

Unique Cellular Characteristics of The Splenic Marginal Zone of The Barki Sheep Make It a Powerful Immunological Defense

Omnya Elhussieny ^{1*}, Mohamed Abdelaziz ², and Eman Khalifa ³

1. Department of Histology and Cytology, Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt.
2. Department of Histology and Cytology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.
3. Department of Microbiology, Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt.

Correspondence*

Omnya Elhussieny, Department of Histology and Cytology, Faculty of Veterinary Medicine, Matrouh University, Matrouh, Egypt.

Email: omnya.elhussieny@mau.edu.eg

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INTRODUCTION

The marginal zone is a uniquely belt-like area encircling the white pulp lymphoid nodules which formed from a diffuse lymphoid tissue and a marginal zone sinus (Mebius & Kraal, 2005). The marginal zone is the front line for immunosurveillance of the circulating blood and their stromal cells are involved in the rapid defense against blood-borne microorganisms and immune cell mobilization (Cerutti et al., 2013). The marginal zone of the spleen was studied in many species for example, and not as a limitation; albino rabbit (Burke and Simon, 1970), the dromedary camel (Camelus dromedarius) (Zidan et al., 2000), human (Polák et al., 2009), horse, cows, carnivores, and pigs (Bacha Jr & Bacha, 2012), other species of sheep and goats (Gnanadevi et al., 2019), and the water buffalo (Bubalus bubalis) (Rashad et al., 2020). Although the stroma of the Barki sheep was studied histologically, immunohistochemically, ultrastructurally, and morphometrically, the splenic white pulp does not incorporate in this study (Elhussieny et al., 2022). So, our present study aims to describe the unique

ABSTRACT

The marginal zone is a unique structure at the marginal area of the splenic white pulp, acting as a transient region where newly arriving cells, antigens, and blood are filtered. The splenic marginal zone of the Barki sheep surrounds the white pulp and is present between the white and red pulp. It is made up of marginal zone sinuses and diffuse lymphoid tissue aggregation peppered with smooth muscle cells and erythrocytes and supported by reticular cells. Rod-shaped endothelial cells seating on an interrupted basement membrane are lining the marginal zone sinus, which is surrounded by the diffuse lymphoid tissue of the marginal zone, which is formed from different stages of maturation of: lymphocytes, plasma cells, dendritic cells, neutrophils, marginal zone macrophages, erythrocytes, platelets, and reticular cells, all of which are supported by a network of reticular fibers and smooth muscles. Motile lymphocytes with pseudopodia are present in the splenic marginal zone of the Barki sheep. Two forms of smooth muscles are also present: solitary and doubled reciprocal smooth muscles, which give the spleen a powerful ability to filter the blood and provide defense against foreign antigens, making it a unique intermediate type of spleen.

Keywords marginal zone macrophages, plasma cell, dendritic cell, motile lymphocytes, smooth muscles

cellular characteristics of the splenic marginal zone of the Barki sheep. This will help us to understand the powerful ability of the spleen of the Barki sheep in immunological defense in addition to its strong ability in restoring and reserving the blood making it a unique intermediate type of spleen.

MATERIALS AND METHODS

Samples Thirty clinically healthy Barki sheep, whose ages range from 6 months to 5 years, were slaughtered for human consumption according to the Egyptian Veterinary Authorities in the abattoir of Marsa Matrouh, Matrouh, Egypt. Fresh tissue samples were obtained from the peripheries, centers, and hilus of the spleen of each animal. The samples were prepared for the histological and ultrastructural examinations as follows: **Light microscopy** The tissue samples were fixed in 10% phosphate-buffered formaldehyde (Rhodes, 2013). The fixed specimens were processed for paraffin sectioning. Serial sections (5µm) were prepared as outlined by Wolfe (2019) and stained with Mayer's hematoxylin and eosin (H&E) stain

