although a few S100+ cells were located in the perivascular compartment close to capillary lumina. The S100+ cells also tended to cluster in association with follicule-like structures (Fig. 1d), but here the immunoreactivity was predominantly localized in the cell nucleus and perinuclear cytoplasm, which is consistent with the diffusible nature of the S100 protein being a member of the Ca2+-binding protein family (Kligman and Hilt, 1988; Allaerts et al., 1990).

Immunolabeling of the anterior pituitary with the MHC-class II marker (OX6) revealed almost the same amount of numerous positive cells as the S100+ cells (Table 1). OX6-labeled cells were distributed throughout the anterior lobe (Fig. 1e). At the light optic level the OX6 mAb intensely stained the cell surface, including one or more processes (Fig. 1f). Using immunoelectron microscopy of rat anterior pituitaries fixed with PLP solution, we were able to distinguish non-granulated cells expressing MHC-class II determinants, whereas no MHC-class II expression was found in the granulated endocrine cells (Fig. 2a), the filamentous folliculo-stellate cells, or macrophages containing a conspicuous lysosomal system (data not shown). At the electron-microscopic level, reaction products were confined to the plasma membrane (Fig. 2b). Some reaction products were seen at tissue edges immediately adjacent to intense-
ly labeled cells. The positive cells had distinctive short, broad cytoplasmic processes and were characterized by possessing very few organelles, various empty vacuoles, and a few lysosome-like structures. The oval nucleus was centrally located with a rim of heterochromatin condensed along the nuclear membrane and a nuclear envelope that exhibited smooth outlines (Fig. 2b). Birbeck granulae (BIRBECK et al., 1961), the hallmark of the Langerhans cell, were not found in the MHC-class II-expressing cells of the pituitary (Fig. 2b). The OX6-immunoreactive cells often contacted the granulated endocrine cells. No specialized cell junctions were observed between these granulated cells and the labeled cells. The dendritic cell marker OKT2 occurred throughout the anterior lobe and was more sparsely present than MHC class II antigen (Fig. 1g; Table 1). OKT2-positive cells had one or more cytoplasmic processes extending between the endocrine cells (Fig. 1h).

Double-immunoperoxidase staining was further performed by using ED1, OX6, OKT2, and SI100 antibodies. It resulted in cells stained either brown, blue (or dark blue), or both. In some instances, however, it was difficult for both antigens to be detected in the same cell. When double-staining with ED1 and OX6 was carried out, positively-stained cells could be classified into the following categories: ED1⁺OX6⁺, ED1⁺OX6⁻, and ED1⁻OX6⁺ (Fig. 3a–c, Table 2).

About 80% of the OX6⁺ cells were also immunolabeled with ED1, while approximately 35% of the ED1⁺ cells were immunostained with OX6. Cells stained by OKT2 and OX6, OKT2⁺OX6⁻, and OKT2⁺OX6⁺ mAb combinations were detected (Fig. 3d, e, Table 2). Nearly 50% of the OX6⁺ cells were immunolabeled with OKT2. Double-staining for SI100 and OX6 resulted in SI100⁺OX6⁻ and SI100⁺OX6⁺ cells (Fig. 3f, g, Table 2). SI100⁺OX6⁻ and SI100⁺OX6⁺ cells were detected with a similar frequency.

In the intermediate zone, a small number of cells labeled with the MHC class II (Ia) antigen (MRC OX6), the dendritic cell antibodies (MRC OX6), and/or the macrophage antibodies (ED1, ED2, or MRC OX2) were located exclusively in the perivascular space of the stromal tissue (data not illustrated; see Tables 1, 2).

