

Hemal Node of Egyptian Water Buffalos (*Bos Bubalus*): It's Role in Erythrophagocytosis

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Abstract

Hemal nodes were obtained from 12 clinically healthy male buffalos aged 2-3 years, their average weight is 500-600kg. Electron microscopy revealed macrophages engulfing intact erythrocytes in vacuoles, or only parts of erythrocytes appeared in the vacuoles. Some electron dense material appeared in the erythrocyte as a lysosomal degradation. Also myeline like figures and residual bodies were seen in macrophages cytoplasm.

plasma cells were observed to be in close association to the macrophages and both cells make a sort of union in some sites. A number of electron dense bodies with variable shapes and sizes were seen in the cytoplasm of the plasma cells near the nucleus. The findings of this study suggests the occurrence of erythrophagocytosis in buffalo hemal nodes. The main cells responsible for this rule were macrophages may be together with the help of plasma cells. No rule could be rec-

orded for other cells as lymphocytes, mast cells, endothelial cells or reticular cells in erythrophagocytosis.

Key words: hemal node, erythrophagocytosis, electron microscopy, macrophage, plasma cell, Egyptian water buffalo.

Introduction

The water buffalo represented an important part of animal production in Egypt. The estimated herd number exceeds 3.6 million heads FAO (2002). It is economically a very important farm animal and genetic improvement of these animals was of economic importance, especially in reproductive performance and quantity of meat and milk as well as diseases and parasite resistance (El-Nahas et al, 1998).

The hemal node (HN) is a hematopoietic and lymphoid organ that was found in some mammals such as humans, rats and ruminants. In ruminants, hemal nodes were located

in the subcutaneous region of the head, the mesenteric region and along the large blood vessels such as the aorta in the thorax and abdomen; however, the distribution and number of hemal nodes in these regions seem to be diverse (Bacha and Bacha 2000, Snider et al, 2003, Casteleyn et al, 2008).

Contrary to their anatomy, the function of hemal nodes had not been fully elucidated. Postulated functions included: erythrophagocytosis, erythropoiesis, platelet formation and, most recently, immune functions (Turner 1971, Cecarelli et al, 1983, Cerutti & Guerrero 2008, Zidan & Pabst 2010, Zidan et al, 2012).

Erythrophagocytosis is currently defined as the process by which an organism removes old or degenerated erythrocytes from the circulation. It is performed primarily by macrophages, mainly in the spleen but also in the liver, lung, bone marrow and, in certain species, in hemal or haemolymph nodes (Cerutti & Guerrero, 2008).

Olah and Toro (1970) observed in rat haemolymph nodes macrophages with abundant rough endoplasmic reticulum, free lysosomes, and numerous phagosomes containing erythrocyte debris had been regard-

ed in erythrophagocytosis. Also Turner (1971) found that the sinuses of rat hemal nodes contained macrophages with abundant haemosiderin in their cytoplasm.

On the other hand, Al-Bagdadi et al. (1986) said that the plasma cell of the hemal node of sheep showed numerous cytoplasmic prolongations suggestive of motility and phagocytic activity, Their irregular perinuclear zones contained granular material of similar density to that of rough endoplasmic reticulum, and some showed morphological evidence of various phases of erythrophagocytosis.

Aim of the work

Recent studies suggested many functions to the hemal nodes of Egyptian water buffalos (*Bos bubalus*) as erythropoiesis, platelet formation, blood filtration, erythrophagocytosis and immunity. In this study we aimed to shed light on the role of the macrophages and plasma cells in erythrophagocytosis to understand of the exact rule of hemal nodes of buffalos and to know if erythrocytes were filtered in hemal nodes or not.

Material and Methods

Fresh specimens of hemal nodes were obtained from 12 clinically healthy male buffalos aged (2-3) years. The animals were slau-

ghtered for human consumption in the abattoir of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Two hemal nodes were obtained from each animal, from the mesenteric and perirectal regions. Each hemal node was examined macroscopically and dissected, then fixed in 10% phosphate-buffered formaldehyde and processed for paraffin sectioning. Sections (4µm) were prepared and stained by using Prussian-blue-stain (Bancroft and Stevens, 1979).

Pieces of 1mm were cut from the hemal nodes and quickly fixed in 6% solution of phosphate buffered gluteraldehyde pH 7.4 for 6 hrs. at 4°C (McDowell and Trump, 1976). After initial fixation, the tissues were washed in several changes of cold (4°C) 0.1 M phosphate buffer every 15 minutes for 2 hrs.

The tissues were post fixed in 1% solution of osmium tetroxide in cold (4°C) 0.1 M buffer pH 7.2 for 2 hrs. Then, they were rapidly dehydrated through ascending grades of ethyl alcohol then transferred to propylene oxide and placed in a 1:1 mixture of propylene oxide and epoxy araldite (Hayat, 1986).

