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FUNCTIONAL COMMUNICATION BETWEEN THE ENDOCRINE AND IMMUNE SYSTEM AT THE PITUITARY : PLEIOTROPISM OR TRANSIENT DIFFERENTIATION STAGES WITHIN THE FOLLICULO-STELLATE AND DENDRITIC CELL GROUP

by

WILFRIED ALLAERTS
DEPARTMENT OF IMMUNOLOGY, ERASMUS UNIVERSITY ROTTERDAM,
P.O. BOX 1738, NL-3000 DR ROTTERDAM (THE NETHERLANDS)

ABSTRACT

Functional communication between the endocrine and immune systems has been described in terms of the common expression of signalling molecules, receptors and coupling proteins. However, information transfer in immune-endocrine interactions may also result from transgression of diffusion barriers in the body by immune effector cells. The presence of myeloid cells in the anterior pituitary (AP) of mouse and rat under non-pathological conditions, illustrates the importance of immune accessory cells in immune-endocrine interactions in the AP. Using double immunogold-labelling of ultrathin cryo-sections of rat AP, we demonstrate the presence of MHC class II^+ $S100^+$ cells with ultrastructural characteristics of pituitary folliculo-stellate (FS) and dendritic cells (DC). Our data suggest the heterogeneity of the group of $S100^+$ FS cells, in that this group may contain a subpopulation of lymphoid DC, which are also present in other endocrine organs. Moreover, our data indicate that dispersed cell populations of the rat AP contain a fraction of immunocompetent cells, that are capable of stimulating T lymphocyte proliferation *in vitro*. The latter cell fraction is also enriched in $S100^+$ and MHC class II^+ cells. Furthermore, in the present study a review is given of the problem of the nomenclature of pituitary folliculo-stellate cells and dendritic cells, whereby special attention is given to the quite different ways in which both cell types are defined.

Key words : anterior pituitary, folliculo-stellate cell, dendritic cell, mixed leukocyte reaction assay, ultrastructural heterogeneity.

INTRODUCTION

Cross-talk between the endocrine and immune systems has been described in terms of a variety of phenomena, occurring at different organismal levels, including the anterior pituitary (AP). Due to the pivotal role of the AP as an endocrine

regulation centre, immune-endocrine interactions at this level may have a profound effect on endocrine regulatory systems. Cross-talk phenomena between the two systems have been characterized in terms of the common expression of signalling molecules (classical hormones, cytokines, growth factors, ...), of receptor molecules, of binding proteins and of other effector molecules ; in addition, the specificity of the information transfer in these immune-endocrine interactions was believed to rely on local signal decoding mechanisms based on target cell specific post-receptor transduction (AUDINOT *et al.*, 1992). On the other hand, it is well known that immune reactions of the body against non-self are exerted by cellular as well as humoral responses, and that diffusion barriers in the body may, in certain conditions, be transgressed by immune effector cells.

The presence of myeloid cells in the AP of mouse and rat under non-pathological conditions (ALLAERTS *et al.*, 1991), suggests a role for immune accessory cells in immuno-endocrine interactions in the AP. Our present data indicate that these normally resident myeloid cells of the AP indeed are immunocompetent cells, and that they are capable of stimulating T lymphocyte proliferation *in vitro*.

Using double immunogold-labelling of ultrathin cryo-sections of rat AP, we demonstrated the presence in the AP of MHC class II-positive and S100-positive cells with ultrastructural characteristics of pituitary folliculo-stellate (FS) cells and dendritic cells (DC) (ALLAERTS *et al.*, 1995 ; in press ; present data). In the present paper we speculate on the heterogeneity of the group of FS cells, an S100-positive resident cell group of the AP with endocrine regulatory function (ALLAERTS, et al., 1990), and suggest that this group contains a subpopulation of DC. DC are a cell group with prominent immune accessory function (INABA and STEINMAN, 1987), they originate from the bone marrow (STEINMAN *et al.*, 1974) and they are present in several endocrine organs under non-pathological conditions (KABEL *et al.*, 1988 ; VOORBIJ *et al.*, 1989 ; ALLAERTS and DREXHAGE, 1994).

The attribution of endocrine regulatory and immune accessory functions to the group of folliculo-stellate and/or dendritic cells, raises the question whether these cells represent a pleiotropic cell group or whether they are a heterogenous group of cells with divergent functional states. We call this the PLEIOTROPISM-HETEROGENEITY problem. Related to this first question is the question of the embryological origin of these cells, and whether the different phenotypes encountered represent ultimate or transient differentiation stages within a cell lineage. As is well known in case of the monocyte-macrophage-dendritic cell lineage, the possible answer to the latter question is provided by the stability of immunohistochemical marker expression and functional activity related to maturation within a given cell lineage. In the subsequent paragraph we will briefly summarize the history of the problem of the embryological origin and immunohistochemical markers of the folliculo-stellate and dendritic cells. The clinical relevance of our findings of myeloid and/of immune accessory cells in the AP is discussed with respect to the role of dendritic cells in the onset of endocrine autoimmune diseases (ALLAERTS and DREXHAGE, 1994).

HISTORY OF NOMENCLATURE

Folliculo-stellate (FS) cells of the AP represent a non-hormone-secreting pituitary cell type with stellate morphology and surrounding follicular cavities (VILA-PORCILE, 1972). Previous studies on intercellular communication between rat pituitary cell types have revealed that FS cells attenuate the action of both stimulatory and inhibitory secretagogues on hormone-secreting cells *in vitro* (BAES *et al.*, 1987 ; ALLAERTS and DENEF, 1989), and therefore FS cells are involved in paracrine communication in the AP. FS cells have also been designated as the local interleukin-6 (IL-6) producing cells of the AP (VANKELECOM *et al.*, 1989, 1993). Some controversy exists whether FS cells originate from the neuroectoderm because of the expression of the S-100 protein (NAKAJIMA *et al.* 1980 ; COCCHIA and MIANI, 1980) or whether precursors of these cells are already present in the stomodeal ectoderm, called Rathke's pouch (GON, 1987). The S-100 protein was first described as a protein characteristic of the nervous system (MOORE, 1965), but thereafter immunohistochemical studies revealed S-100-immunoreactivity in various cell types not derived from the neuroectoderm (TAKAHASHI *et al.*, 1984), nor from the neural crest, as for instance in mesodermal chondrocytes (STEFANSSON *et al.*, 1982). It was suggested that S-100 expression may result from neuroectodermal induction (STEFANSSON *et al.*, 1982), which hypothesis was strengthened by the observation that immunoreactive S-100 in pituitary FS cells is first detected in the marginal cell layers of the pars tuberalis and pars intermedia, which regions are in close contact with the pars nervosa (SHIRASAWA *et al.*, 1983 ; COATES and DONIACH, 1988).

However, a number of studies revealed that S100 protein is also expressed in bone marrow-derived DC such as in the skin Langerhans cell (LC) (COCCHIA *et al.*, 1981), in DC in the airway epithelium (ZEID and MULLER, 1993) and in DC at various locations (UCCINI *et al.*, 1986). DC with the exception of follicular dendritic cells (TEW *et al.*, 1990) are defined as a distinct class of cells originating from a precursor(s) in the bone marrow (STEINMAN *et al.*, 1974). DC display a dendritic morphology and abundantly express HLA-DR (MHC-class II) antigens (NUSSENZWEIG *et al.*, 1981).