**Posterior pituitary**

All the neurohypophyseal microglial cells were labeled with MRC OX42 (Fig. 1c). Light microscopic examination of the tissue stained with the monoclonal antibody OX42 revealed a conspicuous population of immunoreactive microglial cells throughout the neurohypophysis (Fig. 1c). They were often found in pericapillary locations. The microglial cells had small, irregular heterochromatic nuclei and were

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Anterior lobe (Number of cells/mm²)</th>
<th>Intermediate zone (Number of cells/mm²)</th>
<th>Posterior lobe (Number of cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED1 &amp; OX6</td>
<td>+</td>
<td>40.8±31.1a</td>
<td>27.7±20.1</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>70.4±25.4b</td>
<td>31.7±14.3</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>7.8±3.1ab</td>
<td>14.1±10.4</td>
</tr>
<tr>
<td>OX62 &amp; OX6</td>
<td>+</td>
<td>39.1±22.0a</td>
<td>2.7±3.7ab</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>34.0±17.0</td>
<td>17.1±9.9a</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>19.5±6.8a</td>
<td>26.5±12.3a</td>
</tr>
<tr>
<td>SI100 &amp; OX6</td>
<td>+</td>
<td>1.0±1.4ab</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>82.9±14.1a</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>78.1±17.3b</td>
<td>38.6±12.2</td>
</tr>
</tbody>
</table>
bipolar or multipolar cells with processes up to 30 μm in length which branched and had an irregularly stubbled appearance. The microglial cells in the neurohypophysis were similar in morphology to those found in the median eminence and pituitary stalk, except that their processes were far less extended. A small proportion of the neurohypophyseal microglial cells were reactive to the ED1 antibody but were mostly unstained by the ED2 (Table 1). The monoclonal antibody OX6 labeled a small population of cells in the neurohypophysis with a morphology, density, and frequency of distribution different from the cells identified by OX42 (Table 1). Some elongated cells, most prominently along the border with the pars intermedia, were stained by macrophage-associated antibodies (ED1 or ED2) or the antibody directed against the MHC class II (Ia) antigen (OX6). Immunoperoxidase labeling with the S100 antiserum did not detect any positive cells in cryo-sections of the posterior pituitary gland (Table 1).

**DISCUSSION**

Folliculo-stellate cells have been described as non-hormone secreting cells with a stellate morphology in the adenohypophysis (Salazar, 1963; Kagayama, 1965; Vila-Porcile, 1972; Girod et al., 1985; Inoue et al., 1999), that are also immunopositive for S100 protein, which was believed to be a neuroectodermal marker (Cocchia and Miani, 1980; Nakajima et al., 1980). On the other hand, based on their phagocytic and interleukin-6 (IL-6) secreting capacities, folliculo-stellate cells were also suggested to have macrophage-like characteristics (Vankelecom et al., 1989). Moreover, cells characterized by an immunoreactivity to MHC-class II antigen and a dendritic morphology have so far been demonstrated in both human and rat anterior pituitary. Using double immunolabeling of rat anterior pituitaries, Allaerts et al. (1996, 1997a, b) reported an overlap between the populations of MHC-class II-expressing and S100 protein-expressing cells, although folliculo-stellate cells showed only a few gold labels for MHC-class II determinants in spite of numerous gold particles labeling the S100 protein in the cytoplasm. They claimed that MHC-class II-expressing and S100-positive cells showed ultrastructural characteristics that had been previously ascribed to folliculo-stellate cells. In their observations, a 10–20% proportion of the S100-positive cells were immunopositive for the MHC-class II marker OX6 in the rat anterior pituitary. Markers characteristic of dendritic cells such as the L25 antigen and the OX62 antigen are present in anterior pituitaries from human and rat, respectively (Allaerts et al., 1996). In addition recent evidence has shown that the population of folliculo-stellate cells of the anterior pituitary might be heterogeneous (Nakagawa et al., 1985; Sbatbi et al., 1988; Tachibana and Yamashita, 1988; Allaerts et al.,

