Original Research

Enzyme Histochemistry of Spleen of Pig (*Sus scrofa*)

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Rec. Date:	Jul 30, 2019 10:14
Accept Date:	Aug 31, 2019 08:34
DOI	10.5455/ijlr.20190730101415

Abstract

The present study was conducted on the spleen of pig to elucidate the histoenzymic distribution of phosphatases and oxidoreductases. The fresh unfixed spleen samples were immediately collected after sacrifice and were subjected to cryostat sectioning at -20^{0}C with cryostat microtome. The sections were incubated with substrates for demonstration of various enzymes. The study revealed that weak to moderate alkaline phosphatase (AKPase) activity was observed in capsule and trabeculae of the spleen. The AKPase activity was moderate to strong in lymphoid follicle. The ATPase activity was seen moderate to strong in the lymphoid follicle of spleen. Uniform weak Glucose 6-phosphate activity was observed in entire spleen and capsule. Succinic dehydrogenase (SDH) activity was moderate in capsule and lymphoid follicles. Lactic dehydrogenase activity was moderate to strong in capsule and lymphoid follicles. Malic dehydrogenase (MDH) showed weak activity in the capsule and trabeculae. Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-D) and reduced Nicotinamide adenine dinucleotide diaphorase (NADH-D) was moderate in the capsule and moderate to strong in lymphoid follicles. Nonspecific esterases (NSE) activity was moderate in capsule and trabeculae. The presence of variable activity of different enzymes in spleen was correlated with maturation of lymphocytes and development of different metabolic pathways.

Key words: Enzyme Histochemistry, Oxidoreductases, Pig, Phosphatases, Spleen

How to cite: Kaur, H., Singh, O., & Pathak, D. (2019). Enzyme Histochemistry of Spleen of Pig (Sus scrofa). International Journal of Livestock Research, 9(9). 137-142. doi: 10.5455/ijlr.20190730101415

Introduction

The spleen is the largest lymphoid organ and is the primary site for immune cell proliferation and differentiation and is specialized for filtration of blood. It is involved in haemopoiesis and phagocytosis of aged erythrocytes (Das *et al.,* 2005). It is comprised of two functionally and morphologically distinct compartments, the red pulp and the white pulp. The red pulp is mostly involved in blood storage and phagocytosis, the white pulp is the primary region for immune response and B-lymphocyte maturation (Ikpegbu *et al.,* 2014). Dellman and Brown (2006) classified mammalian spleens depending on the type of post capillary vessels into sinusal and non-sinusal type. In majority of domestic animals except dog non

sinusal type of spleen is present. The architectural design of spleen varies due to functional reasons (Alim *et al.*, 2014). A thorough knowledge of enzyme histochemistry will be helpful in explaining the immune response of pigs to varying clinical conditions and also in understanding peculiarities of porcine immune system for applied experimental studies.

Materials and Methods

The present study was conducted on spleen of pig of non-descript breed collected from slaughter house (n=12). The fresh unfixed splenic tissue from pig were immediately collected after sacrifice and stored in liquid nitrogen. These tissues were subjected to cryostat sectioning at -20 ^{0}C with cryostat microtome. The sections of 10 -12 µm thickness were obtained on clean glass slides and were incubated for demonstration of various enzymes as alkaline phosphatase (AKPase), adenosine triphosphatases (ATPase), glucose-6-phosphatase (G-6-pase) by coupling azodye method (Barka and Anderson,1963), succinate dehydrogenase (SDH), malic dehydrogenase (MDH), lactate dehydrogenase (LDH), non-specific esterases (NSE), reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) and glucose-6-phosphate dehydrogenase (G-6-PD) by nitro BT method (Pearse,1972).

Result and Discussion

The histoenzymic activity of different phosphatases and oxidoreductases of pig spleen is detailed below.

Phosphatases

Alkaline Phosphatase (AKPase)

The alkaline phosphatase activity was constantly weak in the capsule of the spleen. The trabeculae showed weak to moderate activity whereas parenchyma of the spleen showed weak activity for AKPase. The activity was moderate to strong in the lymphoid follicles (Fig. 1), however, weak activity was observed in the germinal centers of the lymphoid follicles (Fig. 2). Lymphoid follicles devoid of germinal centers showed moderate to strong activity. The diffused lymphocytes present loosely in the parenchyma of spleen were moderately positive for alkaline phosphatase. The frequent association of the enzyme alkaline phosphatase with monosaccharides in the tissue was observed and may be associated with the transport of nutrients (Kumar *et al.*, 2013).

