In situ demonstration of the activity of 3β-hydroxysteroid dehydrogenase on steroid hormone secreting cells within the paraaortic lymph nodes of golden hamster

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Summary. The in situ activity of 3β-hydroxysteroid dehydrogenase was detected in clustered cells which showed steroid-producing morphology, within the capsule of the paraaortic lymph node. In light and electron microscopic studies, the positive reaction products were detected on intracapsular cell clusters. This result indicates that these unique cells may have a steroid secreting function within the lymph nodes.
Key words. 3β-hydroxysteroid dehydrogenase; steroid producing cell; paraaortic lymph node; golden hamster.

In the female golden hamster, a paraaortic lymph node (PLN) is located in the retroperitoneal adipose tissue, where the renal artery branches from the abdominal aorta. The PLN differs from other lymph nodes in that it has two unique features; its direct connection with both sides of the ovarian bursa through a few lymphatics[1,2], and the existence of intracapsular clustered cells, which show ultrastructural characteristics of steroid hormone producing cells (SH cells), which we previously reported[3]. The former means that the PLN is an ovarian draining lymph node, and may usually accept a large quantity of ovarian exudates, including sex hormones (e.g. estrogen, progesterone), via the lymph flow of the afferent lymphatics. The latter, the identification of the SH cells, is a novel finding in mammalian lymph nodes, because the lymph node essentially works as a peripheral immunological organ, and has no function in the endocrine secretion of hormonal substances, except for various kinds of lymphokines from macrophages and lymphocytes[4]. In the present study, we demonstrate the in situ activity of 3β-hydroxysteroid dehydrogenase (3β-HSD), an indispensable enzyme for the biosynthesis of steroid hormones in mammals, in the SH cells of the PLN of female golden hamsters, using histochemical techniques at the level of both light and electron microscopy.

Materials and methods

Female golden hamsters aged 4–5 weeks were used in this experiment. They were provided by the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were bred in our animal room at a controlled temperature of 24 ± 1 °C and humidity of 55 ± 5%.

Electron microscopy. Before fixation, the hamsters were deeply anesthetized by ether vapor. Individual hamsters were perfused through the left ventricle with cold 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The PLNs were removed and immersed in 2.5% glutaraldehyde in the same buffer for 4 h at 4 °C. After washing with the buffer, the samples were postfixed in 2% OsO_4 for 2 h at 4 °C, and processed for routine transmission electron microscopy. Ultrathin sections were made by Porter-Blum MT IIB ultramicrotome and stained with uranyl acetate and lead citrate, then examined by JEM-1200 EX electron microscope.

Histochemical preparations for light microscopy. Under deep anesthesia, the peritoneal cavities were opened and fresh PLNs and the adrenal glands, used for positive control, were removed. Then they were snap frozen with liquid nitrogen and 20–30-μm sections cut using a cryostat. After air drying, the sections were fixed by acetone for 30 min at − 20 °C. They were subsequently immersed in freshly prepared medium[5] for 1 h at 37 °C, and mounted in glycerine-PBS.

Histochemical preparations for electron microscopy. Under the same conditions as those mentioned above, fresh PLNs and the adrenal glands were removed and immersed in 2.7% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 min at 4 °C. After fixation, the samples were washed with phosphate buffered saline (PBS) several times, snap frozen in liquid nitrogen and cut to give 20–30-μm sections, using the cryostat. The sections were washed in cold PBS for 4 h, and subsequently incubated in freshly-prepared medium of the composition described by Berchtold[6]. Dehydroisoandrosterone (5-androsten-3β-ol-17-one, Sigma, USA) was applied to the substrate of the enzyme reaction. The incubation was performed for 2 h at 37 °C in darkness, with occasional shaking. The control preparations were incubated in a substrate-free medium. After the incubation, the individual sections were quickly rinsed in PBS containing 0.2 M sucrose and postfixed in 2% OsO_4 for 1 h at 4 °C, then prepared for routine electron microscopy. Ultrathin sections were stained with uranyl acetate and examined by JEM-1200 EX.

Results and discussion

Electron microscopic observation showed that the SH cells, which have large nuclei, were located within the PLN capsular walls, surrounded by some fibroblasts with collagen bundles, adipocytes, mesenchymal cells and capillaries, which generally constitute the capsule of a common lymph node (fig. 1a). Most of them were in