They are found in lymphoid as well as non-lymphoid organs and in peripheral blood and lymph (DREXHAGE *et al.*, 1979 ; HOEFSMIT *et al.*, 1982 ; KNIGHT 1984). Although a common progenitor of all cell types representing the DC class has yet to be found (KAMPERDIJK *et al.*, 1994), SANTIAGO-SCHWARZ *et al.*, (1992) have shown that granulocyte-macrophage colony-stimulating factor (GMCSF) in combination with tumor necrosis factor (TNF) induce the differentiation of DC together with monocytes and macrophages (mø) from progenitors in the $CD34^+$ stem cell compartment in neonatal cord blood. It was suggested that DC morphology and marker expression may fluctuate in culture or are transient features acquired by certain cells of the monocyte-mø lineage (SANTIAGO-SCHWARZ *et al.*, 1992). DC may become distinguishable from the monocyte compartment by their weak expression instead of abundant expression of the CD14 antigen (THOMAS *et al.*, 1993). DC also differ from mø's and monocytes by their prominent instead of dim antigen presenting capacity (STEINMANN and NUSSENZWEIG, 1980) and by

their limited instead of abundant (mø) acid phosphatase activity (KAMPERDIJK *et al.*, 1985). Typically, in DC an acid phosphatase spot is found in juxtanuclear position, close to the groove of the indented nucleus (ARKEMA *et al.*, 1991b). Recently also some characteristic, — although not unique —, markers of the DC class have been identified, such as the L25 antigen in the human DC (ISHII *et al.*, 1985) and the OX62 antigen in the rat DC (BRENAN and PUCKLAVEC, 1992).

To conclude, from the present literature survey it appears that, although FS cells and DC share morphological features and may both express the S-100 protein, the two cell types are defined in two quite different manners. FS cells are regarded as an ultimately resident cell type of the AP with enduring regulatory function in the endocrine gland and presumably originating from the (neuro) ectoderm ; DC are regarded as an ubiquitous cell type with possibly transient features and belonging to the bone marrow-derived monocyte-mø lineage. The presence of the latter cell type in the endocrine glands is rather regarded as an expression of the immune surveillance function of these cells, or of the immune accessory potential of these cells which will develop into an ongoing immune response in particular pathological conditions.

MATERIALS AND METHODS

Light Optical Immunohistochemistry

LO immunohistochemistry was performed on cryo-sections of mouse, rat and human anterior pituitaries and paraffin sections of human pituitaries. Human pituitaries were obtained at autopsy from subjects that died from non-endocrine causes. Mouse and rat pituitaries were from Balb/c and C57bl strains and Wistar, BioBreeding (BB) and Lewis strains respectively. Technical details of the immunostaining methods used are published elsewhere (ALLAERTS *et al.*, 1995).

Electron Microscopical Immunohistochemistry

EM immunohistochemistry was performed on cryo-sections of Wistar rat pituitaries fixed in periodate-lysine-paraformaldehyde (PLP) solution (MCLEAN and NAKANE, 1974). Halved pituitaries were fixed for 1 hr on ice, and thereafter were transferred into 2.3 M sucrose in 0.1 M phosphate buffered saline (PBS) on ice for cryo-protection (GRIFFITHS, 1993). After 60-90 min incubation in the cryo-protectant, the tissue blocks were mounted on specimen holders of the Ultracut FC4 ultramicrotome (Reichert Jung, Leica) and plunged by hand into a dewar vessel with liquid nitrogen (LN2).

Immunolabelling of the pituitary tissues was according to the Tokuyasu thawed cryo-section technique (post-sectioning immunolabelling) (GRIFFITHS, 1993) and immunogold staining method (DE MEY *et al.*, 1981). Ultrathin cryo-sections of 80-90 nm were cut with the FC4 ultramicrotome with diamond knife at $-100°C$. Sections were transferred to copper grids that previously had been covered with a

carbon-coated Formvar film (Formvar 1595E ; Merck). To transfer the sections to the grids, a wire loop containing a drop of 2.3 M sucrose was quickly brought into contact with the sections (TOKUYASU method) (TOKUYASU, 1973), thereby preventing the sucrose from freezing, which would hamper the stretching of the sections. Subsequently, the sucrose drop was removed from the sections by laying the grids with the sections faced down on top of a 2 % (w :v) gelatin layer (Merck) in 0.1 M PBS.

Sections were labelled according to the immunogold staining method described in detail elsewhere (ALLAERTS *et al.*, 1995). Primary antisera used were the anti-rat MHC-class II monoclonal antibody (moAb) OX6 (gift of dr. N. Barclay, Oxford, U.K.) in dilutions between 1 :2 and 1 :10 and the polyclonal anti-bovine-S100 (Dakopatt's, Glostrup, Denmark) diluted 1 :100 to 1 :25. Dilutions of antisera and immunogold complexes were made in 0.1 M PBS containing 0.05 % (w :v) of the acetylated and partly linearized bovine serum albumin (BSA) combined with 0.05 % (v :v) Tween-20 (Aurion BSA-C ; Aurion, Wageningen). Goat anti-mouse IgG-25 nm gold complex (Aurion) diluted 1 :20 was used as the first step conjugate. Double immunostaining was performed by first incubating the grids with the OX6 moAb followed by first step conjugate and rinse in PBS with 0.2 % glycine (Fluka), and a second staining sequence with the anti-S100 serum followed by a protein A-15 nm gold complex (Aurion) (diluted 1 :20).

Contrasting and air-drying of the sections was performed according to TOKUYASU's method (1978) for heavy metal staining in an organic polymer scaffold, in order to prevent the collapsing of the sections upon air-drying. After three rinses in distilled water the grids were placed on a drop of filtered 4 % (w :v) uranyl acetate (pH 8.0) in 0.3 M oxalic acid for 3 min, and subsequently placed on a drop of 1.2 % (w :v) low viscosity methyl cellulose (0.25 poise ; Sigma) and filtered 1 % uranyl acetate (pH 4.0) in distilled water for 3 min. The methyl cellulose powder was dissolved at 90°C in distilled water, matured in the refrigerator for 2 days under stirring and then centrifugated at 100,000 g at 4°C in a Sorvall Ultracentrifuge OTD-75 B (Dupont, Wilmington, Delaware, USA). Grids were removed from the methyl cellulose-uranyl acetate mixture using 3 mm wire loops, and excess fluid was removed by carefully blotting the grids on filter paper, yielding a final interference colour of the dried film between gold and blue. Grids were examined in a Philips EM 301 electron microscope.

Functional studies with pituitary Dendritic Cells and Folliculo-Stellate Cells

Populations enriched in FS and dendritic cells were obtained from Wistar rat anterior pituitaries as previously described (ALLAERTS *et al.*, 1993 ; BAES *et al.*, 1987 ; ALLAERTS *et al.*, 1995, in press). Anterior pituitary lobes were enzymatically dissociated into single cells and separated into enriched cell populations by velocity sedimentation at unit gravity in a linear BSA gradient (ALLAERTS *et al.*, 1994), and by a magnetic cell separation system (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) using a moAb against rat MHC-class II determinants (OX6 from Seralab, Sussex, England).

The accessory function of pituitary derived DC or FS cells was evaluated using the autologous mixed leukocyte reaction (MLR) assay, with Wistar rat spleen T lymphocytes as responder cells, and Wistar rat anterior pituitary cells as stimulator cells. DC from Wistar rat spleen, prepared according to the method of KNIGHT *et al.*, (1986) modified after HAVENITH *et al.*, (1992), were used as a positive stimulator cell control population. Concanavalin A (Con A ; Sigma) was used as an internal standard for stimulating T cell proliferation. Pituitary cells were either used as freshly dispersed cell populations, or as enriched cell populations obtained by the BSA gradient sedimentation or MACS separation method. Aliquots of 1.5×10^5 responder T cells in 100 μl volumes were mixed in flat-bottomed microtitre plates with 100 μl volumes of stimulator cells, in a stimulator-responder cell ratio varying between 1 :2 and 1 :32. In some experiments even lower stimulator-responder ratio's were tested. Stimulator cells were X-irradiated for 18 min (2000 rad) before mixing with the T lymphocytes. After 5 days in culture, 0.5 μCi ^{3}H-thymidine was added to each well, and, after 16 hr incubation at 37°C, cells were harvested on filter paper and ^{3}H-thymidine incorporation measured in a liquid scintillation counter (1205 Betaplate, LKB Wallac). Control DC-enriched cell populations were prepared from minced and enzymatically digested spleens. Splenic cell suspensions were cultured overnight at 37°C in a 5 % CO_2 incubator in adherent conditions (KNIGHT *et al.*, 1986), whereafter non-adherent cells were further enriched for DC using a discontinuous Nycodenz gradient (14,5 % w/v Nycodenz, Nycomed As, Oslo, Norway) (HAVENITH *et al.*, 1992). This gradient was centrifuged at 600 g for 10 min, the interphase yielding the DC enriched population. T cell-enriched populations were prepared from Wistar rat spleen according to the method of HAVENITH *et al.*, (1992).

