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Cytochemistry of sheep bone marrow cells

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ABSTRACT

The present study was conducted on bone marrow samples obtained from 15 clinically normal Libyan Barbary sheep. Haemopoietic cells, including those of myelocytic series, erythrocytic series, megakaryocytic series, lymphocytes, plasma cells, monocytes and mitotic cells were identified on the basis of their morphological characteristics in May-Grünwald-Giemsa stained bone marrow smears. Cytochemical reactions of bone marrow cells revealed that all granulocytes, except myeloblasts and basophils, were peroxidase positive. Monocytes were peroxidase negative, but very few of them showed weak activity. Cells of the erythrocytic, megakaryocytic and lymphocytic series were peroxidase negative. Granulocytic series cells were Sudan black B positive, except myeloblasts. Some monocytes were positive with a few scattered black granules, while others were negative. Cells of erythrocytic, megakaryocytic and lymphocytic series were Sudan black B negative. Granulocytes at all stages of development reacted positively to periodic acid-Shiff, except myeloblasts. Monocytes were either completely negative or positive. Some lymphocytes showed a few fine or even coarse periodic acid-Shiff positive granules in the cytoplasm. Megakaryocytes were periodic acid-Shiff positive, while all cells of the erythrocytic series were periodic acid-Shiff negative. There was agreement in the mean values of the percentages of the three cytochemical stains positive cells and May-Grünwald-Giemsa stained bone marrow granulocytes.

Key words: sheep, bone marrow, cytochemistry, peroxidase, Sudan black B, periodic acid-Shiff

Introduction

Approximately 90% of all normal and abnormal peripheral blood and bone marrow cells can be identified by using one of the Romanowsky stains. In the past decades, haematologic morphology has been expanded by the use of cytochemical investigations.

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A variety of intracellular substances, such as peroxidase (KAPLOW, 1965), protein-bound lipids (SONNENWIRTH and JARETT, 1980) and glycogen (DACIE and LEWIS, 1975) can be demonstrated qualitatively by cytochemistry. Cytochemistry aids in defining cellular differentiation, maturation and function and in establishing internationally accepted reproducible standards for the diagnosis of leukemic cells (DACIE and LEWIS, 1975; LI and YAM, 1994). The present study was designed to evaluate cytochemistry of bone marrow cells in 15 clinically normal sheep using peroxidase, Sudan black B and periodic acid-Shiff stains.

Materials and methods

Seven male and eight female clinically normal adult Libyan Barbary sheep were used in this study. The ages of these animals ranged from one to three years. They were medicated with albendazole (two doses at an interval of two weeks) eight weeks before sampling. Skin scraping, faecal samples and blood samples collected from all animals were examined and they were negative for ectoparasites, endoparasites and blood parasites. Complete blood count and differential leukocyte count were performed in EDTA sample obtained from each sheep and the values of haematological parameters were within the ranges of normal values for adult Libyan Barbary sheep (AL IZZI et al., 2004).

Bone marrow samples were obtained from the third or fourth sternebra of each of the 15 sheep while they were restrained in lateral recumbency without sedation. At least seven smears were prepared from the marrow sample obtained from each animal and rapidly dried.

One bone marrow smear from each animal was stained with May-Grünwald-Giemsa stain. Cellular composition of the bone marrow was determined by differentiating 1000 nucleated cells in one smear from each animal; myeloid to erythroid ratio (M:E) was calculated by dividing the number of all cells of the granulocytic series by the number of nucleated cells of the erythrocytic series. Aspirated cells were identified on the basis of their morphological characteristics as described by JAIN (1986).

Cytochemistry of bone marrow cells was determined using peroxidase, Sudan black B and periodic acid - Schiff (PAS) stains in smears prepared from bone marrow samples obtained from the 15 sheep. One bone marrow smear from each sheep was stained for peroxidase activity according to YAM et al. (1971), except that the incubation period used was one minute instead of 30 seconds in order to increase the staining intensity of peroxidase- positive granules. One smear from each animal was stained with Sudan black B stain and another was stained with PAS stain as described by SONNENWIRTH and JARETT (1980). Five hundred nucleated bone marrow cells were differentiated on the basis of their positive or negative reaction in stained smear prepared from a sample

obtained from each animal. Three smears prepared from bone marrow sample collected from each animal were stored for one month at -4 ^{0}C in a light-tight box containing a desiccant capsule. The smears were removed at the end of the storage period and stained with the three cytochemical stains as described previously.

