Journal of Animal Research: v.9 n.1, p. 13-17. February 2019

DOI: 10.30954/2277-940X.01.2019.2

The Spleen Morphology of the African Giant Pouch Rat (*Cricetomys gambianus*-Waterhouse, 1840) from Eastern Nigeria

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Received: 22 Oct, 2018 **Revised:** 14 Jan., 2019 **Accepted:** 15 Jan., 2019

ABSTRACT

The spleen morphology of the African Giant pouch rat from the rainforest vegetative region of Nigeria was investigated to establish is basic biology as there dearth of information on it from available literature. Grossly, the spleen was shaped like an elongated triangle, with the two ventral sides of the triangle forming a hilus at the apex of their both convergence. This hilus served as the site of entry and exist of blood vessels and nerves. Microscopically, the spleen was covered by a capsule of connective tissue. The parenchyma was composed mainly of red pulp and isolated areas of white pulp. The red pulp contained splenic cords, sinusoids and other vessels. The white pulp contained the periarteriolar lymphatic sheath which presented a germinal centre, marginal zone and eccentrically located artery surrounded by small lymphocytes. The microanatomy of the spleen revealed an organ involved in blood storage, blood purification and body immune response. This paper will fill the knowledge gap and serve as baseline data for further investigative research.

Keywords: Spleen, histology, red pulp, white pulp, blood storage.

The spleen is a flaccid bag that serves as a storage site for blood, haemopoeisis and a processing station for the scavenging of aged erythrocytes (Ibe *et al*., 2010). It is the largest lymphoid organ and the most important organ of immunological defense for the blood including phagocytosis and removal of blood parasites (Chen and Weiss, 1973; Pabst, 1993). The mammalian spleen is covered by a capsule of connective tissue (Zidan *et al*., 2000). The mammalian splenic parenchyma consists mostly of red pulp and few areas of white pulp (Corbin *et al*., 2008). The red pulp contains erythrocytes organized into splenic cords and venous sinuses. The splenic cords are composed of reticular fibers, reticular cells, and associated macrophages (Saito *et al*., 1988). The white pulp consists of mainly of B- lymphocytes arranged into periarteriolar lymphocyte sheath –PALS, and macrophages (Bacha *et al*., 1990).

The morphology of the spleen has been documented in the one humped Camel (Zidan *et al*., 2000); mice (Tanaka, *et al*. 1996); man (Satoh *et al*., 1997); in cats and ruminants (Brown & Dellmann, 1976); but there is dearth of information on the morphology of the spleen in the African Giant Pouched Rat (AGR) from the rainforest vegetative (RV) region of Nigeria as that from the guinea savannah vegetative (GSV) region has been documented (Ibe *et al*., 2010).

The AGR is becoming an animal of scientific importance because of its use in land mines and a diagnostic tool for tuberculosis detection (Lindow, 2001; Maggie, 2003; Mott, 2004). Also the AGR has become a ready source of animal protein in several rural communities, hence the possibility of its domestication for intensive production (Ajayi, 1975). The proposal to use the AGR as a research model to replace Winster rat because of its larger size (Dipeolu *et al*., 1981; Olayemi *et al*., 2001), has been faced by the fundamental challenge of dearth of information on its biology from published literature, hence the need to provide the baseline data on this important lymphoid and hemopoietic organ in AGR for further investigative researches especially from the AGR in rainforest vegetative

region of Nigeria. The aim of this work therefore, is to document the basic morphology of the AGR spleen from the rainforest vegetative region of Nigeria, and compare if the difference in vegetative regions have an effect on its anatomy.

MATERIALS AND METHODS

Five adult AGR of both sexes with an average weight of 980g, captured in the wild from Olokoro Umuahia in Abia state, Nigeria from March to November 2012 using metal cage traps were used for the study. Olokoro Umuahia is in the rainforest vegetation of southern Nigeria characterized by heavy rains and thick well grown mangrove forest trees. They were immediately transferred to the veterinary anatomy laboratory of Michael Okpara University of Agriculture, Umudike, for acclimatization. During this period, the animals were fed with grasses, oil palm fruit and water ad libitum.

The rat on the day of sacrifice, the AGR was euthanized with deep inhalation chloroform. The weight of the animal was taken with Mettler balance (Model Ohaus scout PRO-200) with a sensitivity of 0.1gm. Each rat was sacrificed according to Adeyemo and Oke (1990), and placed on dorsal recumbency. The animal was cut open through mid ventral incision from the inguinal region to the mandibular symphysis. The spleen was dissected out and slices fixed in 10% neutral buffered formalin. The tissues were passed through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections 5µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1977). The slides were examined and photomicrographs taken with – Motican 2001 camera (Motican UK) attached to Olympus microscope.

RESULTS AND DISCUSSION

Grossly, the AGR spleen under study was a slender elongated triangular shaped organ with an average length of 5.5cm. The dorsal surface was the broadest surface of the triangle while ventrally, the other two sides of the triangle joined at an apex to form the hilus. At the hilus, the vessels nerves that supply the spleen were seen surrounded by thick accumulation of adipose tissue (Fig. 1).