for general studies (**Bancroft & Layton, 2019**). **Transmission electron microscopy (TEM)**: Fresh tissue samples, about 1mm^3 in size, were obtained from different parts of spleens and were immediately fixed in 3% phosphate-buffered glutaraldehyde at pH 7.2 and stored at 4°C . The specimens were processed in the electron microscope unit at the Faculty of Science, Alexandria University. After fixation, the tissues were washed in several changes of cold (4°C) 0.1 M phosphate buffer every 15 minutes for 2 hours. Then the tissues were post-fixed in a 1% solution of phosphate-buffered osmium tetroxide (2% osmic acid 5 ml and phosphate buffer 5 ml) for 2 hours at room temperature. Then they were rapidly dehydrated through ascending grades of ethyl alcohol series (30, 50, 70, 90, and 100% for 2 changes) for 30 minutes in each. Then transferred to propylene oxide and placed overnight in a 1:1 mixture of propylene and epoxy araldite. Then they were embedded in epoxy araldite. Polymerization of the embedding mixture and the tissue blocks was done in an oven for 5 days as follows: at 35°C for 24 hours, at 45°C for 24 hours, and lastly at 60°C for 3 days. Semithin sections ($1\mu\text{m}$) were cut first and stained with toluidine blue and examined with a light microscope to select the marginal zone areas for the electron microscopic examination. Then the ultrathin sections (60 - 100 nm) were cut with a glass knife on LBK ultramicrotome, then they were stained with uranyl acetate followed by lead citrate (**Graham & Orenstein, 2007**). The sections were examined with transmission electron microscope JEM-1400 PLUS present in the electron microscope unit, Faculty of Science, Alexandria University.

RESULTS

The splenic marginal zone of the Barki sheep encircling the splenic white pulp enclosing the areas between the splenic white and red pulps. It consists of marginal zone sinus and diffuse aggregation of lymphoid tissue peppered with smooth muscle cells and erythrocytes and supported by reticular cells (**Figs. 1&2**). The marginal zone sinus is lined by rod-shaped endothelial cells seating on an interrupted basement membrane (**Fig. 3**) and framing by the diffuse lymphoid tissue of the marginal zone which consists of different stages of lymphocyte maturation (lymphoblasts and lymphocytes) (**Figs. 4, 9, 11&17**), neutrophils (**Fig. 4**), marginal zone macrophages (**Fig. 15**), different stages of plasma cell maturation (plasmablasts, plasmacytes of intermediate or transient characters, and small classic mature plasma cells) (**Figs. 3, 8, 10, 12&13**), different stages of dendritic cell maturation (dendritic cell precursor, dendritic cell of intermediate or transient stage of maturation, mature dendritic cell) (**Figs. 3, 4, 5, 9, 11&12**), erythrocytes, platelets and reticular cells all are upholding by a lattice of reticular fibers and smooth muscles (**Figs. 4, 5, 13&17**). Lymphoblasts (**Figs. 9, 11 &15**) present in the splenic marginal zone of the Barki sheep are characterized by a larger nuclear-cytoplasmic ratio than that of the lymphocytes (**Figs. 3-7, 10&16**) containing 1-2 nucleoli meanwhile mature lymphocytes do not contain nucleoli, and the chromatin in lymphocytes is dense and clumped unlike the chromatin in lymphoblasts.

Some lymphocytes present in the splenic marginal zone of the Barki sheep are characterized by their motility and pseudopodia (**Figs. 5-7&16**). Some lymphocytes inside the marginal zone undergo mitotic division (lymphocyte mitogen) (**Fig. 17**). Marginal zone macrophages with horseshoe shape nuclei engulfing red blood corpuscles are present in the marginal zone (**Fig. 15**). We found different stages of plasma cell maturation including plasmablasts (P2) which are characterized by free polyribosomes, segments of rough endoplasmic reticulum, nucleoli, nuclear pores, and other cellular elements indicative of protein synthesis (**Figs. 4&8**); plasmacyte of intermediate or transient characters (P3) which is characterized by dilated perinuclear spaces and dilated endoplasmic reticulum (**Figs. 9&13**); classic small plasma cell (P4) which display polarized nucleus and cytoplasm and the heterochromatin is distributed in clumps along the inner nuclear membrane this cell is a near-terminal form (**Figs. 3&10**). The splenic marginal zone of the Barki sheep has different stages of dendritic cell maturation: dendritic cell precursor (**Figs. 3, 9, 11&12**) which is characterized by its similarity to the lymphocyte but has a convoluted or star shape nucleus, and the cytoplasm has more numerous mitochondria; dendritic cells at the intermediate stage of maturation have the tadpole-shaped cells and dense granules in the cytoplasm (**Figs. 3, 4&15**); and mature dendritic cells (**Figs. 5, 6& 16**) characterized by its irregular shape and different cell processes and the cytoplasm contains mitochondria, rough endoplasmic reticulum, dense granules, and phagosomes. Smooth muscles present in the marginal zone have two forms solitary smooth muscles (**Figs. 12-15**) and doubled reciprocal smooth muscles (**Figs. 8-10**).