 DOI 10.5455/ijlr.20190730101415

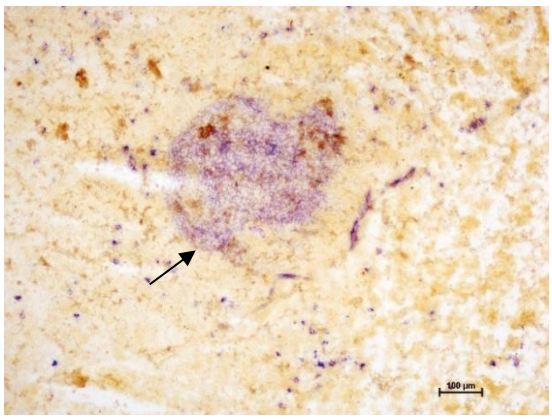

Fig. 1: Cryosection of spleen of pig showing strong AKPase activity in lymphoid follicle (arrow). Azodye X100

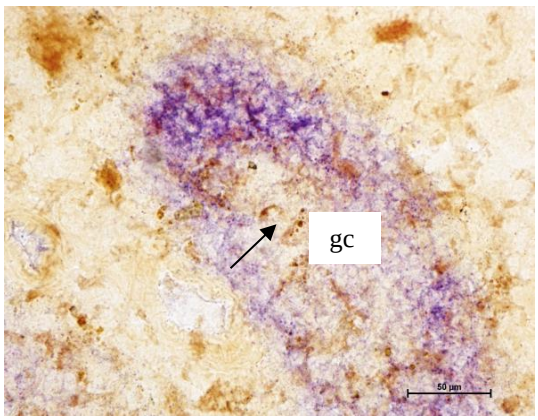

Fig. 2: Cryosection of spleen of pig showing weak AKPase activity in germinal center (gc) of lymphoid follicle(arrow). Azodye X400

Adenosine Triphosphotases (ATPase)

The activity for ATPase in the capsule and trabeculae of pig spleen was weak. The activity for ATPase was moderately to strong in the lymphoid follicle of pig spleen (Fig. 3).

Glucose-6-Phosphatase (G-6-Pase)

Weak to mild activity for Glucose-6-phosphatase was observed in entire spleen (Fig. 4). Glucose-6-phosphatase consisted of amino acids, anchored to the endoplasmic reticulum (ER) and is involved in the release of glucose into the circulation-(Kaur *et al.*, 2018).

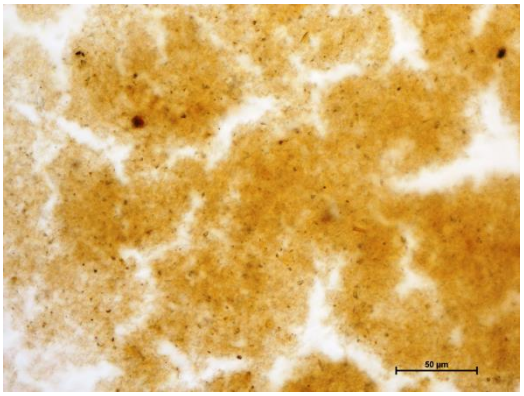

Fig. 3: Cryosection of spleen of pig showing moderate to strong ATPase activity in lymphoid follicle (arrow). Azodye X400

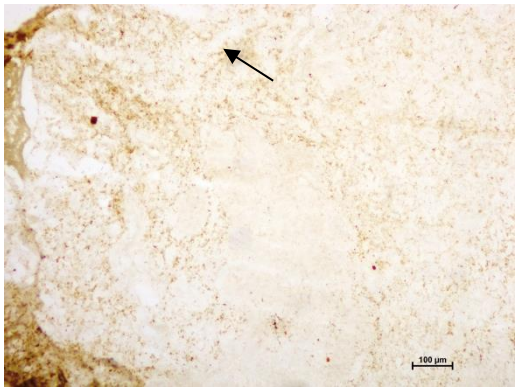

Fig. 4: Cryosection of spleen of pig showing weak activity for Glucose-6-phosphatase (G-6-Pase) in capsule (c) (arrow). Lead nitrate method X 100.

