PLASMA CELL CLUSTERS IN THE INTERSTITIAL TISSUE OF THE TESTES OF *ACANTHODACTYLUS ERYTHRURUS* (REPTILIA, LACERTIDAE)

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INTRODUCTION

Reptiles represent a key group for understanding the evolution of vertebrate immune responses, although some aspects of their immunobiology are still poorly understood (Cohen, 1977; Borysenko, 1978; Cooper, Klempau and Zapata, 1980). The structure of lymphoid organs has been described in some reptiles (Borysenko and Cooper, 1972; Borysenko, 1978). Thymus and spleen appear to be the most important organs in the reptilian immune system (Kanakambika and Muthukkaruppan, 1973; Borysenko, 1976a, b; Cohen, 1977), although lymphoid cells occur in the gut (Zapata and Solas, 1979; Solas and Zapata, 1980) and urinary bladder (LeFevre, Reincke, Arbas and Gennaro, 1973). In addition, ectopic lymphoid tissue has been found in the pancreas of *Psammodromus algirus* (Zapata, Gomariz and San Miguel, 1978), and Kanesada (1956) considered their importance for explaining the phylogeny of vertebrate haemopoietic tissues.

We have accidentally found abundant lymphoid tissue in the interstitial areas of the testes of one apparently healthy, adult lizard, *Acanthodactylus erythrurus*, collected together with other specimens lacking such lymphoid accumulations. In the present work, we describe the ultra-structure of these lymphoid elements and their possible functional significance is discussed in relation to some autoimmune phenomena reported in mammals.

MATERIALS AND METHODS

Ten adult *A. erythrurus* (Reptilia, Lacertilia), collected in Toledo (Spain) at the end of summer (September) were selected for an ultrastructural study of the seminiferous epithelium. One of them, apparently healthy, showing abundant lymphoid tissue in the interstitial areas, was used in the present study. The testes were fixed by immersion in 2·5 per cent glutaraldehyde buffered to pH 7·3 in Millonig liquid, post-fixed in 1 per cent osmic tetroxide in the same buffer, and dehydrated in acetone for embedding in Araldite. Ultrathin sections were obtained with a Reichert OM-U3 ultratome, double stained with lead citrate and uranyl acetate and examined with a JEOL 100-B electron microscope. Semi-thin sections approximately 1 to 2 μm thick were cut from tissues processed for electron microscopy. These sections were stained with an alkaline solution of toluidine blue.

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RESULTS

The testes of *A. erythrurus* contained numerous seminiferous tubules delimited by interstitial areas consisting of connective tissue, blood vessels, myoepithelial cells and Leydig's cells. In these areas, light microscopy showed the presence of lymphoid tissue containing plasma cells, lymphocytes and macrophages (Fig. 1). With electron microscopy, the interstitial lymphoid infiltrates appeared as cell clusters, where plasma cells were the predominant elements, although macrophages and lymphocytes were also present (Fig. 2). The macrophages were large cells with an electron-dense, irregular nucleus, showing a small nucleolus and cytoplasm in which large, electron-dense bodies were abundant (Fig. 3). Many of these bodies suggested a cellular origin and showed phagocytic capacity. The clusters also contained lymphocytes and lymphoblasts. The lymphoid elements exhibited a high nucleo-cytoplasmic ratio with abundant cytoplasmic ribonucleo-proteins but scant membranous components. The nucleus was electron-dense with a large amount of condensed chromatin (Fig. 4).

Mature plasma cells were round or oval and showed abundant condensed chromatin arranged at the nuclear periphery. The cytoplasm was practically filled with very dilated, rough endoplasmic reticulum, containing a moderately electron-dense material. In addition, the cell contained a poorly developed Golgi complex, polygonal electron-dense granules and large mitochondria with a dense matrix (Fig. 5). The clusters also contained immature and degenerating plasma cells. The former exhibited few cytoplasmic granules, a well developed Golgi complex and abundant non-dilated rough endoplasmic reticulum (Fig. 6). The older plasma cells were electron-dense, showed evident degenerative changes, and appeared free among the other cells (Fig. 7) or phagocytosed by the macrophages (Fig. 3).