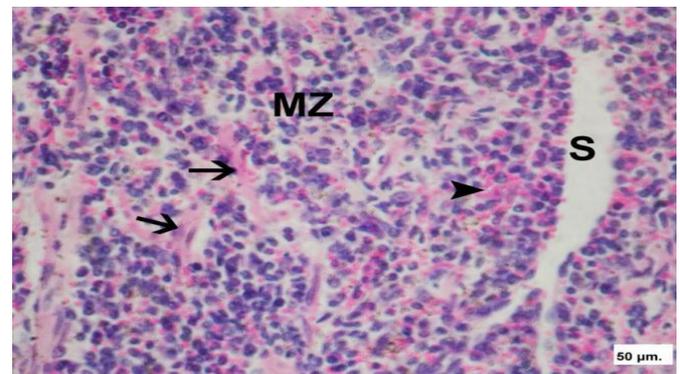


Fig. (1): A photomicrograph of the spleen of Barki sheep showing the marginal zone (MZ) consisting of diffused lymphocytes in addition to erythrocytes (arrowhead) and smooth muscle cells (SM). Note the prominent marginal zone sinus (S). H&E stain. Bar= $50\mu\text{m}$.

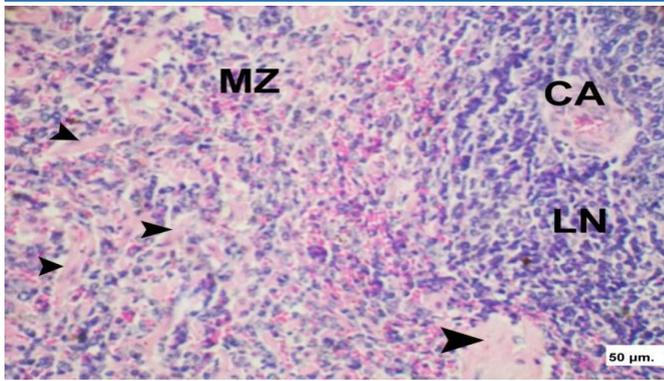


Fig. (2): A photomicrograph of the spleen of Barki sheep depicting lymphoid nodule (LN) contains eccentrically positioned central artery (CA). The marginal zone (MZ) contains diffused lymphocytes, erythrocytes, and smooth muscle cells (arrowheads). H&E stain. Bar=50µm.

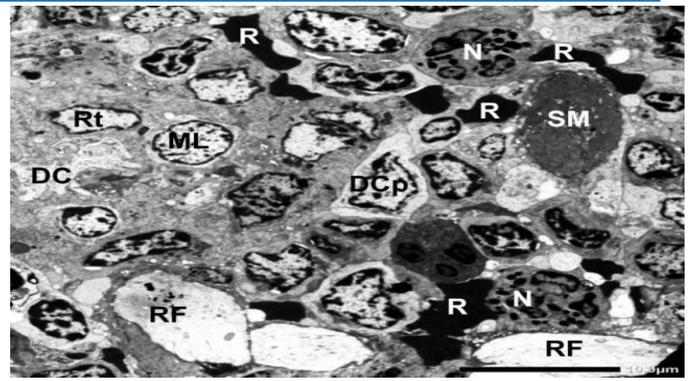


Fig. (5): Transmission electron micrograph of the marginal zone of the spleen of Barki sheep showing that the marginal zone consists of motile lymphocytes (ML), mature dendritic cell (DC), dendritic cell precursor (DCp), neutrophils (N), and erythrocytes (R) all are supported and surrounded by reticular cells (Rt), reticular fibers (RF) and smooth muscles (SM). Bar=10µm.