RESULTS

Presence of myeloid markers in mouse, rat and human anterior pituitary

In Table 1 an overview is given of myeloid markers found in the mouse, rat and human AP using LO immunohistochemistry. These data are collected and adapted from previous publications of our group (ALLAERTS *et al.*, 1991, 1993, 1995). Several of these markers reveal overlap between immunopositive cell populations as previously described (ALLAERTS *et al.*, 1995).

Ultrastructure of S100 and MHC-class II expressing cells in rat anterior pituitary

Ultrathin cryo-sections of rat AP labelled with the OX6 moAb (MHC class II specific) and stained following the immunogold technique, revealed the presence of readily detectable numbers of gold particles on cells with the following characteristics (Fig. 1-4) : $OX6^+$ cells all displayed a narrow cytoplasmic rim around the nucleus and a varying number of cytoplasmic processes. The numbers of gold particles per cell section was rather low, namely of an order of magnitude of 10^1 to 10^2. Background labelling was absent, for granule-bearing endocrine cells were not

TABLE 1

Pattern of LO immunohistochemical staining with moAb's against myeloid antigens present in mouse, rat and human anterior pituitaries

MoAb	*Antigen*	*Reference*	*Presence in pituitary cells*
MOUSE			
BMDM1	aminopeptidase	LEENEN *et al.* 1992	+
M5/114	class II, MHC		+
M1/42	class I, MHC		+
30G12	T200 antigen (common leukocyte antigen)	LEDBETTER and HERZENBERG, 1979	+
N418	CD11c antigen (p 150, 90 antigen)	METLAY *et al.*, 1990	−
RAT			
OX6	class II, MHC	HART and FABRE, 1981	+
OX62	integrin-like antigen (α subunit)	BRENAN and PUCLAVEC, 1992	+
ED1,2	?	DIJKSTRA *et al.*, 1985	+
HUMAN			
CD1a	T6 antigen (common thymocyte antigen)	FITHIAN *et al.* 1981	+
CD14	Phosphatidyl-inositol glycan linked antigen	FREUDENTHAL and STEINMAN, 1990	+
CD45	T200 antigen	FREUDENTHAL and STEINMAN, 1990	+
OKIa	class II, MHC	REINHERZ *et al.*, 1979	+
TB1-4D5	L25 antigen	ISHII *et al.*, 1985	+
RFD1	MHC class II-associated antigen	POULTER *et al.*, 1986	+

labeled with the gold particles. The gold label was frequently found associated with the cell membrane (Fig. 1a), but was also found within the intra-cellular vesicles (ARKEMA *et al.*, 1991a) (Fig. 1b). Exact quantification of the density of gold particles was not possible due to freeze ruptures of the cryo-sections, and due to the fact that high resolution images were selected to reveal the OX6 labelled cells. Therefore, a qualitative description is given.

On the basis of the morphology of the cells, three $OX6^+$ cell phenotypes could be distinguished in the AP. Intermediate phenotypes were also found, therefore the following descriptions are to be interpreted as characteristic rather than distinct phenotypes occurring within the morphological spectrum of this cell group. The first cell phenotype displayed an elongated cell body and nucleus, with one or more long cytoplasmic processes, exceeding the length of the cell nucleus (Fig. 2). A second cell phenotype was found to have a more rounded morphology and many short cytoplasmic processes, often also a heavily indented nucleus and one or more

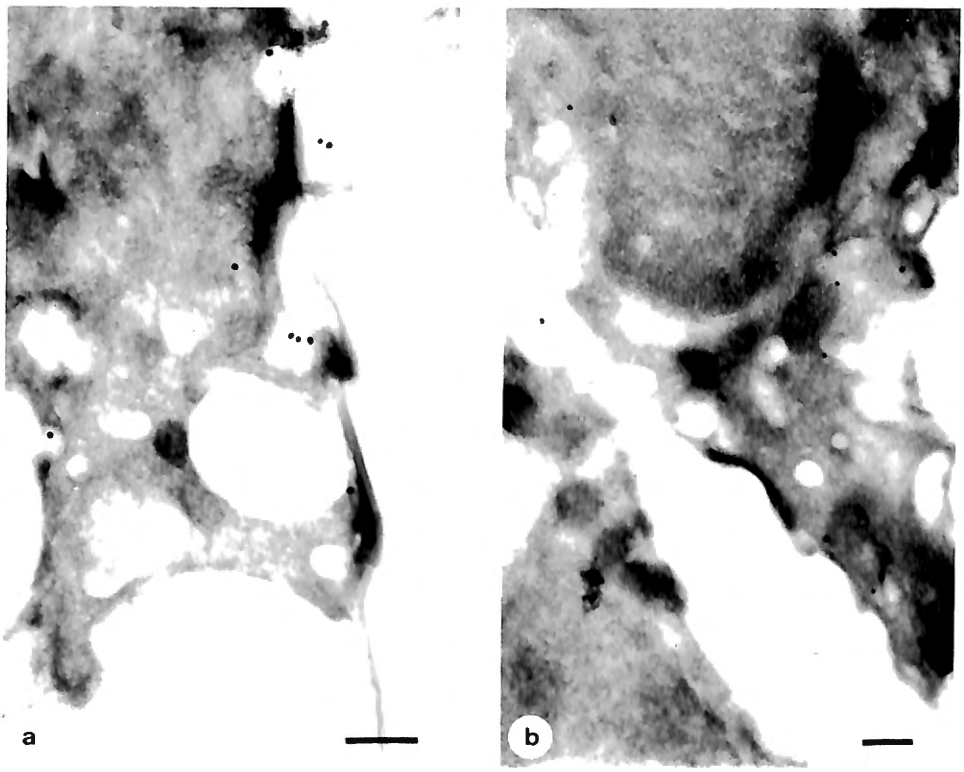


Fig. 1. — Immuno-EM of ultrathin cryo-sections of rat anterior pituitary labelled with the OX6 moAb (MHC-class II specific). The gold label was observed at the cell membrane (3a) (× 58000) or within the intracellular vesicles (3a,b) (× 42000 ; Bars = 0.2 μm).

large vesicles located in juxtanuclear position (Fig. 3) (ARKEMA *et al.*, 1991b). A third cell phenotype with elongated or triangular shape on section was frequently found surrounded by a number of hormone-secreting parenchyme cells (Fig. 4). This cell displayed a more or less indented nucleus, microvillous projections (Fig. 5 a,b), many large intracellular vesicles, including lipid droplets and phagolysosomes, elongated or vermiform mitochondria (VILA-PORCILE, 1972) (Fig. 6a) and a centriole (Fig. 6b). In the AP these characteristics are classically ascribed to FS cells (VILA-PORCILE, 1972 ; SCHECHTER *et al.*, 1988). However, according to STEINMAN and COHN (1973) some of these characteristics may also be found in DC (lipid droplets, microvilli and centrioles), whereas phagolysosomes have been characterized in DC *in situ* (KAMPERDIJK *et al.*, 1985). Birbeck granulae (BIRBECK *et al.*, 1961), the hallmark of the Langerhans cell, were not found in the $OX6^+$ pituitary cells, although they are present in many DC representatives (KAMPERDIJK *et al.*, 1994). The degree of labelling with the OX6 moAb was variable in these three cell phenotypes, but clearly exceeded background, since gold particles were rarely found in a location different from that within these cells.