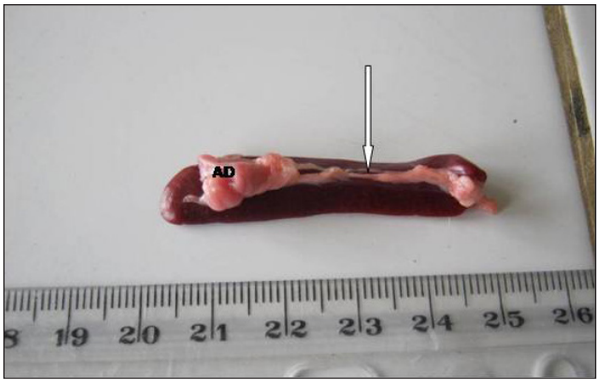

Fig. 1: Ventral view of the AGR spleen. Note the hilus (white arrow) with abundant surrounding adipose tissue AD

Microscopically, mesothelial layer of simple squamous epithelium was seen overlying the splenic capsule. This splenic capsule covered the organ parenchyma (Fig. 2, 3). The capsule was composed of fibrocyte contained in dense regular connective tissue in a circumferential orientation. The splenic trabecullae originated from the capsule and extended radially into the parenchyma partially dividing it into compartments. The splenic parenchyma consisted mainly of red pulp regions and isolated areas of white pulp in the central region (Fig. 3). The red pulp was composed of splenic cords and blood vessels of which sinusoids were very prominent (Fig. 3). The splenic cords consisted of erythrocytes, macrophages, plasma cells, small lymphocytes, reticular cells and granulocytes (Fig. 3).

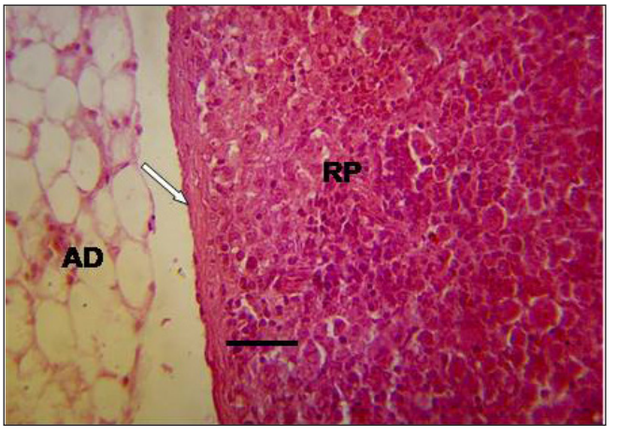

Fig. 2: Transverse section of the spleen showing the capsule (white arrow), and red pulp RP, containing splenic cords. Note the surrounding adipose tissue AD. H&E. (Scale bar = 50µm)

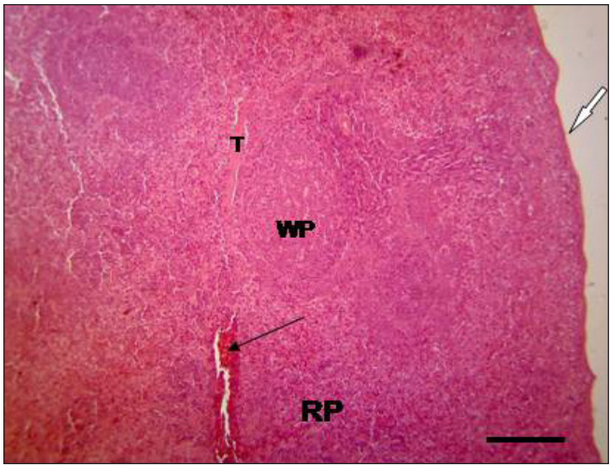

Fig. 3: Section of the AGR spleen showing abundant red pulp RP; sinusoid (black arrow), trabeculla T. Note the few areas of white pulp WP and the capsule (white arrow) - H&E. (Scale bar = 100µm)

The white pulp was composed of the periarteriolar lymphatic sheath, PALS. The PALS contained an ovoid shaped germinal centre containing small lymphocytes; a septum of thin connective tissue separating the germinal centre from the overlying marginal zone a circumferential marginal zone containing small lymphocytes and plasma cells; and an eccentrically located artery surrounded by small lymphocytes (Fig. 4).

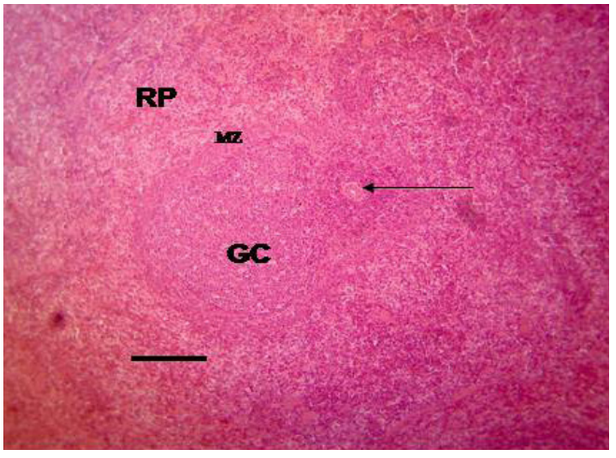

Fig. 4: Section of the AGR spleen showing the white pulp periarteriolar lymphatic sheath containing germinal centre GC, marginal zone MZ, and the eccentrically placed central artery (black arrow). Note the abundant surrounding red pulp RP. H&E. (Scale bar = 100µm)

