A New Type of Cell Death, the Protruded Type, Observed in the Adrenal Gland of Normal Rats

Cleo CHEN-PAN, Yuzuru YAMAMOTO, Yoshihiko ITO, In-Jen PAN¹⁾, and Yoshihiro HAYASHI²⁾

Research Institute for Animal Science in Biochemistry and Toxicology, 3-7-11, Hashimoto Dai, Sagami-hara-shi, Kanagawa 229,

¹⁾Laboratory of Veterinary Clinical Pathology, College of Agriculture and Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa-shi, Kanagawa 252, and ²⁾Department of Veterinary Anatomy, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

(Received 15 May 1995/Accepted 25 November 1995)

ABSTRACT. Apart from apoptosis, a type of parenchymal cell death by the cell protruding into the capillary lumen was observed in the adrenal gland of normal Sprague-Dawley rats. Ultrathin sections were prepared in the conventional manner and were examined by electron microscopy. The protruded cells (p-cells) had the electron lucent cytoplasm and the p-cells with ruptured cell membranes were observed in the capillaries. The egress of p-cells was either through the endothelial gaps, or following the rupture of capillary endothelia. The cytoplasmic matrices of the dying p-cells were seen to scatter in the capillary lumen where the nuclei, mitochondria and granules remained morphologically intact. The p-cells were seen in the capillaries of medulla, but restricted to those of *zona reticularis* in the cortex. — **KEY WORDS:** adrenal gland, cell death, rat.

J. Vet. Med. Sci. 58(4): 373–376, 1996

The parenchymal cell deaths occur in normal and developing tissues of vertebrate and invertebrate animals. They occur also under pathological conditions. Kerr *et al.* [7] proposed that there are two types of cell death, i.e., apoptosis and necrosis. Ales *et al.* [1] emphasized that apoptosis is an emergent scientific hypothesis with few well-defined morphological and molecular characteristics to distinguish it from the necrotic cell death. They also pointed out that the only molecular characteristic commonly associated with apoptosis is the internucleosomal DNA cleavage pattern. However, Corcoran and Ray [4] stressed that necrosis has certain key molecular changes in common with apoptosis, including nuclear Ca^{2+} deregulation and DNA cleavage, on the basis of the examination of alkylation-induced hepatotoxicity *in vivo*.

In an ultrastructural study of various tissues from embryonic and fetal rats and mice, Schweichel and Merker [12] proposed that there are three types of cell deaths in developing tissues, i.e., apoptosis, autophagic degeneration and non-lysosomal vesiculate degradation. Clarke [3] broadened the definition of the third type, non-lysosomal vesiculate degradation, and divided it into two subtypes, i.e., non-lysosomal disintegration and the cytoplasmic type of degeneration. The fate of these dead cells of the former two types [12] is by heterophagia, while disintegration occurs in the non-lysosomal disintegration. Based on a literature search, Clarke [3] suggested that a fourth possibility existed in addition to those of Schweichel and Merker, i.e., the remnants of dead cells might be extruded from the tissue.

In this study, we observed that a mode of cell death, protruded type, occurred in the adrenal gland of the normal rats. The protruded cells (p-cells) egressed from the parenchyma to the capillary lumens through the capillary endothelial gaps. The morphological characteristics of p-cells differed from those of other types, i.e., the rounded and degenerating cells were expelled from the surrounding normal tissue into the extracellular space [5], or dead cells

were ingested by phagocytes, commonly found in the subepicardial space many microns away from the outer surface of the myocardium [9].

A total of 10 male normal Sprague-Dawley rats aged 6–7 weeks were used in this study. In order to obtain a clear view of the protrusion process, adrenal glands were taken immediately after anesthesia of ether, and were immersed for 2 hr at 4°C in a prefixative composed of 2% glutaraldehyde in 0.1 M cacodylate buffer solution on a shaker, followed by postfixation with 1% osmic acid. Following the conventional dehydration, the samples were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with a JEM-1200 EX electron microscope. For the reproducibility, 4 extra male rats with the same age and strain were killed and prepared at Department of Veterinary Anatomy, Faculty of Agriculture, The University of Tokyo. The adrenal glands were fixed in the same manner as above except that the prefixative was at different concentrations and in different type of buffer solutions, i.e., 2% glutaraldehyde in 0.1 M cacodylate buffer, 2% glutaraldehyde in 0.1 M phosphate buffer, 5% glutaraldehyde in 0.1 M cacodylate buffer, and 5% glutaraldehyde in 0.1 M phosphate buffer. The Araldite M was used for embedding.

The characteristics of p-cells seen in the cortex and medulla were the protrusion of parenchymal cells into capillary lumens. Following the rupture of cell membranes, the cytoplasmic matrices of the protruded cells were seen to be scattered in the lumens. Before the protrusion, the cytoplasm of a p-cell was light electron-dense, and the nucleus was neither pyknotic nor fragmented. The dilation of endoplasmic reticulum of p-cell was more conspicuous in the medulla (Fig. 1). Autophagic vacuoles were rarely seen. When the p-cells appeared in the capillary lumen, the nuclear membrane and cytoplasmic organelles, such as secretory granules and mitochondria, remained morphologically intact. Some differences existed in the

Fig. 1. The medulla with the capillary endothelium ruptured at the protrusion site (arrowhead) and the dilated endoplasmic reticulum of chromaffin cell (E). Bar: 2 μ m.

protruding process between the cortex and medulla as follows.