Results

The mean and standard deviation values of bone marrow differential cell count are presented in Table 1. Determination of the cellular composition of the bone marrow of sheep indicated that the bone marrow of sheep was more active in production of erythrocytes than granulocytes. The most outstanding feature of sheep bone marrow was the high number of eosinophils at different stages of maturation.

Cytochemical reactions of bone marrow cells in 15 normal adult Libyan Barbary sheep are presented in Table 1. All cells of the granulocytic series were peroxidase positive (Fig. 1), except for myeloblasts and basophils, which were negative. Very few mature segmented neutrophils were peroxidase negative or with weak peroxidase activity. Monocytes were peroxidase negative, but very few of them showed weak activity. Cells of the erythroid, lymphocytic and megakaryocytic series were peroxidase negative. Very weak peroxidase activity was observed in the granulocytes after one month of storage at - 4 ^{0}C (Fig. 2).

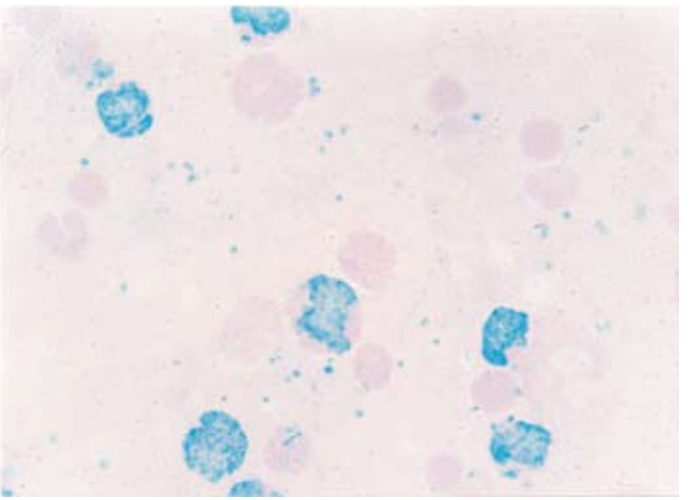

Fig. 1. Peroxidase positive granulocytes in bone marrow smear (Counter-stained with safranin; ×1000)

Table 1. Cytochemical reactions of bone marrow cells in clinically normal sheep

Cell type	May-Grünwald-Giemsa stain Mean (%)	Peroxidase reaction	Sudan black B reaction	Periodic acid-Schiff reaction
Myeloid series				
Myeloblasts	0.96 ± 0.15	-	-	-
Promyelocytes	2.04 ± 0.23	+	+	+
Neutrophilic myelocytes	4.52 ± 0.61	+	+	+
Eosinophilic myelocytes	0.80 ± 0.61	+	+	+
Basophilic myelocytes	0.04 ± 0.08	-	+	+
Neutrophilic metamyelocytes	7.27 ± 1.47	+	+	+
Eosinophilic metamyelocytes	2.16 ± 1.08	+	+	+
Basophilic metamyelocytes	0.04 ± 0.08	-	+	+
Neutrophilic bands	14.68 ± 1.83	+	+	+
Eosinophilic bands	3.20 ± 1.59	+	+	+
Basophilic bands	0.16 ± 0.23	-	+	+
Neutrophils	3.52 ± 1.50	+	+	+
Eosinophils	1.20 ± 0.73	+	+	+
Basophils	0.16 ± 0.19	-	+	+
Total myeloid series	40.75 ± 2.95			
Eythroid series				
Rubriblasts	0.68 ± 0.16	-	-	-
Prorubricytes	1.36 ± 0.29	-	-	-
Basophilic rubricytes	5.08 ± 0.50	-	-	-
Polychromatic rubricytes	31.96 ± 1.91	-	-	-
Metarubricytes	16.65 ± 2.26	-	-	-
Total erythroid series	55.73 ± 2.54			
Myeloid: erythroid ratio	0.73 ± 0.08			
Lymphocytes	1.88 ± 0.59	-	-	+ or -
Plasma cells	0.24 ± 0.15	-	-	-
Monocytes	0.60 ± 0.28	+ or -	+ or -	+ or -
Megakaryocytes	0.32 ± 0.20	-	-	+
Mitotic cells	0.48 ± 0.41	-	-	-