**Fig. 3.** Double-immunoperoxidase staining of rat anterior pituitary. a-c. Cells stained with ED1 (brown) and OX6 (blue). Positively labeled cells can be grouped into ED1*OX6* (double arrowheads), ED1*OX6* (arrow), and ED1*OX6* (arrowhead) cells. d and e. Cells labeled with OX62 (brown) and OX6 (blue). OX62*OX6* (double arrowhead) and OX62*OX6* (arrowhead) cells are detected. f and g. Cells immunoreactive for S100 (dark blue) and OX6 (brown). Two different phenotypes of S100*OX6* (arrows) and S100*OX6* (arrowheads) cells are found. Bars: 20 μm (a, d, f), 5 μm (b, c, e, g)

**Fig. 4.** Diagram illustrating the organization of a dendritic cell (DC), macrophages (Mϕ), and folliculo-stellate cells (FC) in the rat anterior pituitary under normal conditions. *1?* Intermediate phenotypes (S100*OX6* cells) between DC and FC were not identified in the present study. *2* This group (ED1*OX6* cells) is composed of DC derived from the same progenitors as monocytes, immature DC, and MHC-class II-expressing Mϕ.
Several immunohistochemical studies revealed that S100 protein is expressed in Langerhans cells in the skin (Cocchla et al., 1981), in interdigitating cells in the lymph node (Takahashi et al., 1981) and in dendritic cells at various locations (Uccini et al., 1986).

In the present study, however, the OX6-immuno reaction was found in the Langerhans cell of rat skin used, whereas no S100 protein was immunolabeled (unpublished data). Also in this experiment, we were not able to detect S100+OX6+ cells in the anterior pituitary. The immunohistochemical procedure for MRC OX62 revealed the localization of dendritic cells in the anterior pituitary; OX62 detects only a subpopulation of dendritic cells but does not recognize macrophage populations (Brennan and Puklavec, 1992). At the electron-microscopic level OX6-immunoreactive cells can be distinguished from macrophages and folliculo-stellate cells by distinctive short, broad cytoplasmic processes and the rare presence of cytoplasmic organelles including lysosomes, multivesicular bodies, and/or mitochondria. Such morphological characteristics of the cells immunolabeled in the pituitary gland are similar to the la-positive dendritic cells found in other non-lymphoid tissues of rats (Sato et al., 1996, 1998; Sato, 1998). Furthermore, our present results are supported by a report that the folliculo-stellate cells in the mouse pituitary do not show an immunoreactivity to the hemopoietic marker T200 (Alaerts et al., 1991). Bronchioalveolar, well differentiated squamous cell carcinomas and regional lymph nodes to lung tumors contain a high density of S100-positive dendritic cells, while small cell cancer in the lung, poorly differentiated squamous cell carcinoma, and unstimulated lymph nodes do not (Zedd and Müller, 1993). Therefore, the difference between the present results and those reported by Alaerts et al. (1996) may be attributed to the pathological conditions of animals used in the experiment, although we cannot rule out the possibility that some S100-positive folliculo-stellate cells may be immunolabeled with MHC class II (la) antigen in the pituitary gland.

The present investigation provides immunohistochemical evidence for antigen-presenting cells that represent a significant component of the interstitial cells in the rat pituitary gland; the cells are positively stained with MHC class II (la)-specific mAb (OX6). The majority of OX6-immunoreactive cells show a dendritic appearance, characterized by multiple cytoplasmic processes extending between the parenchymal granulated cells in the anterior pituitary. Double-immunoperoxidase staining for OX62, S100, and OX6 results in a large number of OX62+OX6+ and S100+OX6+ cells in the adenohypophysis. An immunohistochemical demonstration of MHC class II antigen or la determinant of the cell surface characterizes cells belonging to the antigen-presenting cell family; under normal conditions, this family comprises macrophages, dendritic cells, and B lymphocytes. The present investigation indicates the presence in the pituitary gland of resident dendritic cells in addition to folliculo-stellate cells and macrophages/microglia under non-pathological conditions. More than 80% of OX6-positive cells in the anterior lobe and 60% of OX6-positive cells in the intermediate zone are also immunoreactive to ED1; OX6-positive cells are scarcely detected in the posterior lobe. ED1 recognizes a CD68-like antigen and the amount of ED1 expressed reflects phagocytic activity (Damoiseaux et al., 1994). Therefore, it appears there is substantial heterogeneity in the dendritic cells with phagocytic activity (Sato, 1998; Sato et al., 1998). In the pituitary gland, ED1+OX6+ cells may be composed of dendritic cells derived from the same progenitors as monocytes, immature dendritic cells, and MHC-class II-expressing macrophages (Fig. 4). The number of OX6+ cells on the double-stained sections is less than that demonstrated by the single-staining; this discrepancy may be explained by technical problems.

