IMMUNOMODULATORY ACTIVITIES OF THE SPLEEN OF THE AFRICAN GIANT (*CRICETOMYS GAMBIANUS)* RAT

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: April 16, 2024 Accepted: June 28, 2024

Abstract: The spleen is the largest secondary immune organ in the body and is responsible for initiating immune reactions to blood-borne antigens and for filtering the blood of foreign material and old or damaged red blood cells. The study investigated Immunomodulatory activities of the Spleen of the African Giant (*Cricetomys gambianus)* Rats. Two African rats with an average weight of 1.3 ± 0.4 kg were purchased from Vom, Jos and were acclimatized for two weeks in the animal house of the department of anatomy used. Their spleens were harvested and their mean weight, thickness, width and length were measured. The spleens were subjected to normal histological and histochemical procedures in which sections of the spleen tissue were divided into three groups; the normal haematoxylin and eosin, best carmine method PAS (periodic acid schiff) for glycogen localization and Alcian blue technique for mucins localization. The average weight of the rats was 1.3 ± 0.4 kg. Mean weight of the spleen, spleen thickness, spleen width and spleen length were 2.3 ± 0.2; 3.1 ± 1.54 mm; 16.5 ± 4.5mm and 56 ± 4cm respectively. The spleen of African giant rat was slightly positive for glycogen when stained with Best carmine method. Similarly, when it was stained with PAS technique, the trabecula was positive for glycogen. On the other hand, when it was stained with alcian blue technique, it was fairly positive for acid mucins. The result of this study has provided for the first time baseline data on the carbohydrates histochemistry of the spleen of the African giant rat which has been lacking and this might be useful in the comparative analysis of the spleen of rodents.

Key words: Histochemical, periodic acid Schiff (PAS), alcian blue, acid mucins, trabecula, haematoxylin and eosin.

Introduction

African giant pouched rat (*Cricetomys gambianus*),a super-smart rat that belongs to an entirely different family (Nesomyidae) from regular house rat (Muridae). Growing to around 0.9 meters long – their tails make up half their length – and 1.4 kilograms in weight, *Cricetomys gambianus* are good-looking and massive (Mott, 2024). They are native to Sub-Sahara Africa, ranging from Angola to Mozambique, where they live in communities of 20 or more, relying on their keen senses of hearing and smell to make up for their relatively poor eyesight. When trained, the African giant pouched rat can be used for the detection of abandoned land mines (Mott, 2004) and tuberculosis (Maggie, 2003) due to high acuity of odour perception.

The rats learn to recognize the presence of TB in samples of sputum and are rewarded when they succeed. The testing process starts when a rat is presented with a row of 10 sputum samples, and when it detects TB the rat hovers over the sample for 3 seconds. The rat's accuracy at detecting TB is almost 100%, but they cannot distinguish between normal and drug-resistant strains (Kizito, 2016).

The spleen is a vital haemopoeitic and immune organ. It is one of the most important immune organs of vertebrates and the principal peripheral lymphoid organ (Rooney *et al*., 2003; Balog *et al*., 2004). It houses some of the body's lymphocytes and initiates cellular immune response and immunological defence through antibody production against blood-borne antigens (Nolte *et al.*,2002; Balogh *et al*., 2004). The lymphocytes are resident in the white pulp of the spleen. The spleen, through the red pulp plays a central role in the filtration of effete blood and foreign materials from the body. The red pulp also serve as a storage site for iron, erythrocytes and platelets and has been implicated to be haemopoeitic in neonatal rodents (Cesta, 2006). The macroscopic features and size of the spleen varies with species (Cesta, 2006).

An essential feature of the mammalian immune system is its ability to initiate different types of responses to foreign matter. The presence of foreign cells or antigens in the body stimulates a highly complex series of immune reactions. The immune responses to invading foreign organisms can be divided into two main types of responses. The first type is innate immune response and the second type is the adaptive immune response. More than 1600 genes are involved in innate and adaptive immune responses (Abbas *et al.,* 2005). These genes are of great importance for sustaining life in a hostile environment.