+ = positive reaction; - = negative reaction; + or - = weak reactivity was observed in few cells while others were negative

Members of the granulocytic series were Sudan black B positive (Fig. 3), except for myeloblasts, which were negative. Neutrophilic granulocytes showed increasing sudanophilia as they matured. Eosinophilic and basophilic granules exhibited variable reactions, some being positive and others negative. Some monocytes were positive with a few scattered black granules, while the others were negative. Cells of erythroid, lymphoid and megakaryocytic series were Sudan black B negative. Similar Sudan black B reactions were observed in smears stored for one month at - 4 ^{0}C.

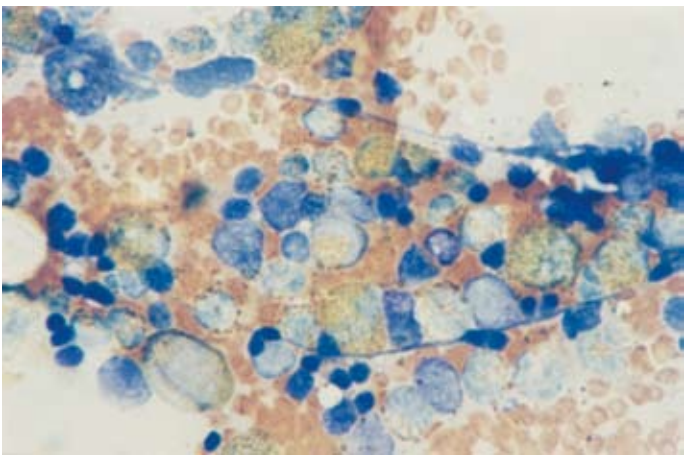

Fig. 2. Very weak peroxidase activity in granulocytes after one month of storage of bone marrow smear (Counter-stained with May-Grünwald-Giemsa stain; ×1000)

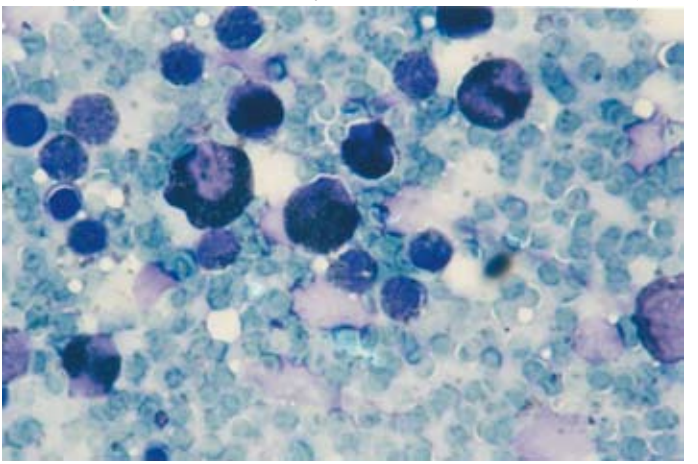

Fig. 3. Sudan black B positive granulocytes in bone marrow smear (Counter-stained with May-Grünwald-Giemsa stain; ×1000)

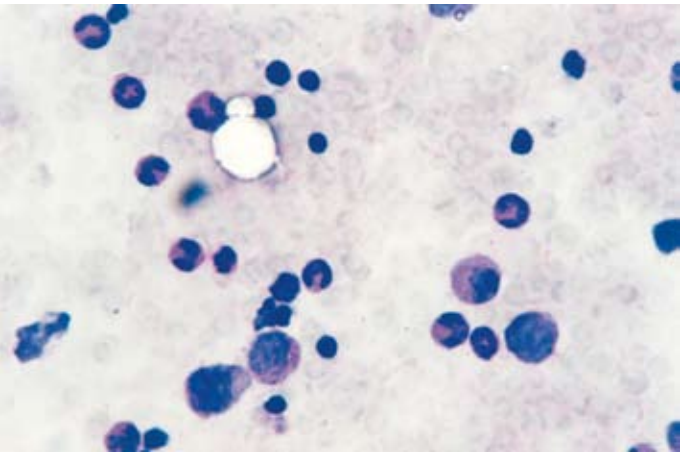

Fig. 4. Periodic acid - Schiff positive bone marrow smear cells (Counter-stained with May-Grünwald-Giemsa stain; ×1000)