Oxidoreductases

Dehydrogenase

DOI 10.5455/ijlr.20190730101415

Succinic Dehydrogenase (SDH)

In the present study weak SDH activity was noted in the capsule and trabeculae of pig spleen. The parenchyma of the spleen was weakly positive for SDH. The activity for SDH was moderate in lymphoid follicles of spleen. SDH is a mitochondrial enzyme that is involved in generation of energy by oxidation-reduction reaction in the cell (Smith, 1969). This enzyme might be associated with oxidation of fatty acids that lead to formation of lipid pigment granules (Smith 1969).

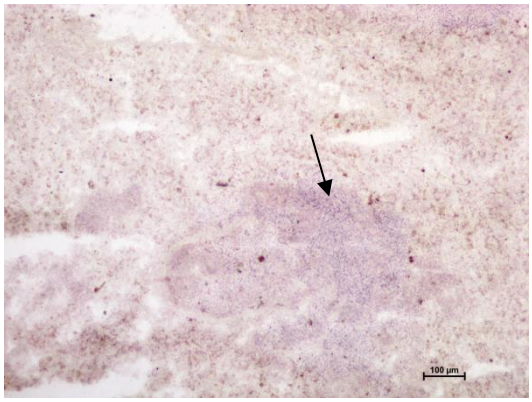

Fig. 5: Cryosection of spleen of pig showing weak to moderate activity of succinic dehyrogenase (SDH) in lymphoid follicle (lf) (arrow). Nitro BT method X 100.

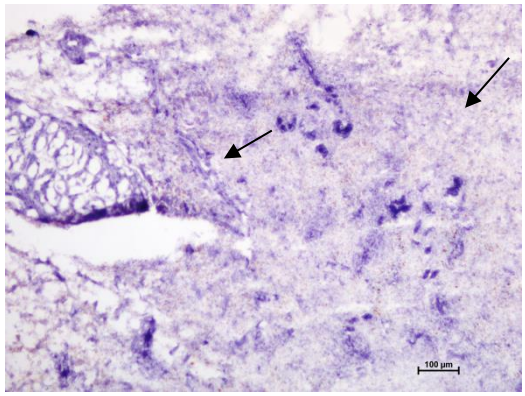

Fig. 6: Cryosection of spleen of pig showing moderate activity for Glucose-6-phosphate dehydrogenase (G-6-PD). Nitro BT method X 100.

Glucose 6 Phosphate Dehydrogenase (G-6-PD)

In the present study, glucose 6 dehydrogenase activity was strong in the capsule and trabeculae. The parenchyma showed moderate G-6-PD activity and lymphoid follicles showed strong activity (Fig. 6). The glucose 6 phosphate dehydrogenase enzyme activity is associated with the pentose phosphate shunt (Fennel and Pearse, 1961). These pentose phosphates might be utilized for nucleic acid synthesis during development. Turkoglu and Aldemir (2003) also reported the Glucose 6-phosphate dehydrogenase (G-6-PD) enzyme activity in all mammalian tissues especially in cytosol and mitochondria.

Lactate Dehydrogenase (LDH)

A moderate to strong activity to lactate dehydrogenase was observed in the capsule of spleen (Fig. 7). However, the lymphoid follicles were moderately positive for LDH (Fig. 8). The diffused lymphocytes arranged loosely in the parenchyma showed weak LDH activity. LDH is an NAD dependent enzyme found in cells in which glycolytic pathway is active. It catalyses the formation of lactate in anaerobic glycolysis and pyruvate in aerobic respiration. The presence of LDH enzyme in spleen suggested the presence of glycolytic pathway in cellular elements especially in the capsule and lymphoid follicles.

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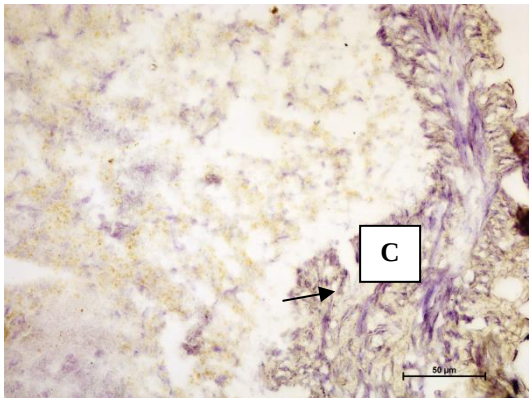

Fig. 7: Cryosection of spleen of pig showing strong activity for Lactic dehydrogenase (LDH) in capsule (c)(arrow). Nitro BT method X 400.