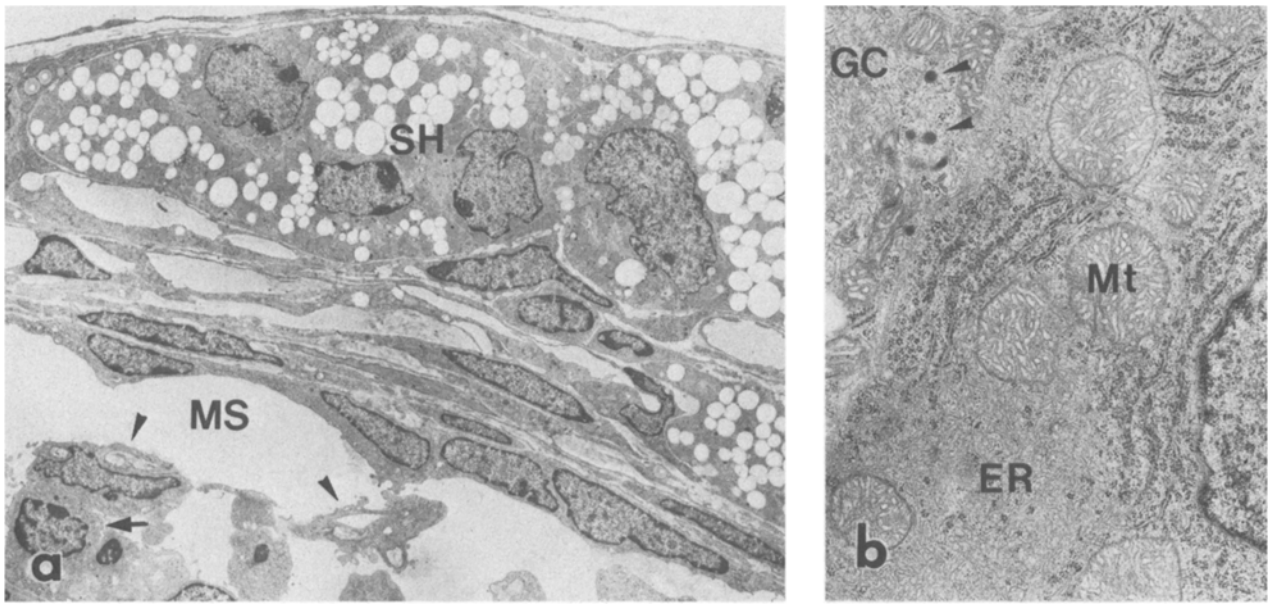

Figure 1. *a* Localization of the intracapsular SH cells (SH) in the PLN of hamster. An arrow indicates the lymphocyte migrating into the marginal sinus (MS) of the node. Arrowheads show reticulum cells. × 3000. *b* High magnification of the SH cell cytoplasm. Tubulovesicular type mitochondria (Mt), well-developed Golgi complex (GC) and smooth endoplasmic reticulum (ER), and some small lysosomes (arrowheads) are seen. × 18 000.

clusters which included 6–8 SH cells, and intermediate or gap junctions were seen between each cell in the cluster. Their cytoplasm showed characteristics of steroid producing cells, such as abundant mitochondria with tubular or vesicular cristae, large amounts of smooth endoplasmic reticulum (ER), well-developed Golgi complexes, and some lysosomes and lipid droplets (fig. 1 b), similar to those of adrenocortical cells in mammals. Occasionally, these cell clusters were observed in the marginal sinus and/or in the peripheral cortex of the node.

Using enzyme-histochemical techniques, obvious 3β-HSD activity could be detected within these lymph nodes (fig. 2); the positive reaction product, deep blue formazan, was distributed on the capsules and peripheral cortexes of the nodes, in a position which paralleled the localization of SH cells observed under electron microscopy (fig. 1). Intensity of the enzyme reaction was stronger in the intracapsular SH cells than in those of the cortex. Observations of serial sections showed that the majority of the cells that reacted positively were detected in the capsule. Ultracytochemically, the reaction products were detected in the form of electron dense particles of copper ferrocyanide, which were distributed around the tubulo-vesicular mitochondria in the smooth ER-developed area of the SH cells (fig. 3 a). These dense reaction products could be observed only in SH cell cytoplasm, not in adjacent fibroblasts (fig. 3 b) or in mesenchymal cells in the capsule. No other lymph nodes, such as renal, mesenteric and some other peripheral lymph nodes demonstrated 3β-HSD activity in either light or electron microscopic investigations. The control

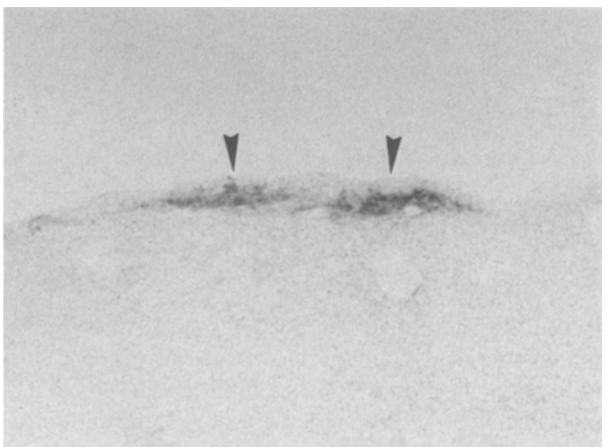

Figure 2. Histochemical demonstration of the 3β-HSD activity in the PLN. Arrowheads indicate the positive reaction products of nitro BT within the intracapsular SH cells of the node. × 150.

preparations lacked positive reaction products within the SH cells (fig. 3 c).