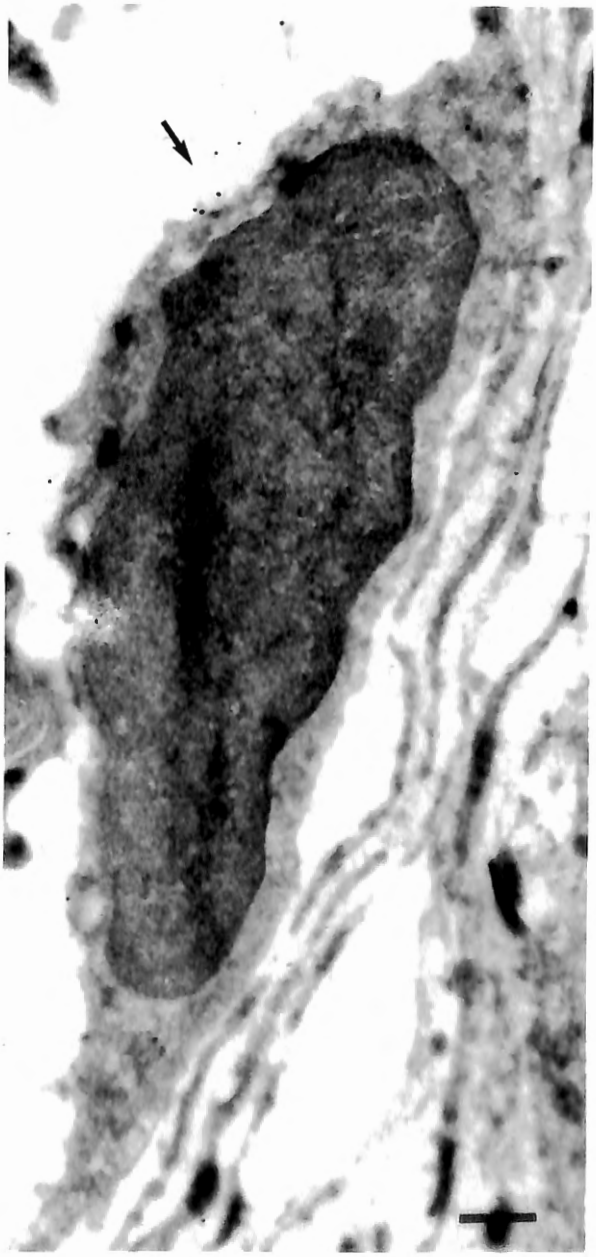


Fig. 2. — Immuno-EM of cryo-section of rat AP labelled with the OX6 moAb : example of *type I* MHC-class II-expressing cell (arrow). The *type I* cell is characterized by an elongated cell body and nucleus, with a narrow rim of cytoplasm and long cytoplasmic processes. This cell is located at the periphery of the epithelial parenchyme cord and is flanked by connective tissue (× 19500 ; Bar = 0.5 μm).

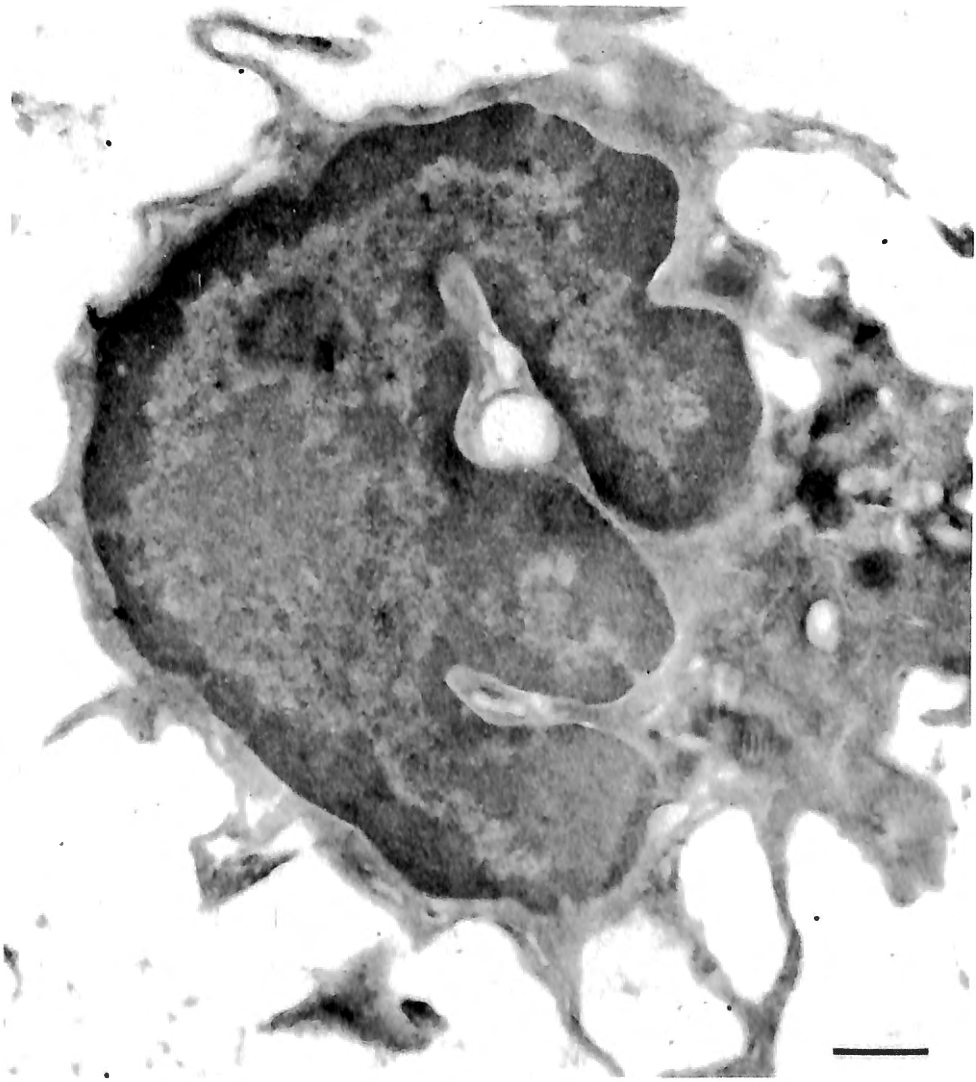


Fig. 3. — Immuno-EM of cryo-section of rat AP labelled with the OX6 moAb : example of *type II* MHC-class II-expressing cell, reminiscent of the « classical » DC. The *type II* cell is characterized by relatively short cytoplasmic processes, a heavily indented but not fragmented nucleus and a few large vesicles in juxtanuclear position, possibly corresponding to phagolysosomes ($\times$ 32000 ; Bar = 0.5 μm).

