Presence of presumptive interdigitating cells in the spleen of the natterjack, *Bufo calamita*

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Summary. The periphery of splenic lymphoid follicles, an area rich in reticulum fibers, contains presumptive interdigitating cells characterized by low electron density, scantiness of cytoplasmic organelles, abundant surface foldings and, sometimes, electron-dense granules of unknown significance.
Key words. Interdigitating cells; spleen; anurans; ultrastructure; lymphoid organs.

Despite current studies of amphibian immunology, little is known about the ultrastructure and histophysiology of the lymphoid organs, especially regarding features related to nonlymphoid components. Widely reported in mammalian central and peripheral lymphoid organs[1], interdigitating cells (IDCs), have also been occasionally found in birds[2,3], reptiles[4] and amphibians[5]. We recently described the histological organization of the spleen of the natterjack, *Bufo calamita* emphasizing both its resemblance to that of *Xenopus laevis* and the presence of giant, dendritic cells in both red and white pulp[6]. In the present study, we describe the ultrastructure of presumptive IDCs, for the first time in amphibians, in the marginal zone of the spleen of *B. calamita*.

Materials and methods. 25 adult natterjacks were collected in León and Madrid, Spain, in October and immediately sacrificed. The spleens, which were removed aseptically, were fixed in toto by immersion in 2.5% glutaraldehyde buffered to pH 7.3 with Millonig solution. They were then postfixed in 1% osmic tetroxide in the same buffer and dehydrated in acetone for embedding in Araldite. Sections 1 μm thick were stained with an alkaline solution of toluidine blue to select appropiate areas. Blocks were sectioned with a Reichert OM-U3 ultratome, and the grids stained with uranyl acetate and lead citrate before examination in a JEOL-100C electron microscope.

Results. The spleen of *B. calamita* consists of white pulp, which is composed of lymphoid follicles and red pulp which is formed by cell cords and blood sinuses, the areas being separated by a continuous layer of reticulum cells. Electron microscopic analysis of the periphery of the lymphoid follicles revealed clusters of electron-lucent, nonlymphoid cells located between medium-sized lymphocytes and lymphoblasts, their morphology recalling that of IDCs in that they presented an irregular-shaped nucleus and an electron-lucent cytoplasm which contained few membra-

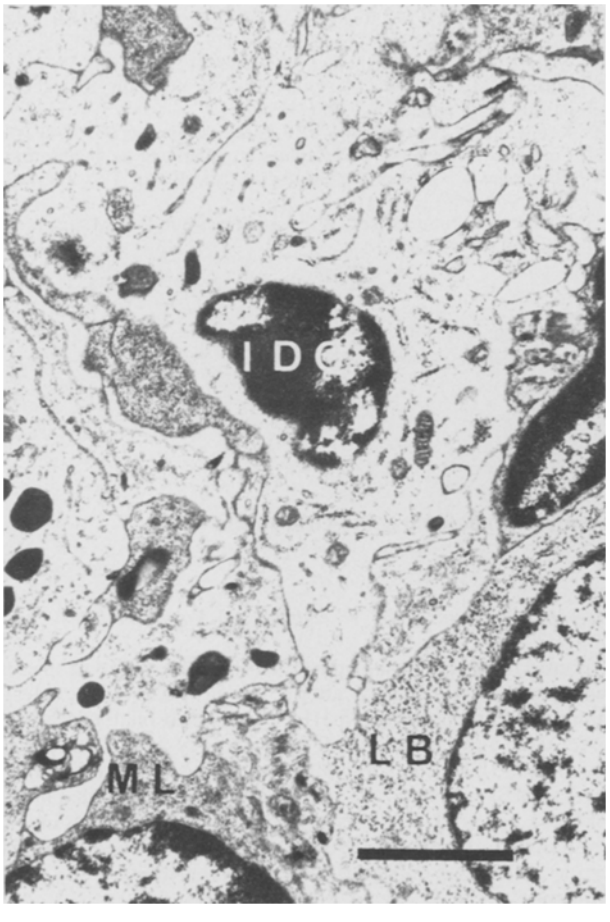

Figure 1. Interdigitating cell (IDC) in the marginal zone of a splenic lymphoid follicle of *B. calamita*. Note the low electron density and scarcity of cytoplasmic organelles. Lymphoblast (LB), medium lymphocyte (ML). Bar = 2 μm.