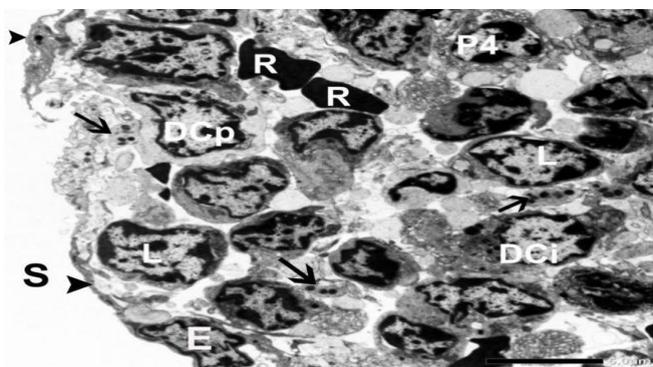


Fig. (3): Transmission electron micrograph of the splenic marginal zone of the Barki sheep showing the marginal zone sinus (S) is lined by endothelial cells (E) resting on an interrupted basement membrane (arrowheads). Observe the aggregation of lymphocytes (L), classic small plasma cell (P4), dendritic cell precursor (DCp), intermediate form of dendritic cell (DCi), erythrocytes (R), and platelets (arrows) in the marginal zone. Bar=5µm.

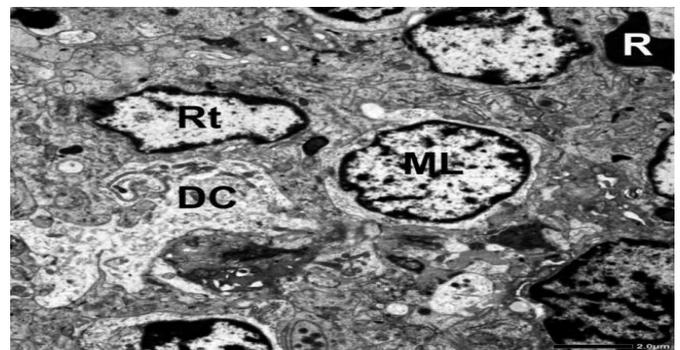


Fig. (6) A higher magnification of the previous transmission electron micrograph of the splenic marginal zone of the Barki sheep shows motile lymphocyte (ML), mature dendritic cell (DC) all are surrounded by reticular cell (Rt). R= erythrocyte. Bar=2µm.

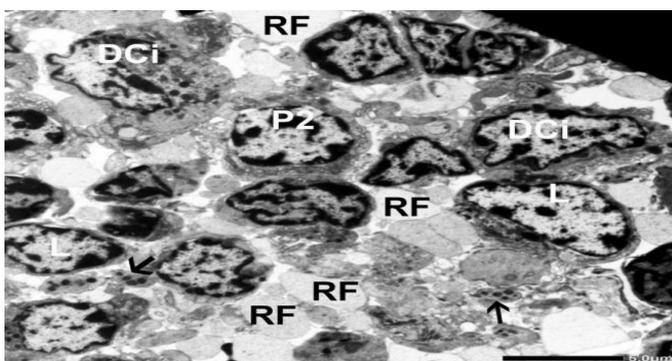


Fig. (4): Transmission electron micrograph of the border between the marginal zone and the lymphoid nodule of the spleen of Barki sheep showing lymphocytes (L), plasmablast (P2), dendritic cells of intermediate form of maturation (DCi), and platelets (arrows) all are supported by a network of reticular fibers (RF). Bar=5µm.