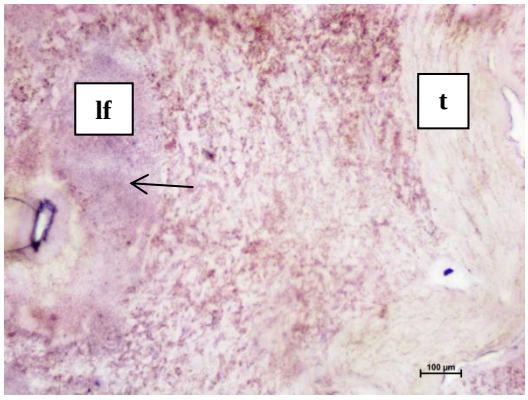

Fig. 8: Cryosection of spleen of pig showing moderate activity for Lactic dehydrogenase (LDH) in lymphoid follicles (lf)(arrow), trabeculae (t) Nitro BT method X 400.

Malic Dehydrogenase (MDH)

The malic dehydrogenase (MDH) activity was weak to moderate in the capsule and trabeculae of the pig spleen (Fig. 9). Weak activity was seen in the parenchyma of the pig spleen. The malic dehydrogenase (MDH) is an enzyme that reversibly catalase the oxidation of malate to oxaloacetate and is a part of metabolic pathways.

Non-Specific Esterases (NSE)

The activity for non-specific esterases (NSE) was seen in the capsule weak to moderately. The activity in trabeculae for non-specific esterases was moderately positive. Strong activity was seen in blood vessels of pig spleen.

Diaphorases

Reduced Nicotinamide Adenine Dinucleotide Diaphorase (NADH-D) and Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-D)

In the present study, moderate nicotinamide adenine dinucleotide diaphorase was observed in the capsule and trabeculae of the spleen (Fig. 9). The activity was weak to moderate in the parenchyma of the spleen. The activity of this enzyme was intense in the lymphoid follicle (Fig. 10). The periphery of the lymphoid follicle showed moderate activity to this enzyme. Connective tissue fibers showed moderate reaction to this enzyme. The cellular elements showed moderate to strong activity. However, intense activity was present in lymphoid follicles.

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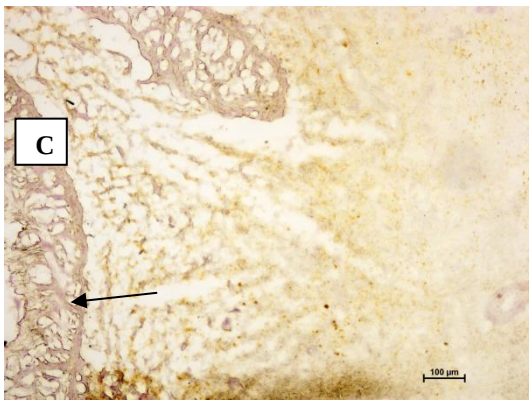

Fig. 9: Cryosection of spleen of pig showing weak activity for Malic dehydrogenase (MDH) in capsule (c) (arrow). Nitro BT method X 400.

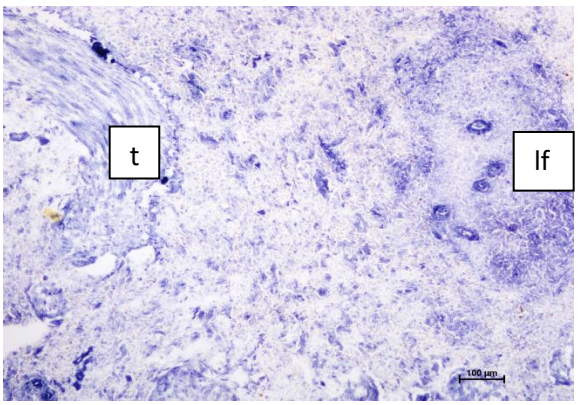

Fig. 10: Cryosection of spleen showing strong activity for Nicotinamide adenine dinucleotide diaphorase (NADH-D) trabeculae (t) and lymphoid follicles (lf). Nitro BT method X 100.

Weak to moderate activity of nicotinamide adenine dinucleotide phosphate diaphorase was present in the capsule and trabeculae of the spleen. In parenchyma, moderate activity of this enzyme was observed. The activity of this enzyme was strong to intense in lymphoid follicles. NADH and NADPH diaphorases are co-enzyme dehydrogenases and acts in the cell as a part of hydrogen transport chain. The enzyme intensity indicated metabolic activity of the cell.

Conclusion

The variable activity of phosphatases and oxidoreductases in spleen of pig reflected the development of metabolic pathways and maturation of cellular elements especially the lymphocytes.

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