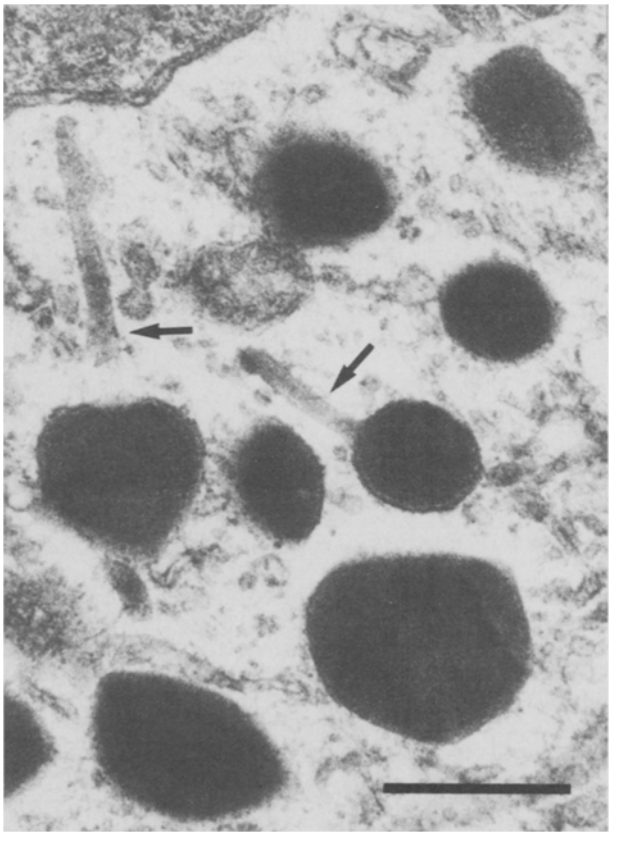

Figure 2. Dumbell-shaped bodies of an IDC. Note the membranous structure of the narrow tail (arrows) and the polygonal shape of the electron-dense heads. Bar = 0.5 μm.

nous organelles, such as rough and smooth endoplasmic reticulum, and small mitochondria (fig. 1). Numerous foldings on the cell surface form an intricate pattern involving cell membranes of neighboring lymphocytes; nevertheless, no cell junctions were found. Some of these cells contained strikingly electron-dense granules, which appeared as dense bodies and/or lysosomes, or exhibited a round or polygonal electron-dense head and a long narrow tail consisting of material with a lower electron density and recalling a multilayer system of membranes (fig. 2).

Discussion. Numerous reports have been made of the presence of interdigitating cells in the central and peripheral lymphoid organs of mammals[1]. Their presence has been pointed out by Kendall and Frazier in some birds[2] and by Fonfria et al. in the spotless starling *Sturnus unicolor*[3]. As for reptiles and amphibians, presumptive pro-IDCs and mature IDCs have recently been found in the thymus and spleen of the turtle *Mauremys caspica*[4], and Plytycz has identified them in the spleen of the frog *Rana esculenta*[5]. No information exists concerning other ectothermic vertebrates.

In order to identify the cells present in the marginal zone of the splenic lymphoid follicles of *B. calamita* as possible IDCs, these morphological parameters have been taken into account; the low electron density of their cytoplasm, the scarcity of cytoplasmic membranous organelles and, most importantly, the numerous foldings on the surface of the membranes, which form a labyrinthine system of interdigitations. These features have all been included in the morphological characterization of mammalian[1] and avian IDCs[3]. This cell-type has been considered a member of the mononuclear-phagocyte system, concerned with the presentation of antigens to T-lymphocytes[7-10]. Remarkably, in the spleen of *B. calamita*, IDCs were more frequent after immunization with sheep erythrocytes[11].

It is difficult to evaluate, however, the presence in these cells of conspicuous cytoplasmic granules. Two types of granules have been described in mammalian IDCs: some are small, electron-dense and lysosomic, while the others are similar to the Birbeck granules of the Langerhans cells of the skin. In the IDCs reported in *B. calamita*, the former type occurs, together with another granular population whose morphology has never been described before. The tails of these granules may bear a certain resemblance to a primitive Birbeck granule or to so-called 'cored-tubules' also described in some Langerhans cells[12], or even to granules described in IDCs of the coecal tonsil of *Sturnus unicolor*[13]. Thorbecke et al.[14], on the other hand, have suggested that IDCs containing Birbeck granules present in mammalian lymph nodes represent mobile Langerhans cells or their descendents, which migrate to the paracortex of the lymph node after antigenic stimulation of the skin, principally in the T-dependent immunocellular reaction. IDCs are involved in the endocytosis of ferritin-antiferritin complexes in the mammalian thymus[15]. In contrast, the electron-dense heads differ totally from granules previously described in macrophages, monocytes, IDCs or Langerhans cells. Further histochemical and immunological characterization is therefore necessary in order for these preliminary results to be confirmed.

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Some species differences in cardiovascular responses to intravenously injected leucine-enkephalin

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Summary. Studies were conducted to determine the cardiovascular responses to leucine-enkephalin (L-enk) in three different species of animals; rabbit, dog and monkey. All animals were anesthetized with pentobarbital sodium after sedation with ketamine. Mean blood pressure (MBP) and heart rate (HR) were simultaneously monitored. The pressor and HR responses to bilateral carotid occlusion (BCO) were determined before injection of L-enk. Increased MBP and HR due to BCO in monkey were significantly greater than in the other two animal groups. Following i.v. injection of L-enk (5–30 μg/kg), a significant fall in MBP occurred in all groups in a dose-dependent manner; however, the time course of changes in MBP in rabbits was significantly shorter than that in the other animal groups. Significant decreases in HR after the injection of L-enk occurred in rabbits and dogs, whereas increases in HR occurred in monkeys. These results show that some cardiovascular responses to L-enk may be species dependent. These different cardiovascular responses to L-enk may be at least partly related to species differences in baroreceptor reflex sensitivity.
Key words. Leucine-enkephalin; blood pressure; heart rate; species differences.

The basic characteristics of cardiovascular responses to opioid peptides have been studied extensively in a number of species including the rat [2,4,11,14-16,18], the cat[5,6,9,14,19], and the dog[7,10,12,13]. Opioid peptides produce changes in systemic blood pressure and heart rate after either intravenous injection[9,12-14,18,19] or administration into the central nervous system[1,5,11,14,19]; however,