Differential Localization of Brain-Type and Epidermal-Type Fatty Acid Binding Proteins in the Adrenal Gland of Mice

XIA YUN,1 MOHAMMAD REZA NOURANI, SOHA ABDELKAWI ABDELWAHAB, NORIKO KITANAKA, YUJI OWADA, FRIEDRICH SPENER,2 HIROO IWASA, AKIRA TAKAHASHI1 and HISATAKE KONDO

Division of Histology, Department of Cell Biology,1 Division of Neuro-Intravascular Disease, Department of Neuroscience, Graduate School of Medicine, Tohoku University, Sendai 980-8575, and
2Department of Biochemistry, Muenster University, Muenster, Germany

YUN, X., NOURANI, M.R., ABDELWAHAB, S.A., KITANAKA, N., OWADA, Y., SPENER, F., IWASA, H., TAKAHASHI, A. and KONDO, H. Differential Localization of Brain-Type and Epidermal-Type Fatty Acid Binding Proteins in the Adrenal Gland of Mice. Tohoku J. Exp. Med., 2004, 203 (2), 77-86 — In immuno-light and -electron microscopy, brain-type fatty acid binding protein (B-FABP) is localized in the sustentacular cells enclosing the chromaffin cells in the adrenal medulla. This represents another new feature commonly shared by the sustentacular cells and ganglionic satellite cells, the latter of which has already been reported to localize this molecule, and suggests a common feature in lipid metabolism shared by the two cells enclosing peripheral neurons and paraneurons. On the other hand, epidermal-type fatty acid binding protein (E-FABP) is localized in two discrete cells in the adrenal gland: the one is a subpopulation of intra-adrenal macrophages which are intensely immunoreactive for F4/80, a marker of macrophages, and are rich in pleomorphic lysosomes. Because of their direct apposition to adjacent cortical endocrine cells and medullary chromaffin cells, the macrophages may be involved not only in phagocytosis of degenerating adrenal cells but also in exertion of some yet unknown effects on the endocrine function of the cortical and medullary cells via humoral factors such as cytokines which have recently been known to be secreted by macrophages. The other is a population of cells having scanty perikaryal cytoplasm poor in organelles and several thinny extended processes in the cortex and exhibiting weak immunoreactivity for E-FABP. The possible natures of these cells immunoreactive for E-FABP are discussed in view of a subpopulation of endothelial cells or the dendritic cells of antigen-presenting property. ——— B-FABP; E-FABP; sustentacular cells; adrenal gland; immunohistochemistry

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Because of their hydrophobic nature, fatty acids should be solubilized and transported by specific intracellular lipid binding proteins, the low molecular mass polypeptides of 14-15 kDa termed fatty acid binding proteins (FABPs). Multiple species of FABPs had been identified and named according to the tissues of their first isolation sites such as heart (H-), epidermal (E-), brain (B-) and adipocyte (A-) types, however, they showed much wider tissue distribution than first thought (Gordon et al. 1983; Alpers et al. 1984; Hunt et al. 1986; Siegenthaler et al. 1993; Feng et al. 1994; Kurtz et al. 1994). Several roles have been assigned to these FABPs such as control of cellular uptake of fatty acids and their subsequent utilization and intracellular compartmentation, modulation of activity of enzymes involved in fatty acid metabolism, protection of cellular membranes and enzymes from detergent effects of fatty acids, and carriers of signaling fatty acids (Glatz et al. 1993; Coe and Bernlohr 1998).

Based on the idea that the exact localization of a given molecule is a first step and great help in understanding its functional significance, we have so far examined the localization of various types of FABPs in a series of studies (Iseki et al. 1988; Watanabe et al. 1991; Owada et al. 1996a, b, 1997, 2001, 2002a; Abdelwahab et al. 2003; Kitanaka et al. 2003). Here we show that B-FABP is localized in the sustentacular cells enclosing the chromaffin cells in the adrenal medulla, while E-FABP is localized in a small number of presumptive dendritic cells distributed throughout the adrenal gland of postnatal mice.