The innate immune response is the first line of defense that limits the spread of infection. Its response to antigen invasion is composed of phagocytic functions, which are rapid and include the mobilization of neutrophils, mast cells, macrophages, and natural killer cells. However, the response of the innate immune system is fast, but not specific and does not produce memory cells.

Adaptive immune responses have long been considered the "territory" of antigenic proteins, whereas carbohydrates are characterized as T-cell-independent antigens that are not typically recognized by the complete adaptive machinery. Nevertheless, there is growing evidence that sugars can also play a critical role in immune recognition. Studies of carbohydrate based vaccines in mice have shown that IgM antibody production dominated the response, although some IgG production has been observed (Barrett *et al.,* 1980). Since the early 1990s naturally occurring glycoproteins (Grundner *et al.,* 2004), glycolipids (Godfrey and Kronenberg, 2004), and even protein-free polysaccharides (Tzianabos *et al.,* 2000) have been shown to be important parts of the adaptive repertoire. For example, the MHC-like protein CD1 has now been shown to present glycolipids in a manner that is analogous to MHC–peptide presentation (Zeng *et al.,* 1997). If

polysaccharides have been shown to be important parts of the adaptive repertoire, then the spleen and by extension all cells of the immune system are likely to have abundant carbohydrate molecules which can be localized through histochemical techniques. For the purpose of this study, the spleen has been chosen because it is an encapsulated and the largest organ of the immune system (Zeng *et al*., 1997).
All carbohydrates have abundant hydroxy and other polar groups and consequently are hydrophilic. The hydroxy groups and the anionic, ester and amide side chains of carbohydrates do not react with formaldehyde, ethanol or other compounds used for fixation and tissue processing. Retention of mucosubstances in fixed animal tissues is due largely to insolubilization of associated proteins by coagulation or covalent cross-linking of nearby protein molecules (Kiernan, 2010). The histochemically interesting parts of sugars are the anionic groups and diols, which are unchanged in paraffin sections. Glycogen frequently diffuses within cells of the liver before being immobilized during fixation by an aqueous formaldehyde solution, giving rise to an artifact known as polarization (Kiernan, 2010). This artifact can sometimes, but not always, be avoided by using a non-aqueous fixative.
Histochemically detectable carbohydrates occur as large molecules on the outside surfaces of all types of cell, as stored or secreted substances within some cells, and in the extracellular matrix. Units (monosaccharides) are joined in chams (Kiernan, 2010). The classification of macromolecular carbohydrates can be quite complicated because it has to take into account materials as diverse as cellulose, gastric mucus and cartilage matrix. The scope of this research work is restricted to only two macromolecular carbohydrates namely: Glycogen and Acid Mucins as no information is available so far on the base-line data of the carbohydrate histochemistry of the spleen of the African Giant Rat (cricetomys gambianus) (Kiernan,2010). Such information if available may be of value in its domestication, breeding and production of the rat.

Materials and Method

Experimental Protocol

Two adult male African giant rats bought in farin gada, Jos were used for the study and taken to the animal house in the Department of Pharmacology, University of Jos. The animals were acclimatized for 3 weeks before commencement of the experiment during which they were physically examined. Water and feed were provided *ad libitum* and they were maintained under constant environmental conditions. They were restrained with a towel soaked in chloroform to render them unconscious. They were weighed using a digital weighing scale and their length measured using a ruler. Thereafter, they were euthanized using chloroform. The carcass was placed on a dissecting board in dorsal recumbence after which incision was made from the linea alba to the anal region. In each of the animals, the spleen was exposed after an exploratory laparotomy. Spleens were carefully harvested and their dimensions and weights obtained using venier caliper and digital weighing scale respectively prior to histological sample collection. The histochemical methods that were applied in this research work is the Periodic Acid schiffs (PAS) and Best carmine method for glycogen localization and the Alcian Blue method for the localization of Acid Mucin.

Histological and Histochemical Procedures.