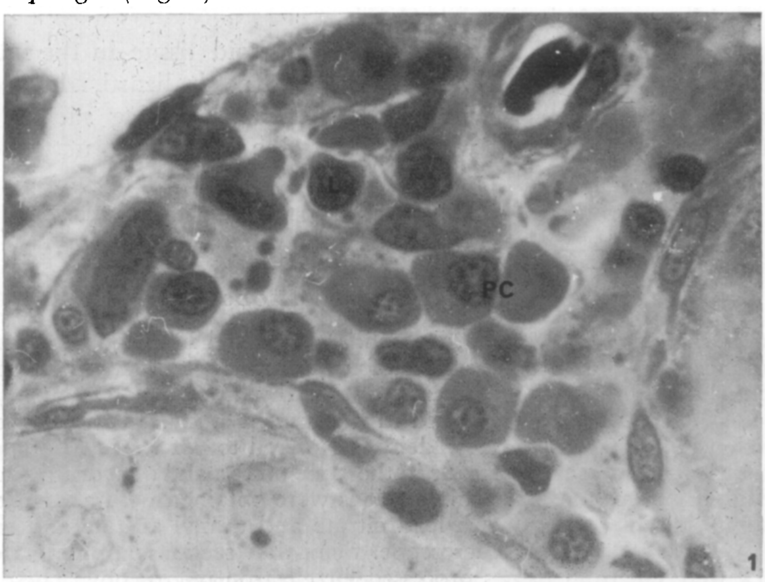

Fig. 1. Testicular interstitial tissue of *A. erythrurus*. Note the presence of plasma cells (PC) and lymphoid elements (L). ×2800.

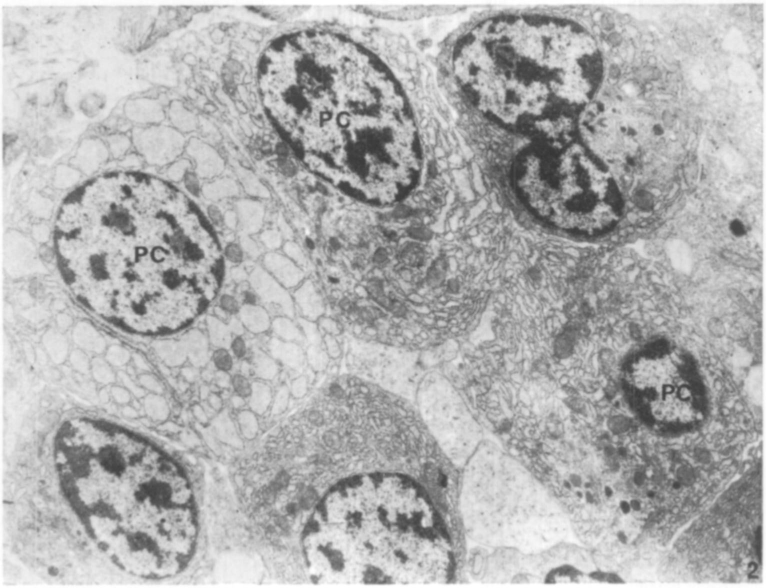

Fig. 2. Ultrastructure of the clusters of ectopic lymphoid tissue present in the testes of *A. erythrurus*. Note the predominance of plasma cells (PC), which show several developmental stages. ×5600.