In this study, we detected 3β-HSD activity in the intracapsular SH cells in the PLN of hamsters at the level of light and electron microscopy. The enzyme 3β-HSD, which catalyzes a dehydrogenation of a 3β-hydroxysteroid to a 3β-ketosteroid, is indispensable for biosynthesis of the mammalian steroid hormones in ovaries and adrenal glands. Although most biochemical pathways of steroid synthesis have become clear of late [7, 8], it has been difficult to obtain in situ evidence of the synthesis or

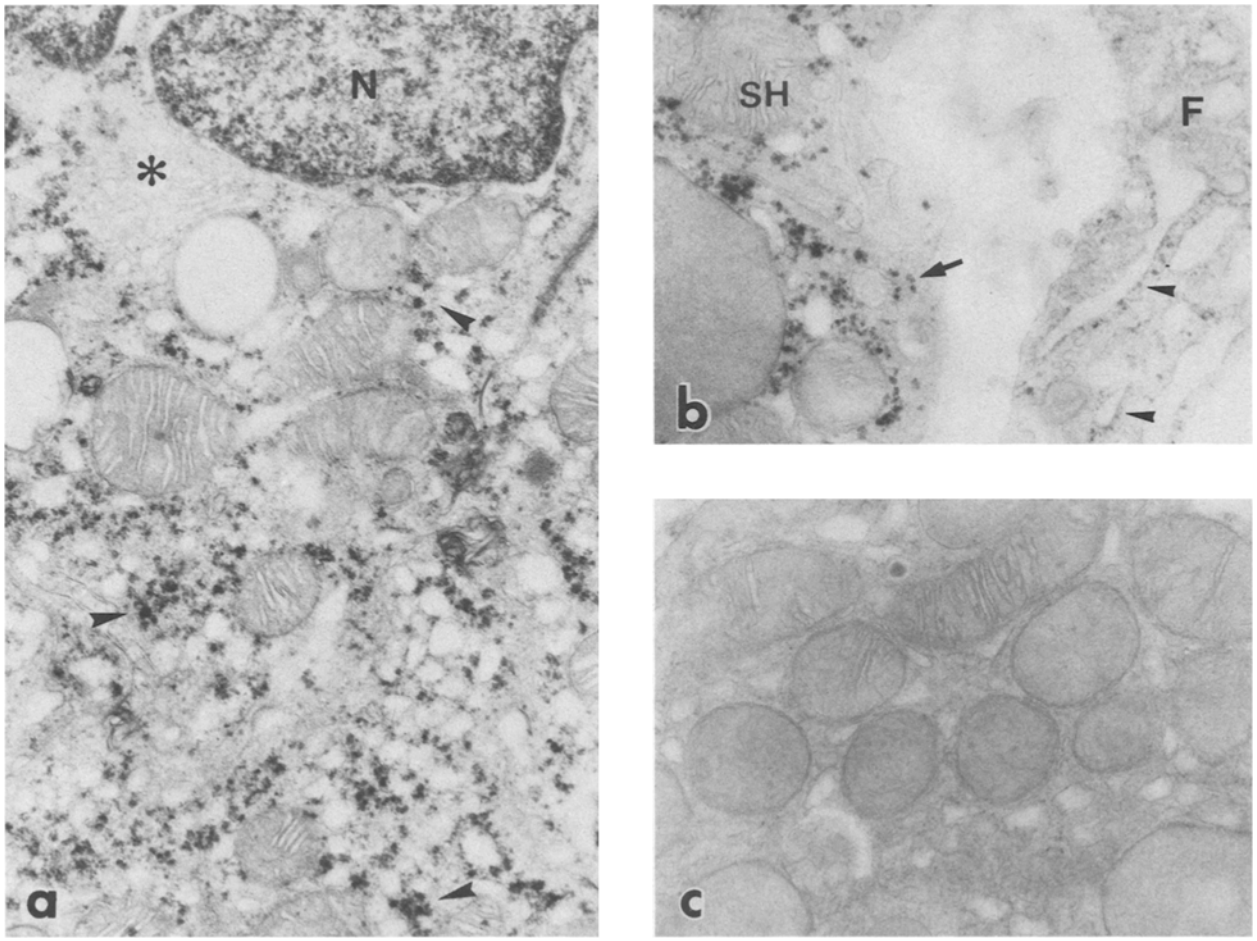

Figure 3. Ultrastructural demonstration of the 3β-HSD activity in the SH cells. *a* Electron dense reaction products were distributed in the perimitochondrial region (arrowheads), not in the Golgi complex (asterisk). N, Nucleus. × 22 500. *b* A part of SH cell cytoplasm (SH) and adjacent fibroblast (F). Note the distinction in size and shape between copper ferrocyanide deposits (arrow) and ribosomes (arrowheads) surrounding ER cistern. × 28 000. *c* Negative control image reacted in substrate-free medium. Compare with fig. 3a. × 28 000.

localization of the steroid hormones with present immunohistological techniques, owing to the impossibility of fixing the hormones[9]. For this reason the detection of 3β-HSD activity offers a reliable way of detecting steroid hormone synthesis.