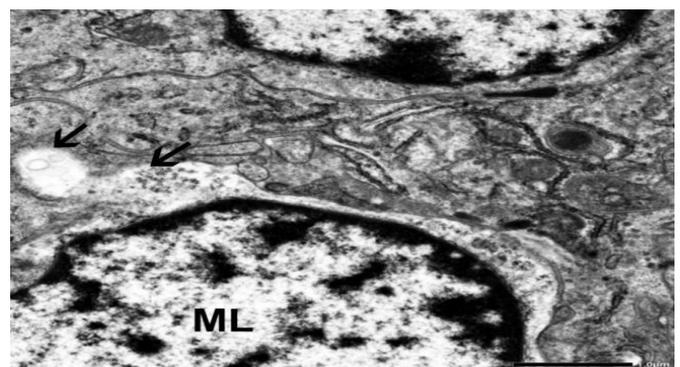


Fig. (7): A higher magnification of the previous transmission electron micrograph showing the motile lymphocyte (ML) with its pseudopodia (arrows). Bar=1µm

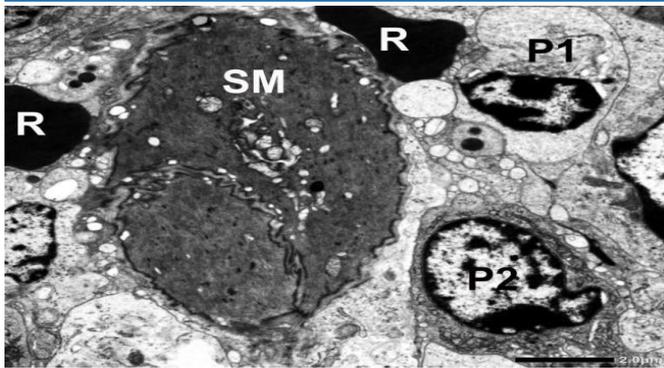


Fig. (8): A higher magnification of figure (41) showing doubled reciprocal smooth muscles (SM) are surrounded by plasma cell precursor (P1), plasmablast (P2), and erythrocytes (R). Bar= 2µm.

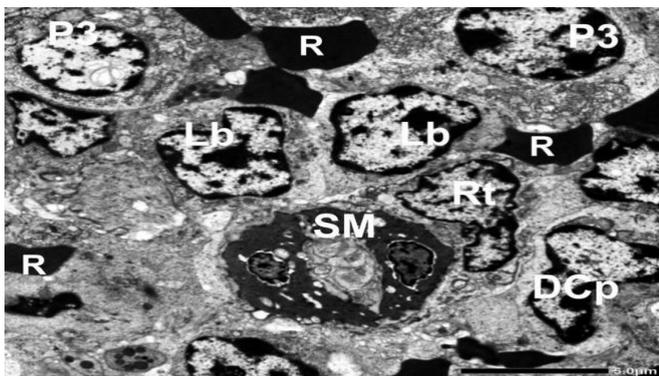


Fig. (9): Transmission electron micrograph of the marginal zone of the spleen of the Barki sheep showing dendritic cell precursor (DCp), lymphoblasts (Lb), plasma cells of intermediate or transient characters (P3), and doubled smooth muscles (SM) surrounded by reticular cells (Rt). Erythrocytes (R) are infiltrated between the cells. Bar 5µm.

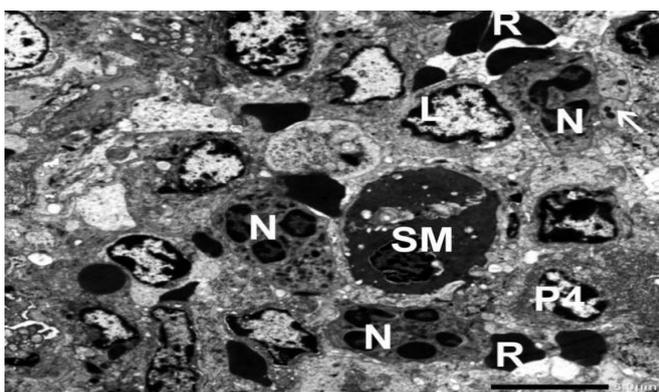


Fig. (10): Transmission electron micrograph of the splenic marginal zone of the Barki sheep showing neutrophils (N), lymphocytes (L), erythrocytes (R), platelets (arrows), classic small plasma cell (P4) are surrounding couple reciprocal smooth muscles (SM). Bar= 5µm.