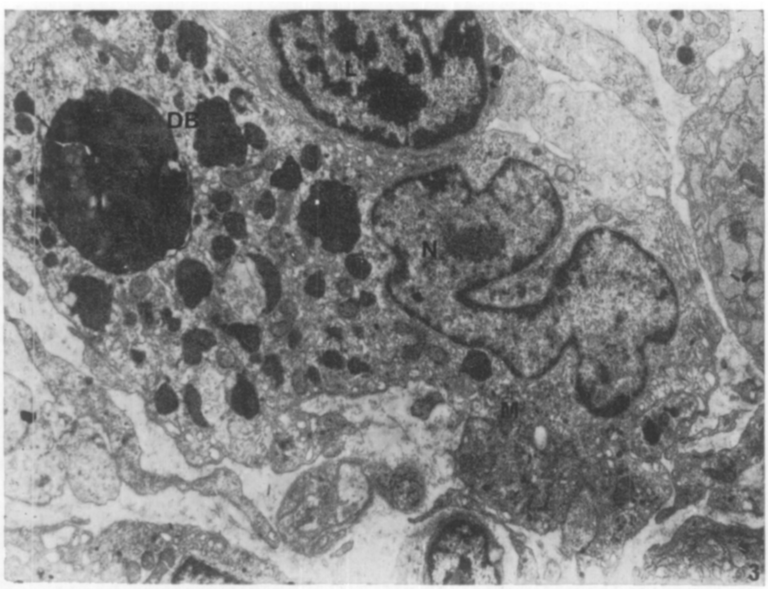

Fig. 3. Presence of macrophages (M) and lymphoid cells (L) in the interstitial tissue of the testes of *A. erythrurus*. Note the irregular nucleus (N) and the presence in the cytoplasm of macrophages of numerous dense bodies (DB), some of which represent degenerated cells. ×8000.

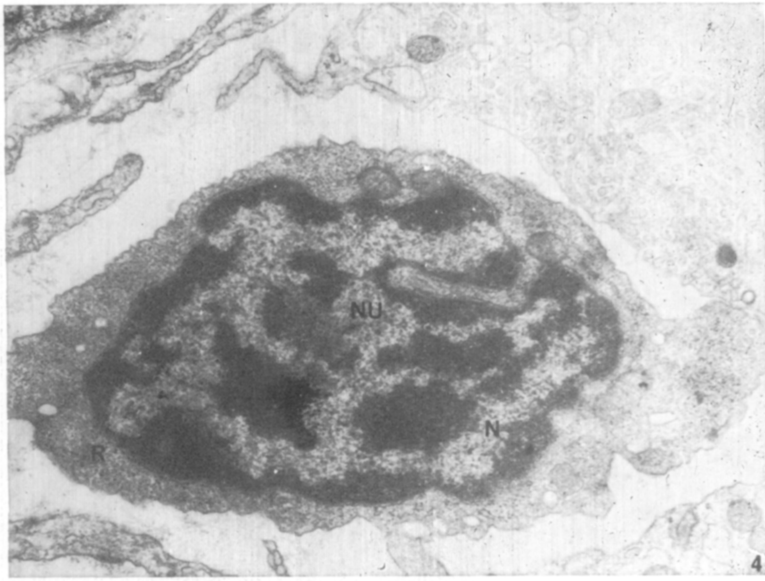

Fig. 4. Lymphoid cell of testes of *A. erythrurus*. The cell shows an irregular nucleus (N) with abundant condensed chromatin and a small nucleolus (NU). The cytoplasm contains numerous, free ribonucleoproteins (R) and scant membranous organelles. $\times$18 400.

Notable features were the existence of gap junctions between neighbouring plasma cells in the clusters (Fig. 8), and the presence of lymphoblasts and mature plasma cells inside the seminiferous tubules, between the developing spermatocytes (Fig. 9). The cells showed an intercellular arrangement in the

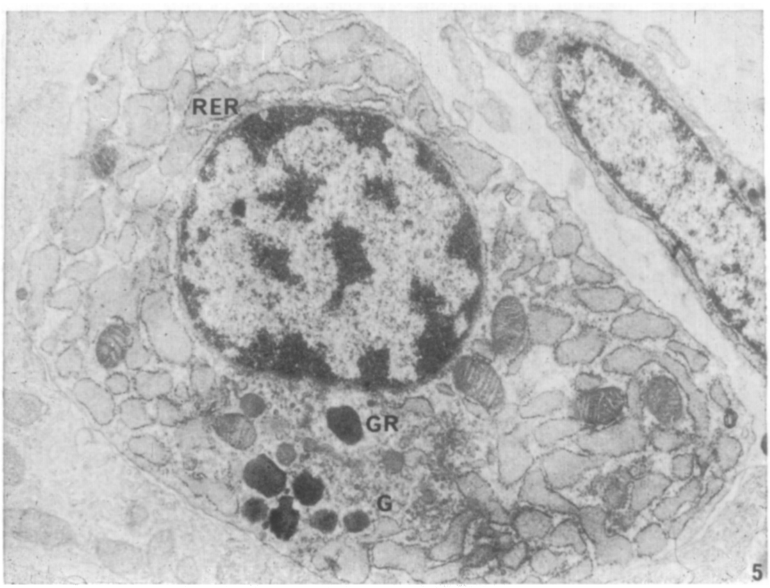

Fig. 5. Mature plasma cell. Note the large development of dilated rough endoplasmic reticulum (RER) and its electron-dense content. Some electron-dense granules (GR) are seen surrounding the Golgi complex (G). $\times$14 400.