**MATERIALS AND METHODS**

Male C57BL/6 mice at the stages of postnatal day (P) 1 week (w), P2w and P7w were used in this study. All experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments of Tohoku University, and were carried out in accordance with the Guidelines for Animal Experiments issued by Graduate School of Medicine, Tohoku University. Mice were perfused under Nembutal anesthesia through the heart with 4% paraformaldehyde/0.1 M phosphate buffer (pH 7.4). The adrenal glands were extirpated and immersed overnight in a phosphate buffer containing 30% sucrose. Antibodies used in this study include E-FABP antiserum at a concentration of 0.5 μg/ml, B-FABP antiserum at a concentration of 2 μg/ml and a monoclonal rat antibody against F4/80 (Serotec, Oxford, UK) at 1:100 dilution. The characterization of anti-E-FABP and anti-B-FABP antibodies were described previously (Kurtz et al. 1994; Owada et al. 2002b). After incubation with the primary antibody, the sections were incubated with biotinylated anti-rabbit secondary antibody for single immunostaining or with a combination of anti-rabbit IgG-Alexa488 and anti-rat IgG-Alexa594 (Molecular Probe, Eugene, OR, USA) for double immunostaining, respectively. The sections for single immunostaining were subsequently visualized using ABC (avidin-biotinylated peroxidase complex) system (Vector Laboratory, Burlingame, CA, USA) with DAB as a substrate. For immunoelectron microscopy, some of the sections were postfixed with 1% OsO4 in 0.1% cacodylate buffer, pH 7.4, for 20 minutes after completion of the ABC procedure. They were subsequently embedded in Epon according to the conventional procedure and ultrathin sections were examined after brief staining with uranyl acetate. For control of E-FABP-immunoreactivity, the adrenal gland of E-FABP-gene knockout mice were immunostained which had been generated and characterized by us (Owada et al. 2002b), while the B-FABP-antibody was immunoabsorbed with its antigen at 10 μg/ml in antiserum for control of B-FABP-immunoreactivity.

**RESULTS**

*Cells immunoreactive for B-FABP in the medulla*

The immunoreactivity for B-FABP was detected in numerous small cells having thin processes in the medulla. They were distributed rather evenly in the medulla and enclosed multiple immunonegative chromaffin cells as groups.
No immunoreactivity was found in the adrenocortical cells, capillary walls, and intra adrenal nerve fibers throughout the gland in immuno-light microscopy (Fig. 1A). The immunoreactive cells in the medulla exhibited ultrastructural features of the sustentacular cells which were characterized by thin cytoplasmic processes enclosing chromaffin cells and nerve terminals with the intercellular space of 15-20 nm and by a relative paucity of cell organelles such as a Golgi apparatus, lysosomes, endoplasmic reticulum and mitochondria (Fig. 1B).

Cells immunoreactive for E-FABP in the cortex and medulla

In immuno-light micropscopy, the immuno-
reactivity for E-FABP was detected in cells of dendritic forms throughout the gland. Two types of the immunoreactive cells were found: the ones were intensely immunoreactive and relatively voluminous, might form small cell groups and were located in the cortex and superficial medulla (Fig. 2A), while the others were less intensely immunoreactive, appeared single and skinny in perikarya, and extended multiple fine processes in the cortex (Fig. 2B). The former were not found to be associated with the vascularization, while most of the latter appeared to extend their fine processes in such a way as to line the capillary lumen. When one and the same section was immunostained with both antibodies against E-FABP and F4/80, a marker of mature macrophages, the intensely E-FABP-immunoreactive cells having relatively voluminous perikarya were co-stained with F4/80, although a substantial number of cells were F4/80-immunoreactive but E-FABP-immunonegative (Figs. 2C-2E). In another word, the cells intensely immunoreactive for E-FABP represent a substantial population of numerous F4/80-immunoreactive macrophages. In contrast, the slender cells showing less intense immunoreaction for E-FABP were not co-stained with F4/80 (Fig. 2E).

In immuno-electron microscopy, the cells intensely immunoreactive for E-FABP were characterized by an immunonegative nucleus and a rich-
ness in membranous organelles such as rough endoplasmic reticulum, mitochondria, lysosomes and pleomorphic phagosomes containing concentrically lamellated structures, but they did not contain chromaffin granules or adrenocortical cell-specific mitochondria (Figs. 3A and 3B). The immunoreactive material was localized in the cytoplasmic matrix among membranous organelles, although the interior of membranous organelles and phagosomes were almost free of the immunostaining (Fig. 3B). The immunoreactive cells and their processes were in close apposition to the medullary chromaffin cells and adrenocortical cells with an intercellular space of 20-50 nm at minimum without any membrane specializations (Fig. 3A), but they never exposed directly to the vascular lumen.