Samples of the spleen were immediately fixed in 10% formalin solution. After dehydration in ascending concentrations of alcohol, the tissues were cleared in xylene, infiltrated with molten paraffin was at 60^{o}c, blocked in paraffin according to standard procedures (Kiernan, 1990) and labeled. Coronal and saggital sections of 5µm each were cut with a rotary microtome.

Haematoxylin and Eosin Staining

The tissues were deparaffinised for 5 minutes using two changes of xylene, washed in water and stained with haematoxylin for 5 minutes. This was followed by washing in water, decolorized with acid alcohol, and then washed in water. They were stained in eosin for 1 minute. In each case (two changes). They were rewashed with absolute alcohol, cleared with xylene (two changes). The sections were mounted on glass slides using DPX mountant. The sections were stained with haematoxylin and eosin (H&E). The tissue slides were observed under a microscope connected to a digital camera.

PAS Technique (Mcmanus, 1946)

Dewaxed sections were brought to distilled water. Duplicate sections were brought to distilled water diastase treatment. The sections were then treated with periodic acid for 5 minutes, followed by thorough washing with several changes of distilled water. They covered with schiff's solution for 15 minutes, followed by washing in running tap water for 5-10 minutes. The nuclei were stained with Harris's haematoxylin, differentiating as appropriate in acid alcohol and blued as usual. They were washed in water and rinsed in absolute alcohol. They were finally cleared in xylene and ready for mounting.

Best Carmine Method (Best, 1906)

Dewaxed test and positive control sections were brought to water, the nuclei were stained well, differentiating in acid alcohol so that the background is clear, washed and blued. The sections were stained with carmine solution for 15 minutes. They washed well in Best's differentiator. This was followed by rinsing in fresh alcohol, followed by clearing in xylene and was ready for mounting.
Alcian Blue Technique for Acid Mucins using Varying PH of Solution.
Dewaxed sections were brought to water, then Alcian blue for 5 minutes, they were washed in water again, followed by counterstaining with 0.5% aqueos neutral red for 2-3 minutes. This was followed by washing in water, rinsing in absolute alcohol and finally cleared in xylene and ready for mounting.

Results

The average weight of the rats used in the study was 1.3 ± 0.4 kg. The means and standard errors of means of spleen weight, thickness, width and length were 2.3 ± 0.2; 3.1 ± 1.54 mm; 16.5 ± 4,5mm and 56 ± 4mm respectively. The spleen of African giant rat is reddish brown in colour and slipper-shaped. It lies on the left lateral aspect of the abdominal cavity, medial to the abdominal wall and lateral to the greater curvature of the stomach. Histologically, the African giant pouched rat spleen is covered by a capsule (plate 1) which has trabeculae that extend into the

parenchyma of the organ. The parenchyma was made up of red pulp separated from the white pulp by a marginal zone (plate 2 and plate 3).

When the spleen of African giant rat was stained using the Best carmine technique, it was slightly positive for glycogen (plate 4). Similarly, when it was stained with PAS technique, the trabecula was positive for glycogen (plate 5 & 6). On the other hand, when it was stained with alcian blue technique, it was fairly positive for acid mucins (plate 7). Plate 8 shows spleen stained using Best Carmine method with diastase which is negative for glycogen.