basal level as well as close to the lumen of the tubule, sometimes close to Sertoli cells (Fig. 10). Lymphoid cells inside the seminiferous tubules did not exhibit degenerative changes, although degenerated spermatids were present.

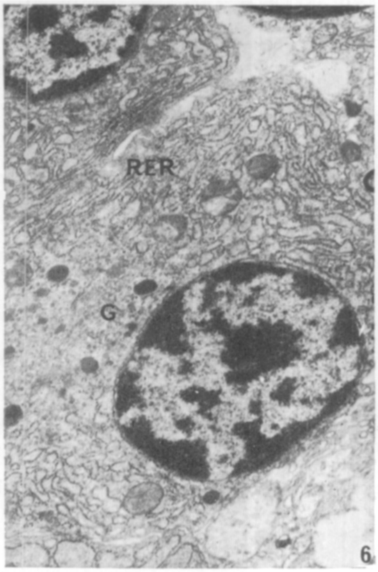

Fig. 6. Immature plasma cell. The rough endoplasmic reticulum (RER) is well developed but its sacculi are not dilated as in the mature stage. Note the large development of Golgi apparatus (G). × 9600.

Fig. 7. Degenerating plasma cell debris free among the connective tissue elements. × 14 400.

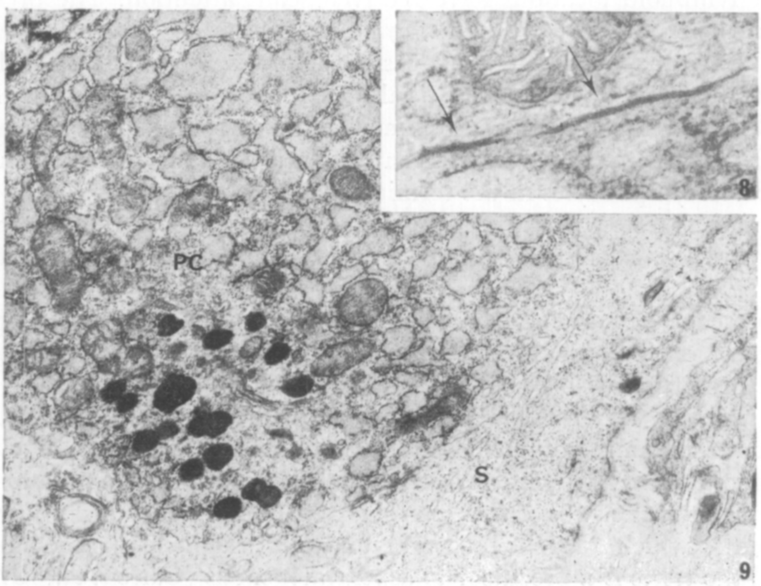

Fig. 8. Gap junctions (arrow) between neighbouring plasma cells, in the clusters of lymphoid tissue found in the testes of *A. erythrurus*. × 48 000.

Fig. 9. Mature plasma cells (PC) among spermatids (S) of seminiferous tubules. Note the intercellular arrangement of infiltrating cells. × 11 200.