In contrast, the slender cells less intensely immunoreactive for E-FABP in the cortex had an oblong nucleus and a scantiness of perikaryal cytoplasm containing a few organelles (Figs. 4A and 4B). The immunoreactive material was localized

Fig. 3. (A) (B) Electron micrographs of intensely E-FABP-immunoreactive cells. Note the richness in membranous organelles including abundance of pleomorphic lysosomes (L). The immunoreaction products are densely deposited in the cytoplasmic matrix, but weakly deposited in perikaryal areas containing a condensation of the pleomorphic lysosomes. The nucleus is free of the immunoreaction. A inset in A represents an higher magnification image showing a direct membranous apposition of the immunoreactive cell with the chromaffin cell. C, chromaffin cell; L, pleomorphic lysosomes; N, nucleus. Bar in A, 2 μm; bar in B, 1 μm.
in the cytoplasmic matrix but not within the nucleus and membranous organelles. Some of the slender immunoreactive cells appeared in single ultrathin sections as lining the sinusoidal capillary lumen: their perikarya were bulged into and exposed directly to the capillary lumen, and they had thin immunopositive cytoplasmic processes to line the capillary lumen together with immunonegative endothelial cells with simple membranous appositions (Fig. 4A). However, others appeared to locate their entire perikarya in the interstitium without direct exposure to the capillary lumen (Fig. 4B). They sent tapering processes among adjacent adrenocortical cells or into subendothelial spaces (Fig. 4B).

Fig. 4. (A) Electron micrographs of a less intensely E-FABP-immunoreactive cell lining the sinusoidal lumen (S) together with immunonegative endothelial cells. (B) E-FABP-immunoreactive cell located in the interstitium among cortical cells (Co) without direct exposure to the sinusoidal lumen. Note the scanty in their cytoplasm and organelles and the extension of tapering processes. The immunoreactive products are present in the cytoplasmic matrix except for the nuclear interior (*). Co, cortical cells; E, endothelial cells; S, sinusoidal lumen; Bars in A and B, 1 μm.
When the adrenal gland from the gene knockout mice for E-FABPs, the details of which had already been reported by us (Owada et al. 2002b), were immunostained with the E-FABP antibody, or the antigen-absorbed serum for B-FABP was applied to sections of the wild adrenal gland, no immunoreaction was detected at a significant level in any portions of the gland (data not shown). These indicate that the immunoreactivity in the satellite cells and that in the cells of dendritic form are due to authentic E- and E-FABPs, respectively.

**DISCUSSION**

With regard to the present finding of B-FABP localization in the sustentacular cells enclosing the adrenal chromaffin cells, the immunohistochemical studies have so far clarified that this molecule is localized in the ganglionic satellite cells of the adult peripheral ganglia as well as the ventricular germinal zone cells and radial glia of the embryonic brain and astrocytes of the postnatal brain (Kurtz et al. 1994). It is well known that both adrenal chromaffin cells and most peripheral ganglion neurons are of neural crest origin (Hamilton and Mossman 1972), that both the sustentacular cells and ganglionic satellite cells commonly share the enclosing feature in relation to the chromaffin cells and ganglionic neurons respectively, and that the sustentacular cells and ganglionic satellite cells exhibit the histological contiguity to Schwann cells enclosing nerve fibers innervating the chromaffin cells and ganglionic neurons (Coupland 1965). Because of the common features, it is generally expected to find constitutive molecules shared by both the sustentacular cells, ganglionic satellite cells and Schwann cells, and a well-known example of the molecules shared by these cells is S-100β (Cocchia and Michetti 1981). Although B-FABP is shown in the present study as another new example of such common molecules in the sustentacular cells and ganglionic satellite cell, a peculiarity of B-FABP is that this molecule is not localized in Schwann cells in normal mice (Kurtz et al. 1994). In this regard, it should be noted that B-FABP expression has been reported to be induced in the Schwann cells of sciatic nerve after nerve injury (Miller et al. 2003) and that the authors have suggested the possible mediation of its expression by the Ras-independent epidermal growth factor receptor signaling. It is possible to speculate that there is a yet unknown difference in the lipid metabolism and signaling between the sustentacular and satellite cells on one hand and the Schwann cells on the other hand, and that B-FABP expression is regulated by the fatty acid environment of given cells.