Immunogold labelling with the polyclonal S100 antiserum of ultrathin cryo-sections (Fig. 5,7) revealed a cell type reminiscent of the third OX6$^+$ phenotype described above (Fig. 4). Immunogold label was more frequently found within the nucleus (Fig. 7, inset) of these cells than within the cytoplasm, although both

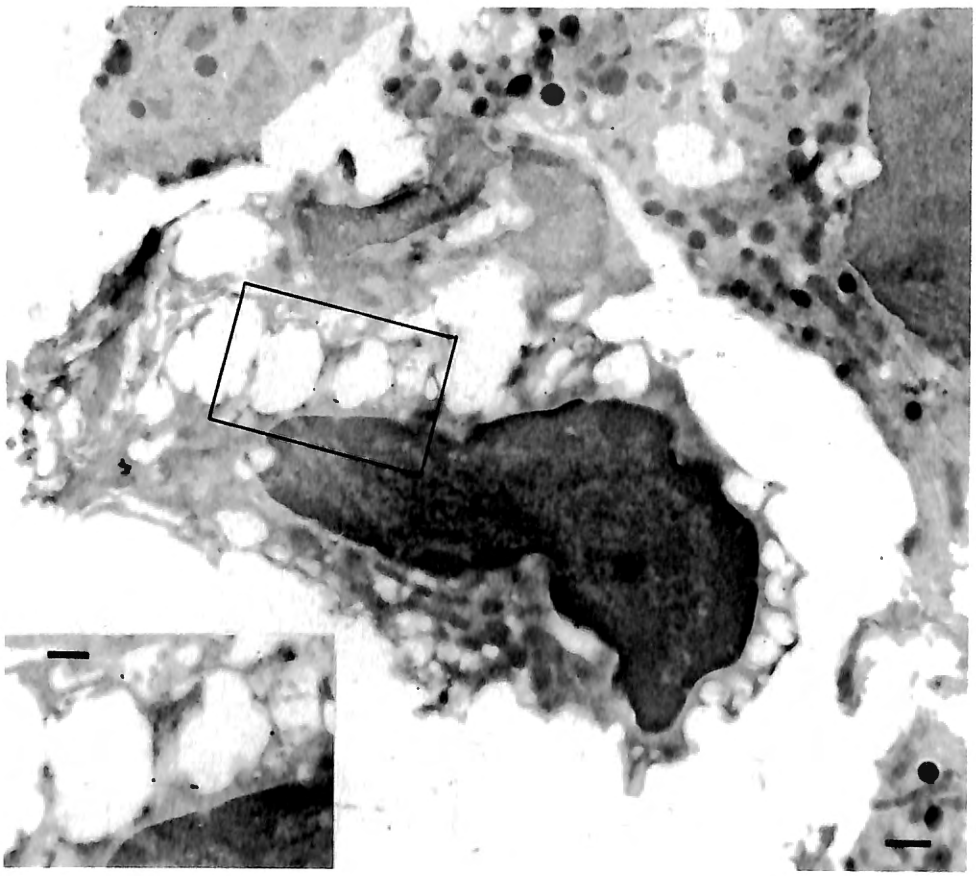


Fig. 4. — Immuno-EM of cryo-section of rat AP labelled with the OX6 moAb : example of *type III* MHC-class II-expressing cell, reminiscent of the « classical » FS cell (× 14000 ; Bar = 0.5 μm). The *type III* cell is characterized by short microvillous projections, many large intracellular vesicles and typical organelles (see Fig. 5, 6). This cell is located in the endocrine compartment and is surrounded by granulated endocrine cells. The inset shows high power magnification (× 31000 ; Bar = 0.2 μm) of some vesicles labeled with the OX6 moAb.

labellings exceeded background. This result corresponds with the LO staining pattern of cryo-sections with the S100 anti-serum (ALLAERTS *et al.*, 1995). Fig. 5 shows a cryo-section of FS cells localized in a cluster at the centre of the endocrine parenchyme compartment, and surrounding a follicle wherein microvillous protrusions are found. The ultrastructural characteristics, immunopositivity for S100 and topographical localization (Fig. 5a), indicate that this cell type corresponds to the classical FS cells, as described by VILA-PORCILE (1972). Recently, using low temperature resin (Lowicryl HM20) immunohistochemistry at the EM level, we could demonstrate $S100^+$ and $OX6^+$ cells with excellently preserved topography reminiscent of FS cells (ALLAERTS *et al.*, 1995).

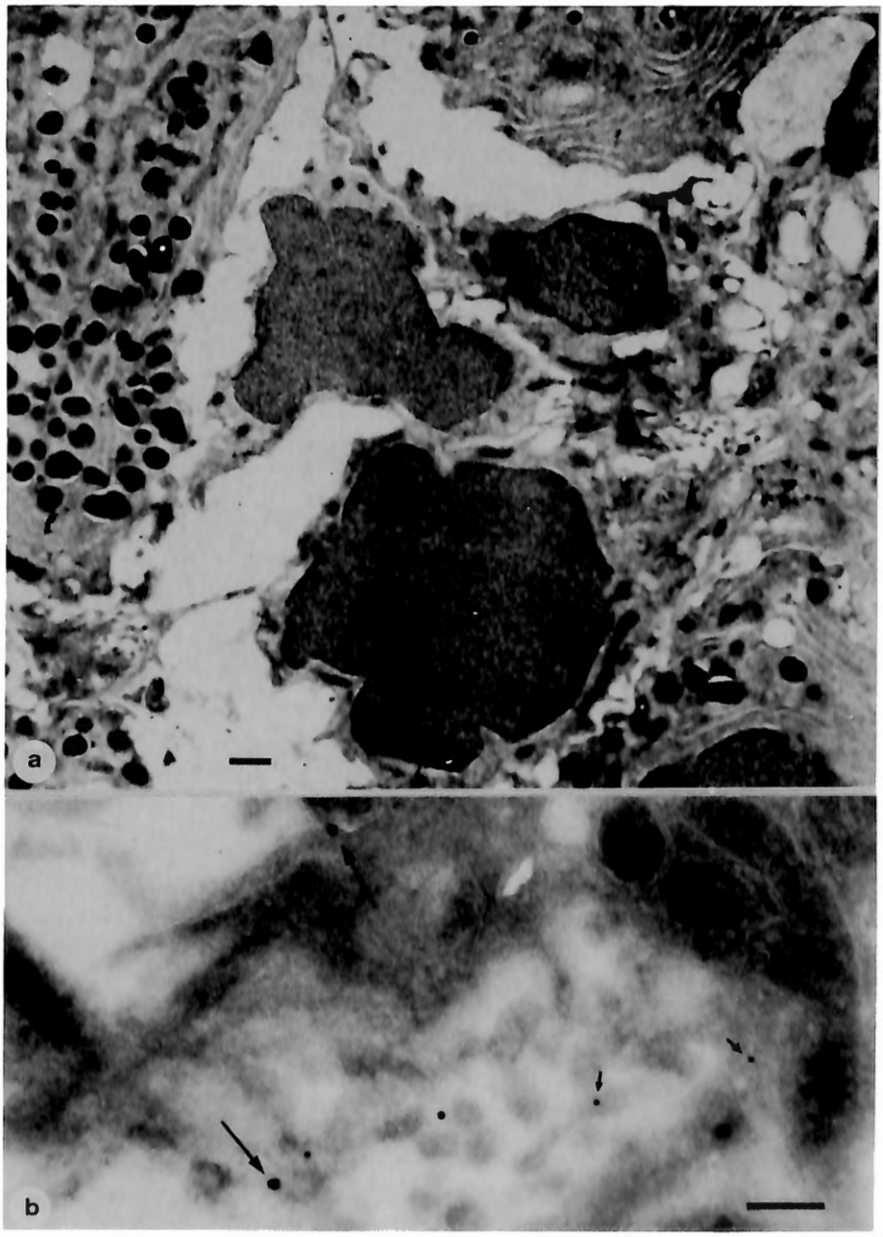


Fig. 5. — Topographical localization of FS cells at the ultrastructural level in cryo-sections of rat AP labelled with the OX6 moAb, showing a cluster of FS cells at the center of a parenchyme cord and surrounding a follicle (arrowhead) (5a) (× 14000 ; bar = 0.5 μm). Microvillous protrusions into the follicle lumen are labelled for the MHC-class II determinants (25 nm gold label ; longer arrows) and the S100 protein (15 nm gold label ; shorter arrows) (5b) (× 64000 ; bar = 0.2 μm).