Semi-thin sections (1µm) were cut firstly and stained with toluidine blue and viewed with light microscope to select the suitable areas for the

electron microscope examination. The ultrathin sections (60-100 nm) were cut by a glass knife with LKB microtome, and then they were stained with uranyl acetate followed by lead citrate (Hayat, 1986). These sections were examined with Joel 100 cx electron microscope operating at 80 Kvs.

Results

Erythrocytes were found inside the cytoplasm of macrophages and in the subcapsular and trabecular sinuses and around the endothelial cells (Fig. 1). Macrophages were large irregular cells containing numerous lysosomes, phagosomes, rough endoplasmic reticulum and mitochondria in their cytoplasm. At the first stage of phagocytosis, intact erythrocyte was seen engulfed by macrophage (Fig. 2) and appeared as if they were intact in the vacuoles. Electron lucent granular material appeared around the periphery and in the center of the erythrocytes. Only parts of the erythrocytes appeared in the vacuoles then, to some extent this electron lucent granular material occupied the whole core of erythrocytes (Fig. 2). Some electron dense material appeared in the erythrocyte as a lysosomal degradation. Also myelin like figures and residual bodies were seen in macrophages cytoplasm (Fig. 2).

plasma cells were observed to be in close association to the macrophages (Fig. 2) and both cells make a sort of union in some sites where their cell membranes become not clear in these areas. Also in paraffin sections stained with Prussian blue staining protocol for iron we could see the bright blue color of the ferric ferrocyanide inside some cells (Figs. 3&4). Plasma cells were oval to irregular in shape with few cytoplasmic processes extending between the surrounding erythrocytes (Figs. 5&6). Their cytoplasm was completely filled with rough endoplasmic reticulum with variable dilations (Fig. 7). Nuclei of plasma cells were oval to irregular in shape with clear nuclear membrane, heterochromatin was clumped near nuclear membrane, and a number of electron dense bodies with variable shapes and sizes were seen in the cytoplasm near the nucleus (Fig. 7).

Discussion

Prussian blue reaction involves the treatment of sections with acid solutions of ferrocyanides. Any ferric ion (+3) present in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called Prussian blue, or ferric ferrocyanide. This is one of the most sensitive histochemical tests and will demonstrate even single granules of iron in blood cells (Cerutti &

Guerrero, 2008) . And this appeared clearly in the present study where macrophages found in the sinuses of hemal nodes were involved in erythrophagocytosis and this appeared clearly by the presence of the bright blue pigments inside some cells. Erythrocytes were found inside the cytoplasm of the macrophages as a whole intact erythrocyte in an intact vacuole or as a parts of erythrocytes with different electron density from electron dense parts to medium and electron lucent parts and this agreed with Turner (1971) who found that the surface membrane extended at a number of sites into long attenuated processes which were wrapped around adjacent erythrocytes. As regard erythrophagocytosis, macrophages with abundant rough endoplasmic reticulum, free lysosomes, and numerous phagosomes containing erythrocyte debris have been observed in rat haemolymph nodes by Olah & Toro (1970).

Myeline like figures were found inside these macrophages but these figures were found occasionally by Turner (1971) and instead he found many large rounded osmiophilic structures which contained small granules , which were not in the macrophages of buffalo hemal nodes of this study. So macrophages in the sinuses of buffalo hemal

nodes were actively included in erythrophagocytosis and breaking down of erythrocytes to ferritin and myelin like bodies. Turner (1971) added that macrophages were capable of engulfing particles in the sinuses and then migrating to the edge of the germinal center.

Our findings in this study agreed also with Cerutti & Guerrero (2008) that corroborate the performance of erythrophagocytosis in bovine haemal nodes. They observed large quantities of haemosiderin, the pigment produced by degradation of haemoglobin, in both the sinuses and the cytoplasm of some macrophages; and transmission electron micrograph images showed numerous macrophages and polymorphonuclear cells with erythrocyte debris in their cytoplasm or actually in the act of erythro-phagocytosis. While in haemal nodes of goats, Ezeasor et al. (1989) observed erythro-phago-cytosis by endothelial cells, reticular cells, lymphocytes and eosinophils as well as by macrophages. But in the present study in buffalo hemal nodes only erythro-phagocytosis by macrophages could be seen.

Ezeasor et al. (1989) and Cerutti & Guerrero (2008) could not observe erythrophagocytosis by plasma cells, as Al-Bagdadi et al. (1986) did in hemal lymph nodes of healthy

sheep. In this study we could not prove that also, but there is a possibility for that indicated by the modified shape of plasma cells and their location in association with the erythrocytes and macrophages. Also the cytoplasmic processes extending from plasma cells in between the erythrocytes may postulate its role in erythrophagocytosis.