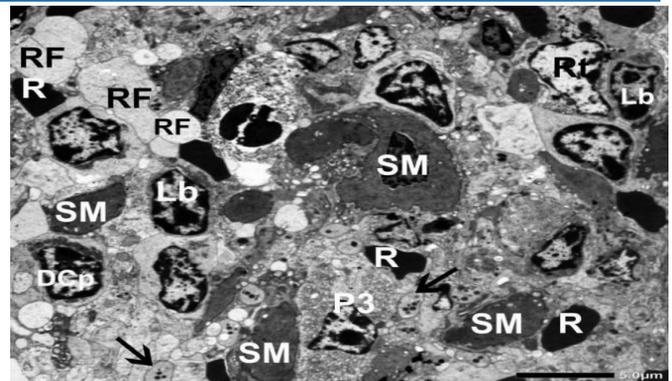


Fig. (11): Transmission electron micrograph of the marginal zone of the spleen of Barki sheep showing solitary smooth muscles (SM) reticular cells (Rt) and their secreted reticular fibers (RF) are surrounding and supporting the lymphoblasts (Lb), dendritic cell precursors (DCp), plasmacyte of intermediate or transient characters (P3), platelets (arrows), and erythrocytes (R). Bar= 5µm.

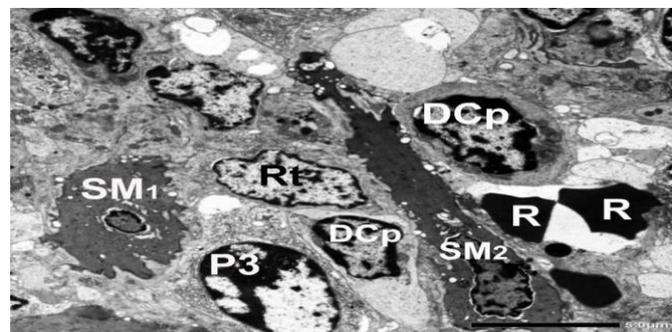


Fig. (12): Another transmission electron micrograph of the splenic marginal zone of the Barki sheep showing cross-sectioned (SM1) and longitudinally sectioned (SM2) solitary smooth muscles and reticular cells (Rt) are supporting and surrounding the plasmacyte of intermediate or transient characters (P3), and dendritic cell precursor (DCp). R= erythrocyte. Bar= 5µm.

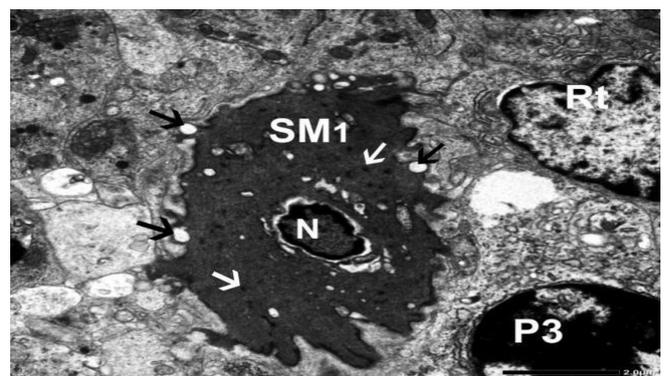


Fig. (13): A higher magnification of the previous transmission electron micrograph showing cross-sectioned solitary smooth muscle cell (SM1) with dense bodies (white arrows) and caveolae (black arrows). N= nucleus of the smooth muscle. Observe the plasmacyte of intermediate or transient characters (P3), and reticular cell (Rt) surrounding the smooth muscle. Bar= 2µm.