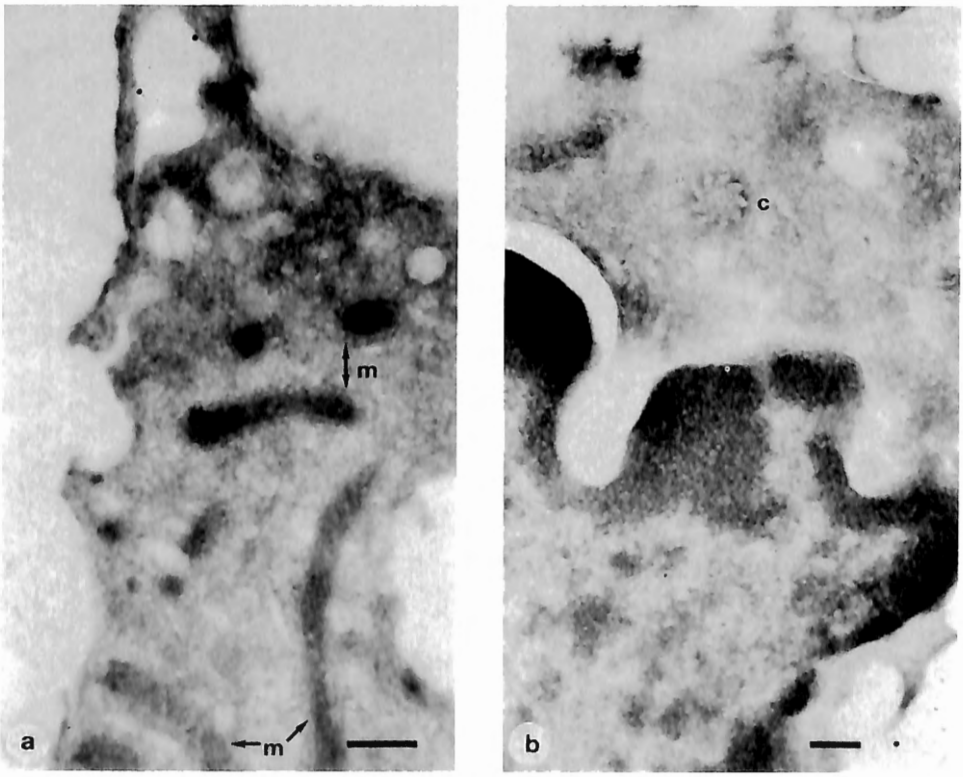


Fig. 6. — Immuno-EM image to demonstrate the ultrastructural characteristics of FS cells in cryo-sections of rat AP labeled with the OX6 moAb. The cells are labeled for the MHC-class II marker, and display long vermiform mitochondria (m) (6a) (× 58000) and a centriole (c) (6b) (× 42000) as described by VILA-PORCILE (1972) (Bars = 0.2 μm).

Further, using a double immunogold-labelling procedure with gold particles of 15 nm and 25 nm diameter respectively, we were able to identify cells with the third phenotype described above bearing both the S100 protein and the OX6 antigen (Fig. 5b, 8a). The larger gold particles labelling the MHC-class II molecules were found at the cell membrane and within the cytoplasm, whereas the smaller gold particles labelling the S100 protein were more numerous within the cell nucleus (Fig. 8a). Some protruding microvilli found within the follicular lumen of an FS cell cluster were labelled with the MHC-class II marker and the S100 protein (Fig. 5b). Occasionally, also cells with the second phenotype (cfr. above) were found immunopositive for both the S100 protein and the OX6 antigen (Fig. 8b).

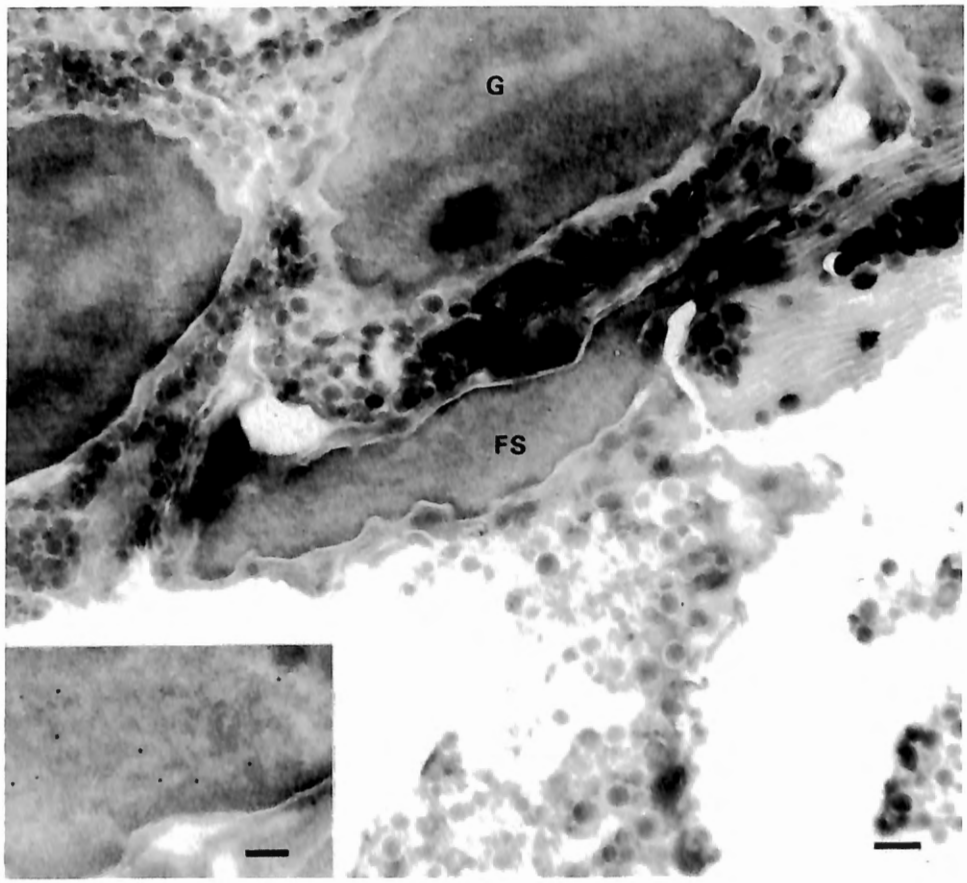


Fig. 7. — Immuno-EM of cryo-section of rat AP labelled with the polyclonal anti-S100 serum : example of FS cell (FS) surrounded by granulated endocrine cells (G) (× 14000 ; bar = 0.5 μm). The FS cell nucleus is abundantly labelled with the S100 antibodies (high power magnification in inset) (× 31000 ; bar = 0.2 μm).

Mixed leukocyte reaction assay with pituitary Dendritic Cells

In Fig. 9a the autologous stimulation of T lymphocyte proliferation by pituitary cells is shown. A cell population obtained from the interphase of the Nycodenz gradient corresponding with a DC enriched fraction of splenic cells and consisting of 34 % $S100^+$ and 25 % $OX6^+$ cells was capable of stimulating T lymphocyte proliferation for a stimulator-responder ratio as low as 1 :160. On the other hand, in the pellet phase of this Nycodenz gradient (remaining pituitary cell population with only 2 % $S100^+$ and 8 % $OX6^+$ cells) a stimulation was only observed for ratios larger than 1 :20.