In the neurohypophysis, three morphologically distinct types of glial cells—protoplasmic pituicytes, fibrous pituicytes, and microglial cells—comprise the cellular elements interspersed among terminal arborizations of the hypotalamuc neurosecretory neurons which secrete vasopressin, oxytocin, and their associated neurophysins (Dellmann, 1973; Wittkowski, 1986). OX42 immunolabels the plasmalemma of all microglial elements, but does not label the fibrous or protoplasmic pituicytes or endothelial cells. According to reports by Mandel and Morris (1995), microglial cells in the neurohypophysis of rats were well labeled with OX42, but most of them were unstained by the ED1 or ED2 antibodies. A small subset of the microglia in other regions of the CNS appear to be ED1-immunoreactive (Perry and Gordon, 1991; Sato et al., 1996). Some OX42+ cells appear to be immunoreactive for ED1, and others to be immunonegative in the posterior pituitary examined in the present experiment (Table 1). The majority of macrophages are weakly la positive compared with dendritic cells, and the density of la is not sufficiently high for these cells to function as accessories or stimulators (Klinkert et al., 1982). It has been suggested that the amount of la expressed might be critical for accessory or stimulatory processes (Nussenweis et al., 1980). Dendritic cells are professional antigen-presenting cells and are able to initiate an
immune response (Klinkert et al., 1981, 1982; Steinman, 1991). In contrast to macrophages and other antigen-presenting cells, dendritic cells are able to activate naïve T cells (Inaba and Steinman, 1984). The immunocytologic features of dendritic cells include their irregular outline, bean-shaped eccentric nucleus, absence of acid phosphatase (or the enzyme concentrated in a juxtanuclear spot), and strong surface expression of MHC class II (Ia) antigens (Van Voorhis et al., 1982; Austyn, 1987; Knight and Stagg, 1993). In the neurohypophysis, the dendritic cell is not a main component of the interstitial cell.

REFERENCES


VAN VOORHIS, W. C., M. D. WITMER and R. M. STEIN- 
MAN: The phenotype of dendritic cells and macro- 

VILA-PORCILE, E.: Le reseau des cellules folliculo-stellaires 
ettes follicules de l'adenohypophyse du rat (pars dis- 

WIKTOR-JEDRZEJCZAK, W., A. BARTOCCH, A. W. JR, FER- 
rante, A. AHMED-ANSARI, K. W. SELI, J. W. POLLARD 
and E. R. STANLEY: Total absence of colony-stimulat- 
ing factor in the macrophage-deficient osteopetrotic 
(op/op) mouse. Proc. Nat. Acad. Sci. USA 87: 4828-4832 

WITTKOWSKI, W.: Pituitocytes. In: (ed. by) S. FEDOROFF and 
A. VERNADAKIS: Astrocytes Vol. 1. Academic Press, 

YOSHIDA, H., S. I. HAYASHI, T. KUNISADA, M. OGAWA, S. 
NISHIKAWA, H. OKAMURA, T. SUDO, L. D. SHULTZ and 
S. I. NISHIKAWA: The murine mutation 'osteopetrosis' 
(op) is a mutation in the coding region of the macro- 
phage colony stimulating factor (Csfm) gene. Nature 

ZEID, N. A. and H. K. MÜLLER: S100 positive dendritic 
cells in human lung tumors associated with cell 
differentiation and enhanced survival. Pathology 25: 