The plasma cell contact with the erythrocyte indicates the possibility that surface receptors on the plasma cell initiate recognition of the cell to be engulfed (Al-Bagdadi et al, 1986). They proved that erythrophagocytosis by plasma cells from hemal lymph nodes of healthy sheep can occur. This could be interpreted as a normal function of plasma cells in healthy animals which results in the removal of aged or impaired erythrocytes from the circulation. While other investigators have reported phagocytic activity of plasma cells only in pathological conditions (Abramson et al, 1970, & Butterworth et al, 1953).

There is a functional relationship existing between plasma cells and macrophages for a possible passage of materials from the macrophage to the plasma cell (Thiery 1962, Nossal et al, 1968). In our study we found a site relationship between both cells where plasma cells were always found close to macrophages and there is some-

what a loss of cell membranes of both cells at sites of attachments, which may suggest a great role of plasma cells in helping macrophages doing erythrophagocytosis. Al – Baghdadi et al. (1986) found close contacts and occasionally a fusion between the plasmalemma of a mast cell and that of a plasma cell. This might indicate a transfer of material between the mast cell and the plasma cell. It is believed that the purpose of contact between the plasma cell and macrophage is to carry out immunospecific functions, which means an antigen transfer (Schneider ,1979). While, this contact was not observed in the present work. Meanwhile, presence of cytoplasmic granules of variable electron density , shapes and sizes in the cytoplasm of plasma cells mainly near the nucleus may suggest presence of phagosomes or secondary lysosomes containing parts of degraded erythrocytes and may support its role in erythrophagocytosis. [Al-Baghdadi et al. (1986) supported this idea in hemal nodes of sheep based also on presence of granular material of similar density to that of rough endoplasmic reticulum, in their irregular peri-nuclear zones.

Ezeasor et al. (1989) concluded the participation of small lymphocytes in erythrophagocytosis in the lymph nodules. Nuclei of those lympho-

cytes that had phagocytized erythrocytes were indented so as to accommodate the erythrocyte-containing heterophagic vacuoles. They added that although the macrophages are the major cells engaged in erythrophagocytosis they provided evidence of erythrophagocytosis by endothelial cells, reticular cells, small lymphocytes and eosinophils, but in our study we could not find any erythrophagocytosis in those cells which may reveal the absence of their role in this process. Megakaryocytes were rarely seen in buffalo hemal nodes, although Folse et al. (1971) did observe them in bovine hemal nodes. And this may suggest that buffalo hemal nodes were incorporated in erythrophagocytosis more than that in erythropoiesis .

Conclusion

The finding of this study suggests the occurrence of erythrophagocytosis in buffalo hemal nodes. The main cells responsible for this role are macrophages with the help of plasma cells. No role could be recorded for other cells as lymphocytes , mast cells, endothelial cells or reticular cells in erythrophagocytosis.