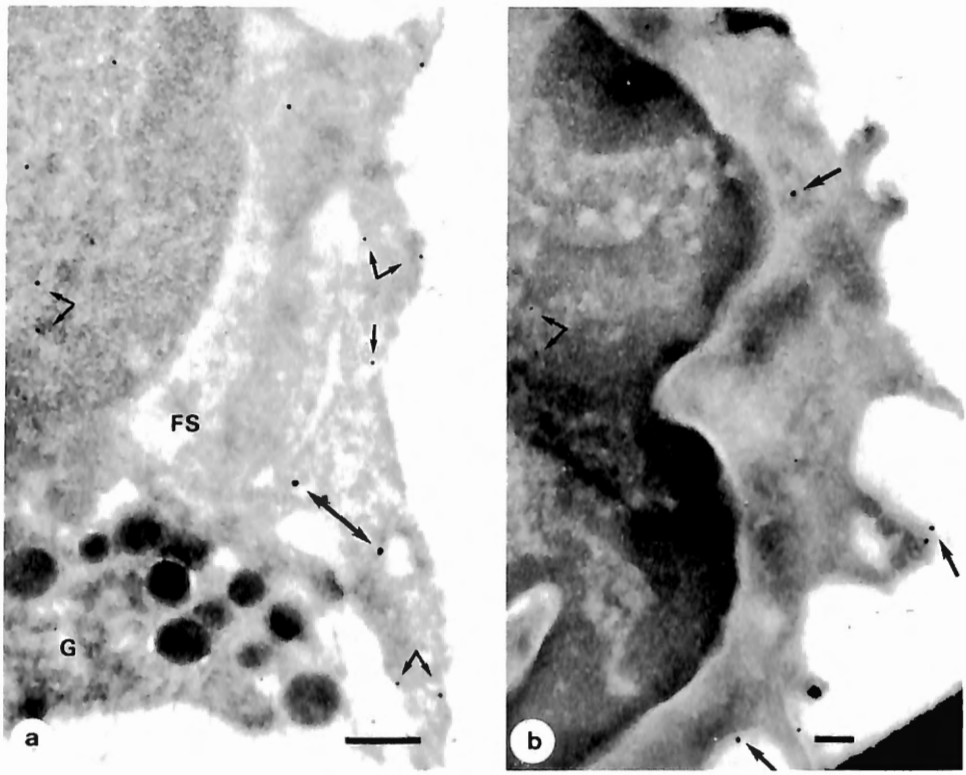
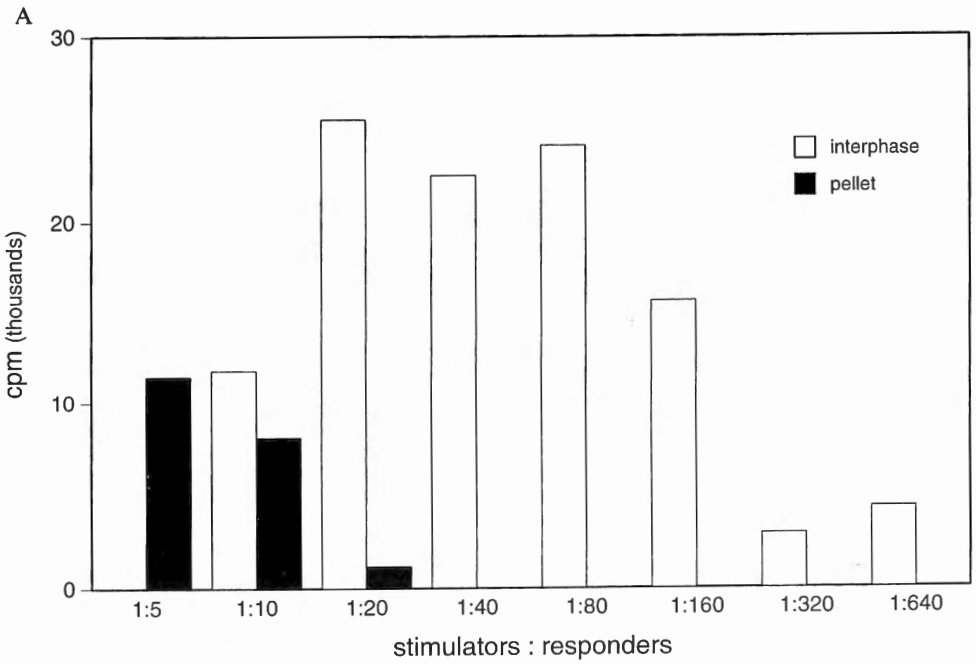


Fig. 8. — Double immuno-labelling of ultrathin cryo-sections of rat AP with the OX6 moAb (25 nm gold label; longer arrows) and the anti-S100 serum (15 nm gold label; shorter arrows). A FS cell (FS), reminiscent of the *type III* MHC-class II-expressing cell is shown in 8a : the cell is abundantly labelled for the S100 protein and weakly labelled for the MHC-class II determinants (× 60000). A *type II* cell is shown in 8b, labelled for the MHC-class II determinants, with only very little label for the S100 protein (× 32000). G : fragment of granulated endocrine cell (Bars = 0.2 μm).

The autologous stimulation of T lymphocyte proliferation by pituitary cells obtained by BSA gradient sedimentation is shown in Fig. 9b and c. Original populations of dispersed pituitary cells called « originals », and cell populations obtained by velocity sedimentation at 1 g (cfr. above) were able to stimulate T lymphocyte proliferation for stimulator-responder ratios varying from 1 :5 to 1 :320 (Fig. 9b). At the maximally effective stimulator-responder ratio, the cell populations of the sedimentation gradient were more effective in stimulating T lymphocyte proliferation as compared to the originals (Fig. 9c). The combined fractions 1 and 2 were the most potent stimulatory fraction in this assay (Fig. 9c). The magnitude of maximal T lymphocyte proliferation, moreover, is highly correlated with either the proportional number of MHC-class II expressing cells or the proportional number of $S100^+$ cells (unpublished observations).


A
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cpm (thousands)
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1:40
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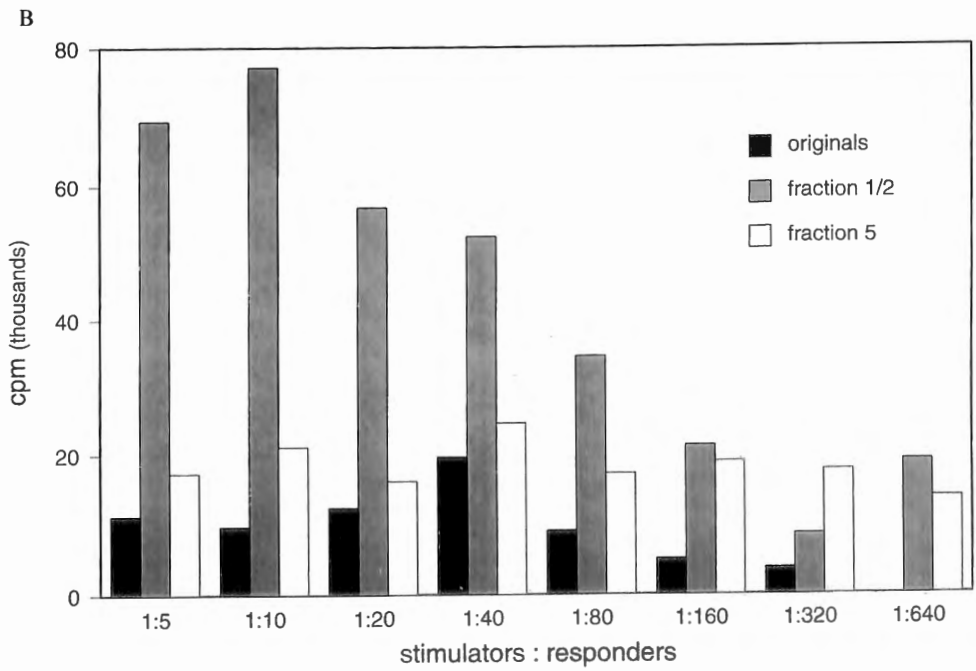
1:320
1:640
stimulators : responders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stimulators : responders