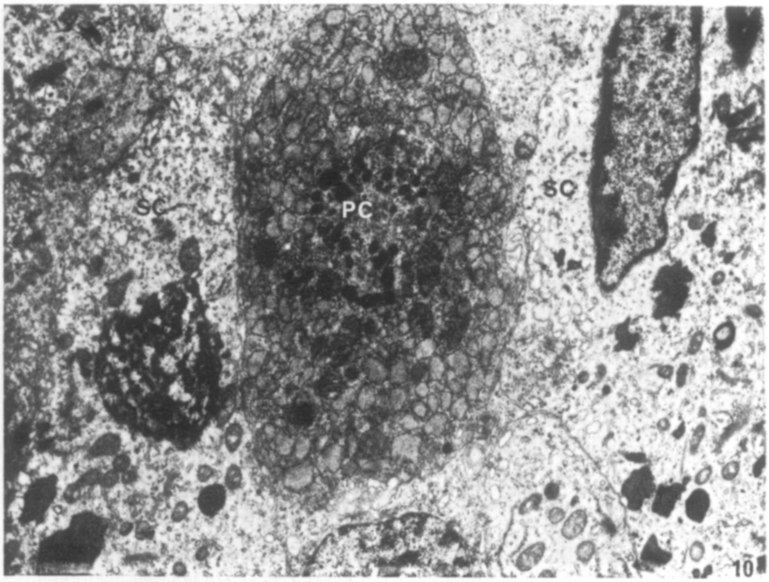

Fig. 10. Mature plasma cell (PC) in the central area of aseminiferous tubule of *A. erythrurus*. The plasma cell is next to Sertoli cells (SC). ×8000.

DISCUSSION

The structure of reptilian tissue was described by Borysenko and Cooper (1972) and by Borysenko (1978). With the exception of thymus and spleen, the other lymphoid locations have received scant attention. The presence of lymphoid tissue in the testes of *A. erythrurus* is notable because of the large number of plasma cells and the invasion of the seminiferous epithelium by lymphoid elements. In the interstitial tissue of human and animal testes, fibroblasts and macrophages are common, and occasional sparse plasma cells, mast cells and lymphocytes have been reported (Ohata, 1979). Dym (1974) also observed lymphocytes in the terminal portions of the seminiferous tubules of the monkey testes, and macrophages with actively ingested spermatozoa were seen in the excurrent ducts of short-term vaso-ligated birds (Aire and Heath, 1977).

The presence of lymphoid tissue in the testes of *A. erythrurus* suggests an evolutionary explanation for autoimmune diseases and especially for autoimmune orchitis. Carlo, Hagopian, Jackson, Limjuco and Eylar (1976) reported massive cell infiltrations in experimentally induced orchitis. The infiltration of inflammatory cells consisted predominantly of lymphocytes and other mononuclear cells with some polymorphonuclear cells. Invasion of the seminiferous tubules was often seen in agreement with our observations. Such events, which preceded aspermatogenesis, suggest that cellular infiltration of the testes is a precursor to aspermatogenesis (Carlo *et al.*, 1976). Our results might correspond to a precocious stage of the disease, where the cell invasion has not yet produced degeneration of seminiferous tubules, though degenerating spermatids were present. These degenerative processes might be related to lymphoid infiltrates, although seasonal degeneration of spermatids has been reported during the sex cycle of these lizards (see review by Fox, 1976).

Similar cell infiltrates have been reported in other autoimmune diseases of man. Prineas (1979) described perivascular infiltrates of lymphocytes and plasma cells as a histological feature of the discrete lesions that develop periodically in different parts of the brain and spinal cord. He stated that the predominant elements were clusters of plasma cells where plasmacytes are in contact by cell surface junctions which are similar to the observed infiltrates in the testes of *A. erythrurus*. Gap junctions were also found between the plasma cells of the testes interstitial tissue of *A. erythrurus*. Prineas (1979) suggested that these clusters may represent the major site for antigen processing and antibody production within the central nervous system of patients with multiple sclerosis.

Although our results are only preliminary, we believe that there is sufficient evidence, from a morphological view-point, to relate them to certain autoimmune diseases. If these data are confirmed by controlled experimental immunological investigations, reptiles may be an excellent experimental model for investigating the evolutionary origins of autoimmune diseases.

SUMMARY

The nature of ectopic lymphoid tissue present in the testes of 1 of 10 lizards *A. erythrurus*, was investigated by electron microscopy. Lymphoid clusters, consisting mainly of plasma cells, occurred in the interstitial tissue among the connective tissue elements, and some lymphocytes and mature plasma cells infiltrated the seminiferous tubules. The possible functional significance of these accumulations is discussed in relation to autoimmune diseases of man.

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