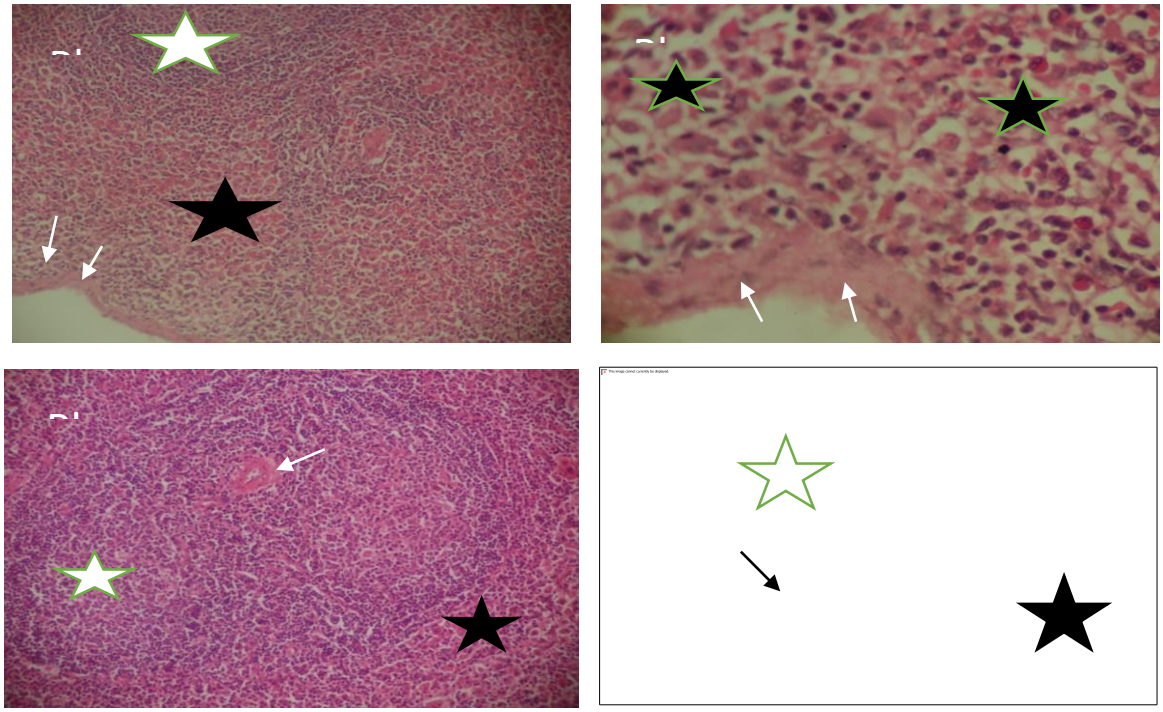

Plate 1- (Haematoxylin and Eosin Technique): Photomicrograph of the Spleen of African giant rat showing the capsule (white arrows) is intact. Black stars showing the red pulp.

Plate 2- (Haematoxylin and Eosin Technique): Photomicrograph of the Spleen of African giant rat showing the capsule (white arrows) at a higher magnification is intact and the red pulp indicated by the black star

Plate 3- (Haematoxylin and Eosin Technique): Photomicrograph of Spleen of African giant rat showing the central artery (white arrow) seen within the white pulp (white star). Black star indicates the red pulp.

Plate 4- (Best Carmine Technique): Spleen of African giant rat stained using the best carmine method was slightly positive for glycogen Black arrow=central artery, black star=red pulp and white star=white pulp.