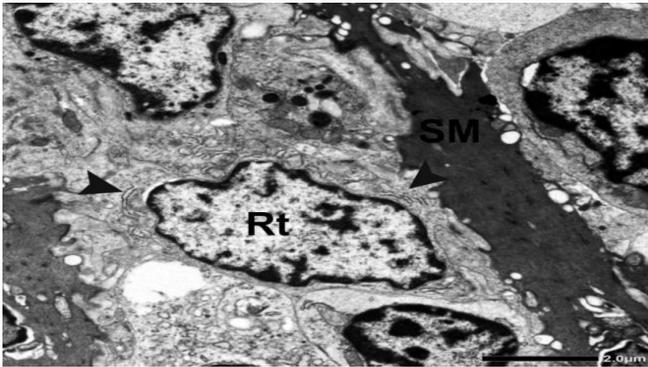


Fig. (14) Another higher magnification of figure 48 shows that the cytoplasm of the reticular cells (Rt) which are present in the marginal zone is filled with Golgi apparatus (arrowheads). SM= solitary smooth muscle. Bar=2 μm.

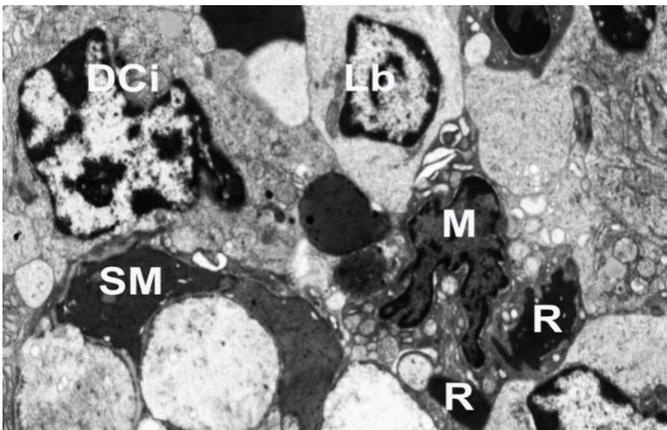


Fig. (15): Transmission electron micrograph of the splenic marginal zone of the Barki sheep denoting dendritic cell of the intermediate or transient stage (DCi), lymphoblast (Lb), marginal zone macrophage (M) with horseshoe shape nucleus, and engulfing red blood corpuscles(R). Note solitary smooth muscle (SM) is suspended among them. Bar= 2 μm.

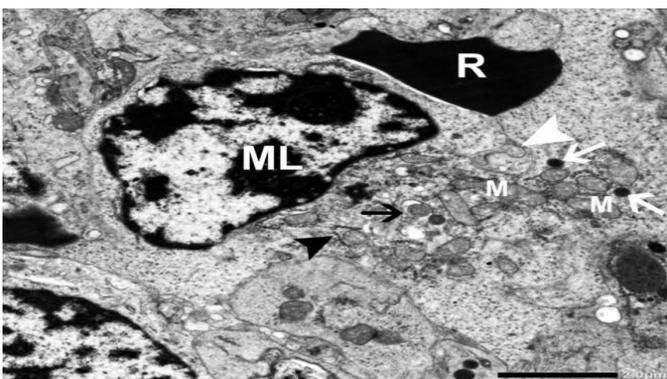


Fig. (16): Transmission electron micrograph of the splenic marginal zone of the Barki sheep depicting motile lymphocyte (ML) with its pseudopodia (white arrowhead) and the cytoplasm of dendritic cell contains mitochondria (M), rough endoplasmic reticulum (black arrowhead), dense granules (white arrows) and phagosome (black arrow). R= erythrocyte. Bar=2μm.

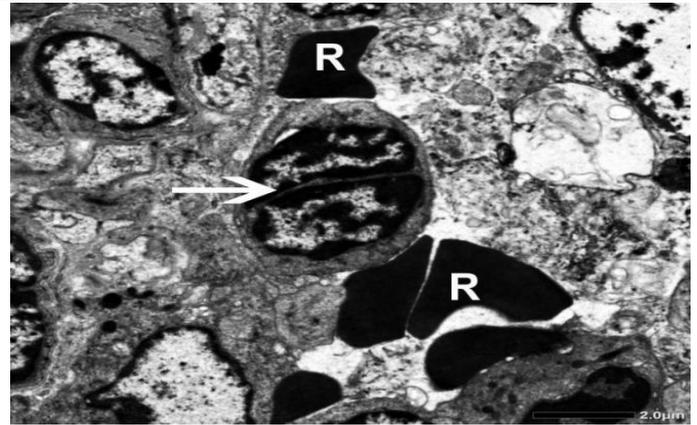


Fig. (17): Transmission electron micrograph of the splenic marginal zone of the Barki sheep showing lymphocyte during its division (lymphocyte mitogen), lymphoblast in the upper left corner. R= erythrocytes. Bar= 2μm.