Cortex: The protrusion was limited in *zona reticularis*. Similar to white blood cells migrating through the capillary wall, protruded cells either egressed through the endothelial gaps or through cytoplasm near the nucleus of the endothelium (Figs. 2, 3 and 4). Subsequently, the cell membrane ruptured, and the cytoplasmic matrix scattered into the blood stream (Fig. 3). During the protrusion, the cell nucleus was deformed by passing through the ruptured endothelium (Fig. 4). The size and electron density of the nucleus of p-cell in the capillary lumen were the same as those of surrounding cells. There was only one protruded cell observed in each plane of section of a capillary. The ruptured capillary endothelium at the protrusion site remained *in situ* (Fig. 4).

Medulla: The whole medulla was subjected to the protrusion process. The capillary endothelium at the protrusion site became ruptured. The neighboring chromaffin cells with dilated endoplasmic reticulum gathered closely to the degenerated endothelium. During the protrusion, the basement membranes of the protruded chromaffin cells remained *in situ*. Plural cells were visible in each plane of the capillary sections (Fig. 5).

There are two kinds of cells, ultrastructurally dark and light cells, in *zona reticularis*. In the past, there were considerable differences in interpretation of the function of these cells [11]. Based on the morphological evidences we have found in this study, it seems that the light cells are indicative of degenerating cells.

In the collagen fibrillar framework of rat adrenal gland as described by Kikuta *et al.* [8], a collagenous, fibrillar plexus of an irregular, single cell-sized compartment was observed in *zona reticularis* of the cortex, and a basket-like fibrillar sheath enveloping clusters of chromaffin cells was observed in the medulla. Distinctive and architectural differences in the cortex and medulla could explain our observations that different numbers of protruded cells existed in these two layers of the adrenal gland.

Although the perfusion fixation maintains a better morphological structure of the tissues than the immersion fixation, it cannot be certain that the subtle environment in sinusoidal capillaries of the adrenal gland as shown in Figs. 2 and 3 will not be affected by the former fixation. In other words, any possible influence mechanically and/or chemically caused by the perfusion treatment should not be eliminated, i.e., the capillary gaps may be closed, or the partial protruded portion of p-cells may be drew back or ruptured. Taking these into account, we selected the immersion fixation for this study. The phenomenon of cellular protrusion was reproducible in the blocks embedded in Araldite M. Idelman [6] described that the cellular debris, which originated from chromaffin cells or cortical cell fragments, appeared in the sinusoidal capillaries of *zona reticularis* of rat adrenal cortex. He suggested that this debris might be a phenomenon of the holocrine secretion [6]. Furthermore, the artifacts in the adrenal cortex stated by Idelman [6] were not observed in this study.

Among the criteria of necrotic cells described by Wyllie *et al.* [13], the death of protruded cells shared only a few morphologic traits with necrotic cells, i.e., the rupture of cell membrane and dilation of endoplasmic reticulum. Following the rupture of cell membranes the p-cells simply released the cytoplasmic contents into the blood stream, while necrotic cells induced a secondary process. The margination of chromatin, as seen in necrotic cells, did not occur in p-cells. According to the morphological classification of Clarke [3], the p-cells reported herein resemble atypically the cytoplasmic degeneration, i.e., the dilation of endoplasmic reticulum.

Wyllie [14] found apoptosis occurring in *zona reticularis* of rat adrenal gland. The death of pheochromocytoma PC12 cells, induced by withdrawal of the nerve growth factor, was studied *in vitro* by Bartistatou and Greene [2] and Mesner *et al.* [10]. These cell deaths could be distinguished by DNA fragmentation. The death of protruded cells we observed was a silent pattern, the discrimination of which was not as easy as that of apoptosis, nevertheless, it could be recognized morphologically by transmission electron microscopy. Furthermore, the occurrence of protruded cells seemed to be related to the architecture of tissues like adrenal cortex and medulla, i.e., those rich in sinusoidal capillaries as shown in this study. It is considered that the protruded cell plays a role in cell kinetic processes of the adrenal gland.

Figs. 2, 3. Zona reticularis of the cortex. The protruded cell (E) (p-cell) egressed through the endothelial gap (arrowhead) and the cytoplasmic content of a ruptured cell membrane released into the capillary lumen. Cytoplasmic content of the p-cells was visible in the capillary lumen of lower-left of the Fig. 2. The mitochondria and secretory granules remained intact in Fig. 3. Bar: 2 μ m (Fig. 2); 500 μ m (Fig. 3).

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Fig. 4. *Zona reticularis* of the cortex. Nucleus of p-cell (E) deformed and egressed through the ruptured endothelium. Bar: 5 μ m.

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Fig. 5. Capillary lumen in the medulla. Five p-cells appeared in the blood stream. The nuclei with membrane were neither pyknotic nor fragmented, and cytoplasmic contents were scattered in the lumen. Bar: 5 μ m.