The enzyme activity in the PLN of hamsters was demonstrated in the intracapsular site of the node where the cluster of SH cells could be observed in our electron microscopic study (figs 1 and 2). Ultracytochemically, the reaction products were detected in the perimitochondrial smooth ER region of the cytoplasm of the SH cells (fig. 3). Previous histochemical studies of 3β-HSD activity in ovaries or adrenal glands have reported that enzyme activity was localized in the mitochondria[10,11] and smooth ER[6,12], and biochemically in the microsome-fraction[13], which caused some confusion. However, recent immunohistological studies using an anti-3β-HSD antibody resolved the discrepancy by showing that the enzyme was distributed in the area of smooth ER around the mitochondria of the cytoplasm of bovine adrenal cortical cells[14], which agrees with our histochemical data for the SH cells. In mammalian steroidogenic cells, pregnenolone, one of the substrates of 3β-HSD, is believed to be produced from cholesterol, through the side chain cleavage system within mitochondria, so it is not surprising that the 3β-HSD activity is detected mainly in the perimitochondrial region of the SH cell. The evidence, including ultrastructural findings, strongly suggests that the SH cells produce a steroid hormone within the PLN of the hamster.

The influence of steroid administration on the immune system has frequently been studied in vitro; suppressive effects on the differentiation of macrophages from monocytes[15], effects on migration of macrophage[16,17], and further, an enhancement of lymphocyte traffic[18,19] and of antibody response to sheep red blood cells[20,21] have been reported. Koyama et al.[22] recently indicated that the density of murine epidermal Langerhans cells depends on testosterone, inducing a higher immune response in females than in males. However, there have been no morphological studies dealing with the in vivo physiological relations between steroid producing cells

and immune organs, such as the SH cells within the PLN. In the previous report [3], we made a suggestion about the origin of the SH cells, that is, that the cells are probably differentiated from mesenchymal cells in the capsule of the PLN as the result of a stimulation with ovarian exudate via directly connected lymphatics, following ovulation. Recently, Kochhar [23] reported that steroid hormones induced a sister-chromatid exchange in mammalian cells, suggesting that the environment in the cells was more mutagenic. Thus it may be that steroid hormones produced by the SH cells play an important role in the rearrangement of immunoglobulin genes due to antigen-stimulated B lymphocyte maturation in the follicle of the PLN. Although we were able to demonstrate 3β-HSD activity in the SH cells within the PLN, no direct evidence linking the PLN immune functions and the physiological role of SH cells was obtained. For the present, the biological significance of the SH cells remains unknown; further studies are needed to elucidate the problem of function, especially concerning the effect of the steroidogenic SH cells on the immune cells of the PLN.

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Effect of human serum on alkaline phosphatase induction in cultured human tumor cells

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Summary. The continuous cell lines T 24 and HT-29, derived from human bladder and colon carcinomas, produce term-placental and intestinal alkaline phosphatase, respectively. Growth in hyperosmolar medium or exposure to prednisolone or sodium butyrate induces increased enzyme levels, and combinations of inducers elicit synergistic activity increases. The effect of the inducing agents is strikingly diminished when cells are grown in the presence of high concentrations of human serum, and the synergistic increases are essentially abolished. Major human serum protein fractions do not affect alkaline phosphatase induction.
Key words. Alkaline phosphatase; human serum; cultured tumor cells; enzyme induction.

Alkaline phosphatases (ALPs) (orthophosphoric-monoester phosphohydrolase (alkaline optimum) EC 3.1.3.1) are glycosylphosphatidylinositol-anchored cell membrane metalloenzymes [1]. At least three major, easily distinguishable enzyme forms (term-placental, intestinal and liver/bone/kidney or tissue-unspecific) have been identified in humans [2]. ALPs are among the substances produced ectopically by many human cancers [3] and are synthesized by certain tumor-derived cell lines [4]. Previous studies [5] have shown that primate sera, but not sera from other mammals reversibly depress ALP activity in HeLa cells and their derivatives in a concentration-dependent fashion, without affecting the enzyme characteristics. Here we compare the effect of human serum (HS) on