DISCUSSION

Our study reported that the splenic marginal zone of the Barki sheep surrounds the splenic white pulp enclosing the areas between the white and red pulps, thus the splenic marginal zone of the Barki sheep is similar to that of the rodents, rabbits (*Snook, 1950*), and the dromedary camel (*Zidan et al., 2000*) but differs from that of the human where it surrounds only the lymphoid nodules (*Steiniger, 2015*) and the water buffalo where it present between the lymphoid nodules and the red pulp (*Rashed et al., 2020*).

The splenic marginal zone of the Barki sheep contains a marginal zone sinus which is lined by rod-shaped endothelial cells resting on an interrupted basement membrane, thus came in the accordance with (*Burke and Simon, 1970*) who reported that these interruptions in the basement membrane provide a passage of cells from the white pulp into the marginal sinus and vice versa.

The marginal zone of the spleen of the Barki sheep is characterized by the presence of marginal zone sinus in addition to the presence of smooth muscle cells which give the spleen of the Barki sheep a powerful ability to filter the blood and defense against foreign antigens making it a unique intermediate type of spleen having a powerful ability to filter the blood and making a strong immune response in addition to its powerful ability in restore and reserve the blood.

The marginal zone sinus of the Barki sheep is surrounded by a diffuse lymphoid tissue of the marginal zone which consists of different stages of lymphocyte maturation, neutrophils, marginal zone macrophages, different stages of plasma cell maturation, different stages of dendritic cell maturation, erythrocytes, platelets and reticular cells all are supported by a network of reticular fibers and smooth muscles. This came in the accordance with *Cerutti et al. (2013)* who concluded that lymphocytes present in the marginal zone are a type of B cell (Marginal-zone B cell) created there and are capable of binding IgM-antigen complexes, and *Nutt et al. (2015)* who reported that mature B cells have three major subsets; Follicular B cells which present in the lymphoid nodules, B1 cells which present in the peritoneal and plural cavities and at mucosal sites, and the marginal zone B cells which about the marginal sinus of the spleen where they are ideally placed to encounter blood-borne pathogens and particulate antigens. *Charles and Thor (2013)* reported that macrophages transported the blood-borne antigens trapped in the marginal zone to the periarterial lymphatic sheaths (PALS) which was

an environment rich in recirculating lymphocytes and dendritic cells. **Kraal and Mebius (2006)** concluded that macrophages present in the marginal zone are well prepared to recognize pathogens and filter the blood under the virtue of unique combinations of pattern recognition receptors. They interact with the marginal zone B cells which can be present only and able to react rapidly to bacterial antigens. This combination of strategically located cells is important in defense against blood-borne pathogens.

The presence of different stages of plasma cell maturation and dendritic cell maturation indicates the high activity of the marginal zone in the production of antibodies and the powerful presentation of antigens, respectively

CONCLUSION

The marginal zone of the spleen of the Barki sheep is characterized by the presence of marginal zone sinuses in addition to the presence of smooth muscle cells, which give the spleen of the Barki sheep a powerful ability to filter the blood and defend against foreign antigens, making it a unique intermediate type of spleen having a powerful ability to filter the blood and make a strong immune response in addition to its powerful ability to restore and reserve the blood. The present study revealed four stages of maturation of the plasma cells: Stage 1: The plasma cell precursor, which is the B lymphocyte. Stage 2: Plasmablast, which is the post-germinal center B lymphocyte. Stage 3: Plasmacyte of intermediate or transient characters. Stage 4: Classic and typical plasma cells. The presence of four stages of plasma cells in the marginal zone indicates that the Barki sheep's spleen has higher immunological activity. The current study found multiple subsets of dendritic cells in intermediate stages of maturation as well as typical mature dendritic cells, indicating active antigen presentation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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