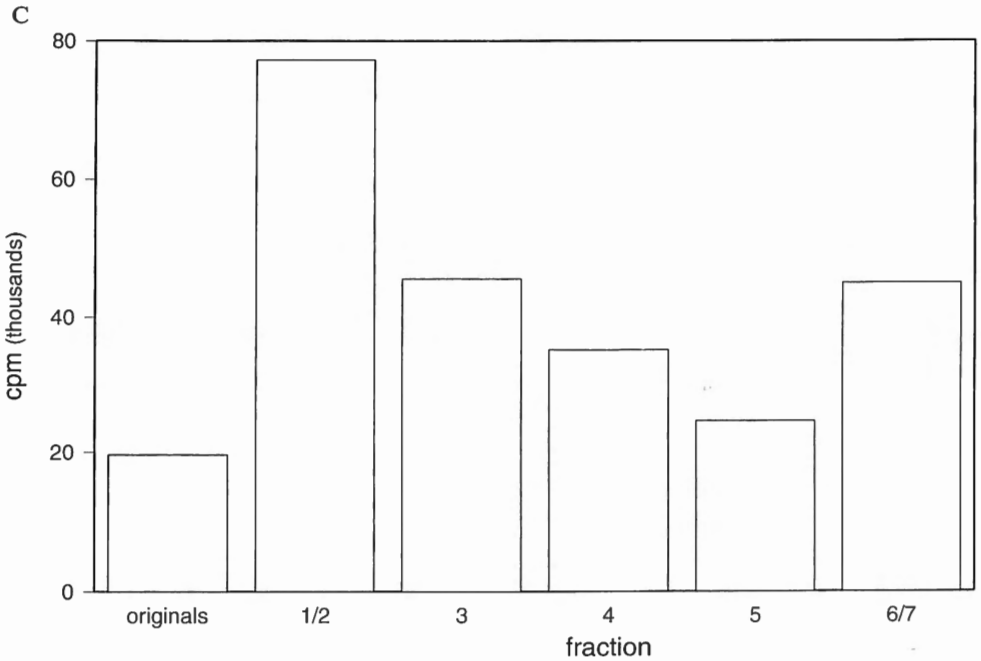

Fig. 9. — Mixed leukocyte reaction assay with pituitary dendritic cells. A. T cell proliferation induced by pituitary cells separated by Nycodenz gradient in stimulator : responder ratio between 1 :5 and 1 :640. B. T cell proliferation induced by pituitary cell populations (original population, BSA gradient fractions 1 plus 2 and BSA gradient fraction 5). C. T cell proliferation induced by pituitary cell populations at maximally effective stimulator : responder ratio (representative experiment out of 3 similar experiments).

DISCUSSION

In the present paper a review is given of the myeloid markers found in mouse, rat and human anterior pituitary as previously described (ALLAERTS *et al.*, 1991, 1993, 1995). We previously treated the problem of the (partial) overlap between the different cell populations immunopositive for markers of the monocyte-macrophage-dendritic cell lineage (ALLAERTS *et al.*, 1995). The present ultrastructural investigation of $S100^+$ and MHC-class II expressing cells in the rat AP is suggestive for the occurrence of three distinct immunopositive phenotypes within the morphological spectrum of folliculo-stellate (FS) and/or dendritic cells (DC) in the AP. Hitherto, this cell group has been regarded as either a resident pituitary cell population (folliculo-stellate cells ; VILA-PORCILE, 1972) or as a lymphoid cell group which may also occur in almost any non-lymphoid organ (dendritic cells ; STEINMAN and COHN, 1973, KLINKERT *et al.*, 1982). Moreover, no conclusive evidence has been brought forward with regard to the embryological origin of the FS cell group (ALLAERTS *et al.*, 1995), although a myeloid origin of the DC cell group is commonly accepted (KAMPERDIJK *et al.*, 1994). The heterogeneity of the FS cell group

has already been proposed, based on ultrastructural (VILA-PORCILE, 1972) or immunohistochemical findings (TACHIBANA and YAMASHIMA, 1988 ; NAKAGAWA *et al.*, 1985). The presence of DC markers in the mouse, rat and human AP, the fact that $S100^+$ FS cells and MHC class II expressing DC of the rat AP co-sediment within the upper gradient fractions of a velocity gradient (ALLAERTS *et al.*, 1993) and the occurrence of double immunolabelled cells at the EM level (ALLAERTS *et al.*, 1995 ; present data) are suggestive for a (partial) overlap between the two cell groups. Moreover, the present finding of an immune-accessory function of pituitary cells *in vitro*, which is most prominent in the S100-enriched and MHC-class II enriched cell population obtained by gradient sedimentation, demonstrates that these cells are immunocompetent cells comparable to lymphoid DC. Because of the lower numbers of DC marker expressing cells in the AP compared to the numbers of $S100^+$ FS cells, the overlap between the two cell groups is only partial, and this fact adds to the heterogeneity of the FS cell group (ALLAERTS *et al.*, 1995).

It remains to be elucidated whether the heterogeneity of the FS cell group as revealed by ultrastructural and immunohistochemical characteristics is also a functional heterogeneity. To date, no information is available concerning the stability of the immunohistochemical markers and functional states of the FS cell group during normal and pathological development. The possibility of transient differentiation stages within the FS and DC group is substantiated by the occurrence of intermediate forms between the FS and DC phenotype, that also express immunohistochemical markers of the two cell groups (present data).

The presence of DC in the AP, that moreover may act as immune-accessory cells, raises the question of the functional significance of this cell group. We previously demonstrated using an *in vitro* culture system that FS cells communicate with granulated cells of the pituitary parenchyme (BAES *et al.*, 1987 ; ALLAERTS and DENEF, 1989) and FS cells are the likely producers of IL-6 in the AP (VANKELECOM *et al.*, 1989, 1993). It was suggested that FS cells may exert an endocrine regulatory function as well as an « immune defense » function (ALLAERTS *et al.*, 1990). A remarkable synchronization in the rat AP during development of IL-6 producing (CARMELIET *et al.*, 1991) and $S100^+$ (SHIRASAWA *et al.*, 1983) and MHC-class II expressing cells was previously observed (ALLAERTS *et al.*, 1993). In the AP of an autoimmune-prone rat model, the BB rat, an increased number of $S100^+$ and MHC class II expressing cells was found, compared to the Wistar and Lewis rat AP (unpublished observations). Whereas in the thyroid (VOORBIJ *et al.*, 1990) and pancreatic islets (VOORBIJ *et al.*, 1989) of the BB rat DC play a crucial role in initializing autoimmune thyroiditis or insulitis, respectively, it is unknown whether BB rats may also develop an autoimmune adenohypophysitis. Autoimmune hypophysitis has been described in humans (MAUERHOFF *et al.*, 1987) and an animal model of adenohypophysitis resulting from injecting AP homogenates in the rat was developed by LEVINE (1967) (reviewed in ALLAERTS and DREXHAGE, 1994). Recently, it was reported that injection of the S100β specific T cell lines activated with the S100β protein in naive Lewis rats resulted in experimental allergic encephalitis (KOJIMA *et al.*, 1994). The involvement of DC in the initiation of endocrine autoimmune disease (KNIGHT *et al.*, 1988) opens a new perspective on the immune sur-

veillance function of the FS and DC cell group in the AP, apart from their role as IL-6 producing cells (VANKELECOM *et al.*, 1989). The expression of IL-6 mRNA and IL-6 receptor mRNA in the rat pituitary (SCHÖBITZ *et al.*, 1992 ; VELKENIERS *et al.*, 1994), thereby has been interpreted as part of an inflammation process with a central pituitary effect (SCHÖBITZ *et al.*, 1992 ; REICHLIN, 1993), or was interpreted as the manifestation of local paracrine or autocrine communication processes, which may dysfunction in pituitary adenomas (TSAGARIKIS *et al.*, 1992 ; VELKENIERS *et al.*, 1994).

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