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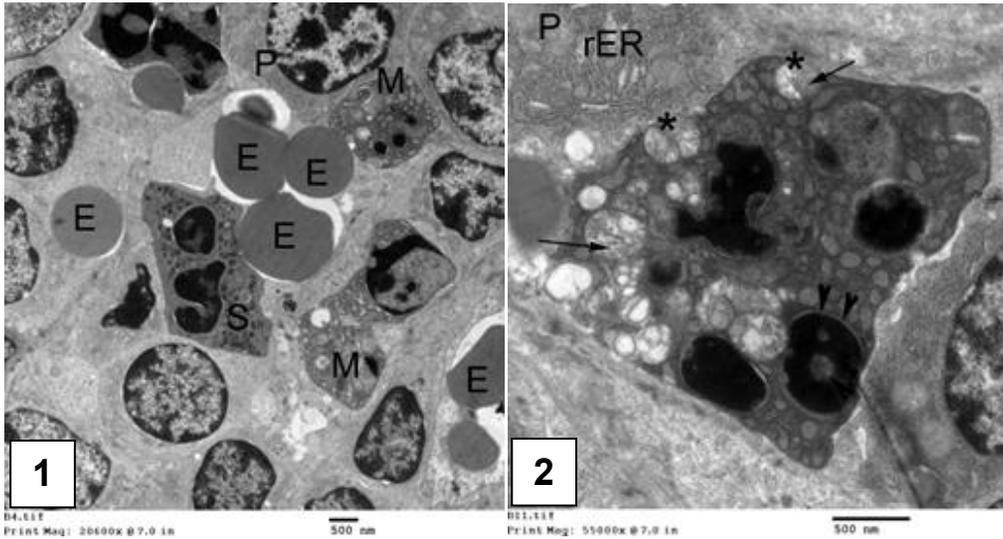
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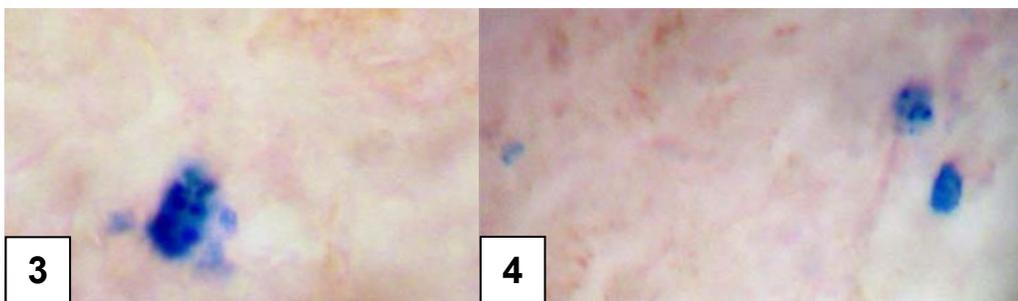
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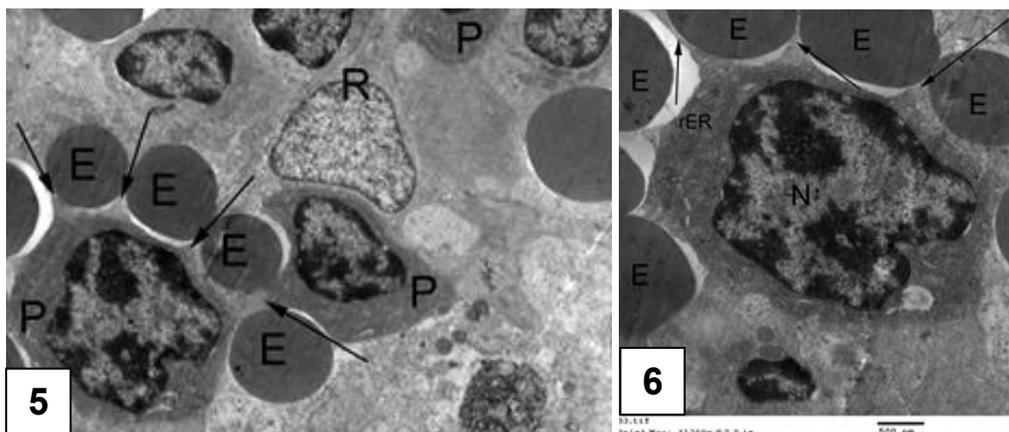
(Fig.1): Transmission electron micrograph of buffalo hemal node showing erythrocytes (E) intermingled with macrophages (M), eosinophil (S), and plasma cell (P). (x1500).

(Fig.2): Transmission electron micrograph of a macrophage showing the presence of electron lucent granular material at the periphery and center of the phagocytosed erythrocyte (arrow heads) and myeloid figures (arrows). Note the neighbor plasma cell (P) with a well-developed rough endoplasmic reticulum (rER) and sites of cytoplasmic contact between it and the macrophage (asterisk) (x4000).



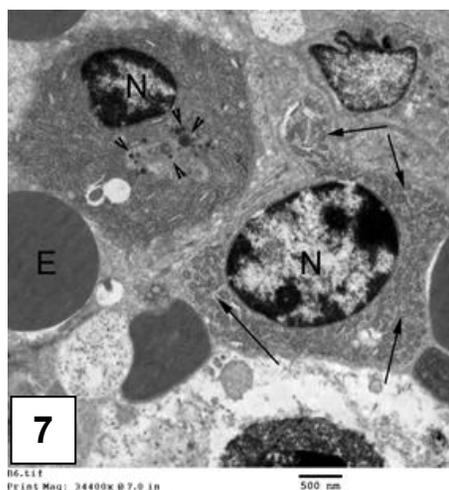
(Fig.3): photomicrograph of the hemal node macrophage inside the sinus showing the bright blue prussian blue pigments (ferric ferrocyanide) inside it. Prussian blue stain X1000).

(Fig.4): photomicrograph of two the hemal node macrophage inside the sinus showing the Prussian blue pigments inside it. Prussian blue stain X1000).



(Fig.5): Transmission electron micrograph of plasma cells (P) of buffalo hemal node engaged to the erythrocyte making depressions in the cytoplasm of plasma cells, Note the cytoplasmic processes of the plasma cell in-between the erythrocytes (arrows). Reticular cell (R) . (x1500).

(Fig.6): Higher magnification of the previous photo showing processes of plasma cell (arrows) extended between erythrocytes.The cytoplasm of plasma cell was filled with rough endoplasmic reticulum(rER) and the nucleus (N) is with prephiral clumps of condensed chromatin. (x3000).



(Fig.7): Transmission electron micrograph of two adjacent plasma cells in buffalo hemal node depicting dilated rough endoplasmic reticulum (arrows) and some electron dense granules with different shapes and sizes in the cytoplasm (arrow heads). Nuclei(N) and (E) erythrocyte . (x 2500)