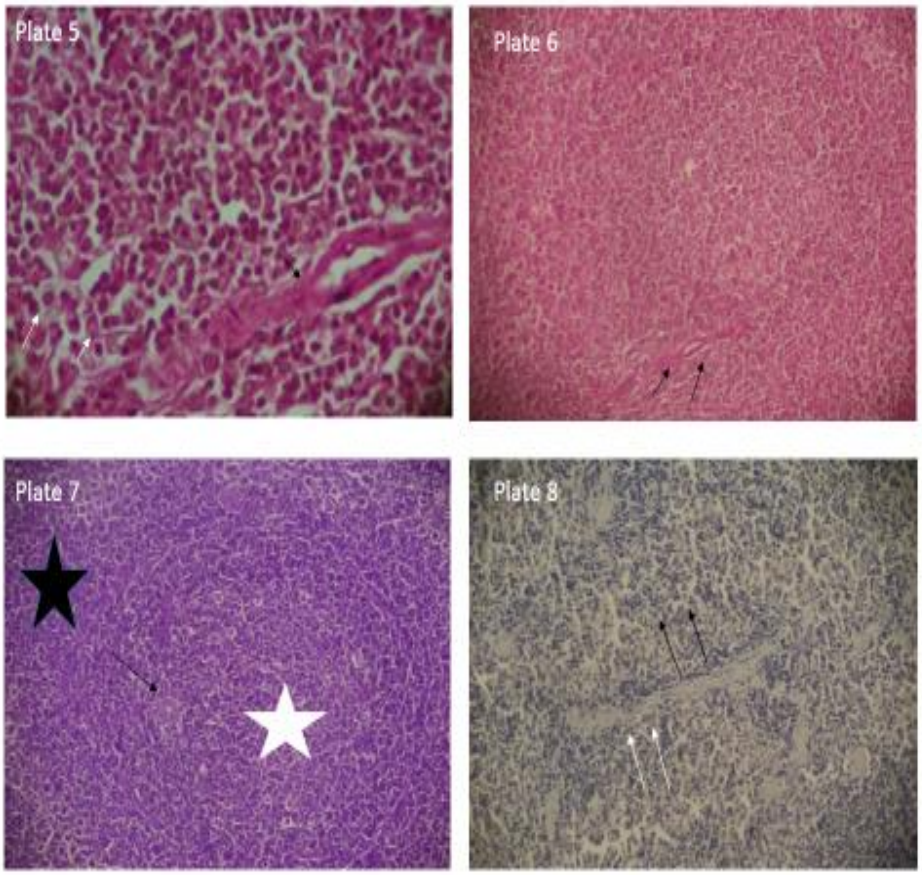

Plate 5-(PAS Technique): Spleen of African giant rat stained using the PAS Technique shows the trabecula (black arrow) at a higher magnification is positive for glycogen as demonstrated by this technique and also the splenic cells being intact.
Plate 6- (PAS Technique): Spleen of African giant rat stained using the PAS Technique shows the trabecula (black arrow) is positive for glycogen as demonstrated by this technique.

Plate (Alcian Blue Technique): Spleen of African giant rat stained using the Alcian Blue Technique was fairly positive for acid mucins. Black star=red pulp, white star=white pulp and black arrow=central artery.
Plate 8- (PAS Technique with diastase): Spleen stained using the PAS Technique with diastase was negative for glycogen.white arrows= trabeculae, Black arrows=Splenic cells.

Discussion

Carbohydrates play important roles in development regarding their number and complexity on cell surfaces glycoconjugates which function in cellular communication (Gabius, 2000).

The present study revealed small amount of glycogen and acid mucins in the spleen of the African giant rat (Cricetomys gambianus) use for the study.This is no surprise as the spleen does not store glycogen and most mucins are secreted as principal components of mucus by mucus membranes or are secreted to become components of saliva. The glycogen was more conspicuous along the trabeculae (plate 5 & 6).

Since the present study revealed small amount of glycogen, their role in immune recognition is minimal. Their presence in the spleen of the AGR may also indicate a kind of adaptation in the role played by the spleen in phagocytosis, erythrocyte storage, immune responses and haematopoiesis. The works of Jorns *et al.* (2003) revealed that cellular interactions within the immune system are in part mediated via carbohydrate rich coat of the cell membrane, the glycocalyx of which the terminal carbohydrates residues are of particular functional importance. These investigations were carried out on the thymus, bursa of fabricus, spleen and bone marrow of 2- and 30- day old chickens by lectin histochemistry. In the thymus, mannose as well as N-acetyl-glucosamine (glc NAC)- specific lectins labelled macrophages, epithelial reticulum cells and lymphocytes within the cortex. In the bursa of fabricius, the brush border of the lining epithelium, the macrophages and the endothelium were labeled by a broad spectrum of lectins (Jorns *et al.*, 2003).

The follicle associated epithelium was labeled by a broad spectrum of lectins. Epithelial cells that separated the cortex from the medulla and large mononuclear cells in the cortex were only being labeled by N-acetyl-galactosamine (gal NAc)- specific and glcNAc- specific lectins respectively. In the spleen, lymphocytes of the peri-ellipsoid lymphocyte sheaths and macrophages of the red pulp were more intensively labeled in the 2- day old chicken than in the 30-day old chicken, indicating changes in glycotope expression during post hatching development (Jorns et al., 2003).