With regard to the nature of the cells intensely immunoreactive for E-FABP, because of their double-immunostaining with F4/80 antibody, they are regarded as a subpopulation of intra-adrenal macrophages whose abundant existence has already been described by previous authors (Mausle 1974; Magalhaes and Magalhaes 1980, 1984; Hume et al. 1984; Gonzalez-Hernandez et al. 1994). The occurrence of E-FABP in such a substantial, but not all, population of the macrophages suggests that E-FABP may be involved in some specific roles, but not general to the intra-adrenal macrophages. Considering their direct apposition to adjacent cortical endocrine cells and medullary chromaffin cells, the macrophages may be involved not only in phagocytosis of degenerating adrenal cells but also in exertion of some yet unknown affects on the endocrine function of the cortical and medullary cells via humoral factors such as cytokines or eicosanoids which are secreted by macrophages (Currie et al. 2000).

The nature of the slender cells less intensely immunoreactive for E-FABP in the cortex, is enigma. Because of the absence of immunostaining for F4/80 and paucity of cell organelles including lysosomes in their cytoplasm, it is clear that the cells are discrete from the macrophages. Several interpretations are possible: the one is that the immunoreactive cells represent a subpopulation of fibroblasts because of their location. However, there have so far been no reports de-
scribing the occurrence of E-FABP-immunoreactive fibroblasts in other tissues and organs. Another possible interpretation is that the cortical endothelial cells are heterogeneous in terms of fatty acid-related activities, and a type of the endothelial cells, which are highly involved in the activities, exhibits E-FABP-immunoreactivity, and that the immunoreactive cell profiles in the interstitium represent tangential section profiles of the immunoreactive endothelial cells. The occurrence of E-FABP immunoreactivity in endothelial cells has been reported in some organs (Masouye et al. 1997). The other possibility to be considered is that the immunoreactive cells are the dendritic cells involved in the antigen presentation because of their dendritic morphology. There have been several studies indicating that the dendritic cells are present in the interstitium of most organs of so-called non-immune nature excluding the brain, and that their occurrence is in general at a frequency so low that this has posed a major impediment to their detection (Bell et al. 1999). The dendritic cells can usually be characterized by a high-level expression of class II MHC molecules. Because of the fact that the antigenicity of class II MHC molecules is in most cases preserved at sufficient levels for immunohistochemistry only by acetone fixation, there has been few information on the fine structural features of the dendritic cells. By partially overcoming these impediments, the occurrence of the dendritic cells have recently been described in a light microscopic study of the adrenal gland (Sato 1998). The similarity of the present skinny cells showing less intense immunoreactivity for E-FABP to the MHC II-immunoreactive dendritic cells in the adrenal cortex in terms of the cell shape, size and distribution, is in favor of the presumption that the present E-FABP-immunoreactive cells of dendritic forms are the dendritic cells although its confirmation remains to be done by simultaneous examination of expression of class II MHC antigen although it is actually difficult as discussed above. In this regard, we have recently disclosed that E-FABP-immunoreactivity is localized in the dendritic cells in the splenic white pulp, but not in the splenic marginal zone (Kitanaka et al. 2003). It is known that the dendritic cells in the splenic white pulp except for those in the follicles are positive for CD8, a surface antigen, while those in the marginal zone are CD8(−) and much more numerous than the former (Leenen et al. 1998). The rather frequent appearance of some of the immunoreactive slender cells in direct contiguity to E-FABP-immunonegative endothelial cells and lining the capillary lumen suggests that the cells under discussion could move away constantly and frequently from the capillary lining into the luminal blood flow or into the interstitium. This movement would be understandable if the cells under discussion be the dendritic cells which belong to a hematopoietic lineage (Bell et al. 1999).

In order to further understand the functional significance of B-FABP in the sustentacular cells and that of E-FABP in adrenal macrophages and presumptive dendritic cells or endothelial cells in the adrenal cortex, the analyses of phenotypes of these respective cells from B- and E-FABP gene knockout mice recently generated by us (Owada et al. 2002b) is crucial, which is underway in our laboratory.

References