This current investigation is aimed at providing the normal morphology of the AGR spleen from the Nigerian rainforest vegetative region. The triangular shape of the AGR under study will enable for easy expansion of the spleen when engorged with blood. This triangular shape observed is different from the slender shape reported in the AGR from the GSV region (Valli *et al*., 2002; Ibe *et al*., 2010), but a triangular shaped spleen has been reported also in mice, rat and musk shrew (Fukuta *et al*., 1982; Cesta, 2006), while a bean shaped spleen has been documented in the alligator and guinea fowl (Rooney *et al*., 2003; Onyeanusi, 2006). The presence of abundant surrounding adipose tissue around the hilus indicates that this region is a site for metabolite storage in this species.

The mesothelial cells observed is an investment of the peritoneum that covers the organ capsule (Cesta, 2006; Onkar and Govardhan, 2013). This mesothelial cells may serve as ready source of fibroblast for wound repair in case of injury to the spleen. The connective tissue fibres of the capsule is for protection of the splenic parenchyma and also serves as the origin of the trabeculla network that partially compartmentalizes the organ parenchyma (Corbin *et al*., 2008; Onkar and Govardhan, 2013). The trabecullae seen served as the anchorage for vascular and nervous supply into the spleen parenchyma. A connective tissue capsule of only collagen and fibrocytes as seen in this study has been reported in other rodent (Valli *et al*., 2002) but splenic capsule containing collagen, elastic fibres and smooth muscle cells have been reported in humans and dogs (Onkar and Govardhan, 2013), an elasto-fibroleiomyocytic tissue in the capsula-trabecullae system was reported in Whale spleen (Nakamine *et al*., 1992) but a double layered capsule of outer collagen connective tissue and inner smooth muscle cell have been documented in one humped camel (Zidan *et al*., 2000), dog and cat (Dellman and Brown, 2006) and this was associated with blood storage type spleen. The absence of smooth muscle cells in the capsule observed in this study is consistent with findings in other rodents (Valli *et al*., 2002), and it is related to reduction in the contractile ability of the rodent spleen.

The splenic parenchyma comprising mainly red pulp with less developed trabecullae reflects need for more blood storage in the mammals (Banks, 1993; Corbin *et al*., 2008), thus indicating the AGR spleen under study is of the storage type. The splenic cords comprising erythrocytes, plasma

cells and macrophages reflects the need for efficient body defense through cell and humeral mediated mechanism by phagocytic action of the macrophages and the antibody production by the plasma cells . The abundant plasma cells may be associated with more priming and differentiation of the B-lymphocytes due to exposure to antigens in the RV habitat.

The isolated regions of white pulp consisting of mainly PALS observed in this study have been reported in the rats and camel, whereas the lymphoid follicular type was documented in humans (Steiniger *et al*., 1997; Zidan *et al*., 2000). These white pulp regions are the major site of immune defense in the spleen. The presence of germinal centres in the white pulp reflects previous exposure to infectious agents (Samuelson, 2007). In this study the marginal zone contained small lymphocytes and plasma cell but a marginal zone rich in macro-phages and eosinophils has been reported in the Antarctic seals (Schumacher and Welsch, 1987). The marginal zones serve as the transition area between the white pulp and the red pulp, the site of heavy blood flow, purification and filtration (Zidan *et al*., 2000; Onkar and Govardhan, 2013). The septum surrounding the germinal centre serves as barrier protection the differentiating germinal centre lymphocytes from injurious substances that will affect it proper development especially from the nearby central artery. This septum has been reported in other animals where it is related to the migration of lymphocytes from the white pulp into the marginal zone (Fukuta *et al*., 1982). The presence of eccentrically placed central artery as seen in this study has also been reported in the human and dog spleen (Onkar and Govardhan, 2013). The lymphocytes surrounding the single central arteries were seen at the periphery of the white pulp, unlike what was observed in the AGR from guinea savannah vegetative region with numerous centrally located arteries (Ibe *et al*., 2010). This is a morphological adaptation to increase interaction between the lymphocytes of the PALS and antigens in the substance of the closely associated red pulp. This will increase its efficiency to defend the body against pathogenic organisms in the splenic blood, hence help the AGR to better survive in this RV region of Nigeria.

CONCLUSION

This report presents the spleen morphology of the AGR from the RV region of Nigeria. The anatomy is very similar to mammalian spleen of the storage type with abundant red pulp and less developed trabecullae. The triangular shape differed from the slipper shape of the AGR from guinea savannah vegetative region. The presence of singular peripherally located central artery also differed. The presence of abundant macrophages and plasma cells reflects and adaptation for greater survival in the rainforest region with a greater relative humidity for optimal challenge by infectious agents. The study has been able to observe morphologic difference which may have been influenced by the prevailing environmental factors

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