Thus cells of the avian immune system were as rich and diverse in their lectin binding site as their mammalian counterparts indicating that similar carbohydrates lectins interactions between cells and matrices take place in birds as well (Jorns et al., 2003).

In an attempt to study some histochemical aspects of carbohydrates, proteins and lipids in the swim bladder development of sea bream, Sparus aurata and sea bass, Dicentrarchus labrax, Dinis(1997), did not observe any important differences in the histochemical distribution of glucidic, proteic and lipidic macromolecules during the swim bladder development, he discovered though, that glycoproteins containing mannose and/or glucose sugar were present in the swim bladder (gas gland, wall and rete mirabile) of both species, but glycogen was observed only in the gas gland of Sparus aurata swim bladder (Dinis, 1997). The presence of glycogen may only be an indication of its relevance as an energy reserve for the swim bladder in its role of maintaining the buoyancy of Sparatus aurata in contrast to the amount of glycogen present in the spleen of the AGR which may play a small role in immunity.

The swim bladder is an internal gas-filled organ that contributes to the ability of many bony fish (but not cartilaginous fish) to control their buoyancy, thus to stay at their current depth without having to waste energy in swimming. This role played by the swim bladder will require energy and so the presence of carbohydrates in this organ is understandable as it provides the energy needed for its proper functioning (Dinnis, 1997).

Dullman *et al*, (2000), in their study of histochemistry of sub compartmentalization of the white pulp in rat spleen, using a broad panel of lectins, reported that the sub compartmentalization of the white pulp is due its specific stromal cells and migrating subtypes of lymphocytes, which is as result of carbohydrates residues of cell membranes and extracellular matrices involved in cell-cell and cell-matrix interactions. They further reported that splenic macrophages, which were also demonstrated by perls' Prussian blue reaction were labeled selectively by most mannose specific lectins and gave the characteristic distribution patterns in all splenic (sub) compartments. The result of this study is in disagreement with our present findings which revealed small amount of glycogen and acid mucins in the spleen of AGR considering the fact that carbohydrates plays a role in the characteristic pattern of the white pulp which plays a vital role in immunity.

The greatest concern in this present study is the quantity of these carbohydrates molecules in the spleen of the African giant rat (AGR) which was seen to be far below expectations considering the lifestyle of this animal and other capabilities of the animal like its ability to detect Tuberculosis in sputum of affected victims which does not completely agree with the work of Dullman and his co-authors.

Considering the African giant rat's lifestyle of running away from predators to hide in burrows, sewages, drainages, and its habit of feeding on materials which might predispose it to bacteria and other microbial infection, it was expected that the spleen of these animals should have rich concentrations of glycogen and acid mucins which would help their systems in immune recognition, it was not seen to be so, and this was not in agreement with the works of John and Spicer (1967) where rich secretions of mucins in bovine, ovine and porcine submandibular and sublingual glands were discovered

The two major storage site of glycogen are the liver and skeletal muscle. This might explain why very small amount of it were found in the spleen of the African giant rat (plate 5 & 6). The trabeculae carry nerves, arteries and veins. The high concentration of the glycogen along the trabeculae might be an indication of the high level of activities of blood transportation to and from the spleen within these vessels. It is in contrast to the study conducted by Austin *et al*.(2013) who found that glycogen storage disease type I is associated with menorrhagia.

Conclusion

The low carbohydrate secretions in the spleen of the African giant rat (AGR) clearly indicates little influence of carbohydrate on their immune system. In order words, carbohydrates play minimal role in immune recognition. The result of this work shows that the role of immune recognition is largely played by the cells of the white pulp and red pulp i.e., immune recognition takes place at the cellular level with little contribution at the histochemical level. Considering the low carbohydrates secretions in the organ of this animal, one wonders how they cope with many cellular processes like disease, growth and development since carbohydrates play a critical role in these cellular processes.

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