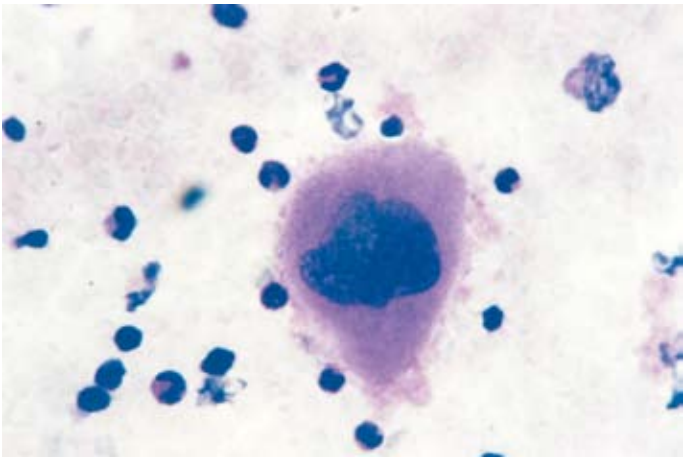

Fig. 5. Megakaryocyte with positive periodic acid - Shiff reaction (Counter stained with May-Grünwald-Giemsa stain; ×1000)

Granulocytes at all stages of development reacted positively to PAS (Fig. 4), except myeloblasts. Promyelocytes and myelocytes contained a few positively-staining granules but the cytoplasm stained diffusely pale pink. Metamyelocytes, band cells and segmented neutrophils became progressively more positive due to dense, coarse and

closely placed granules. Eosinophils contained finely granular PAS-positive material that did not involve the specific granules. Some of the lymphocytes showed a few fine or even coarse PAS-positive granules arranged in rings parallel to the periphery of the cytoplasm. Monocytes were either completely negative or positive with fine or coarse red granules. Megakaryocytes were PAS-positive, showing a diffuse cytoplasmic activity, with occasional large PAS-positive granules (Fig. 5). All cells of erythrocytic series were PAS-negative. The storage of bone marrow smears had no effect on the intensity of PAS stain.

The mean, standard deviation and range values of the percentage of May-Grünwald-Giemsa stained and positively reacting bone marrow cells to different cytochemical stains are summarized in Table 2. The mean values of the percentage of positive cells and May-Grünwald-Giemsa stained bone marrow granulocytes were almost similar

Table 2. Mean values of positively reacting bone marrow cell to different cytochemical stains in clinically normal sheep

Type of stain	Mean ± SD (%)	Range (%)
May-Grünwald-Giemsa	40.75 ± 2.95*	36 - 44*
Peroxidase	39.25 ± 4.26	33 - 45
Sudan black B	40.25 ± 5.12	32 - 45
Periodic acid-Schiff	40.75 ± 1.92**	39 - 44**

*Values of myeloid series in May-Grünwald-Giemsa stained smears; **Megakaryocytes were not included

Discussion

The mean values of bone marrow differential cell count and myeloid to erythroid ratio were within ranges established previously for clinically normal Libyan Barbary sheep (AL IZZI et al., 2005).

Cytochemistry is the use of special stains in the microscopic examination of cellular constituents, such as enzymes, lipids and carbohydrates (LI and YAM, 1994). These stains may distinguish normal cell types and identify the lineage of poorly differentiated blast cells.

In this study, cytochemistry of bone marrow cells in 15 adult Libyan Barbary sheep was evaluated using peroxidase, Sudan black B and PAS stains.

All cells of the granulocytic series, except myeloblasts and basophils, were peroxidase positive. Peroxidase positive reaction was observed in human bone marrow granulocytes (DACIE and LEWIS, 1975; SONNENWIRTH and JARETT, 1980). Auer rods in leukemic

blasts are nearly always peroxidase positive. This positive reaction is useful in the investigation of acute leukemia (DACIE and LEWIS, 1975). Peripheral blood granulocytes, except basophils, in dogs, cats, cattle and horses were peroxidase positive (RASKIN and VALENCIANO, 2000). Very few sheep bone marrow monocytes showed weak peroxidase activity similar to human monocytes. Weak peroxidase activity was observed in bovine bone marrow monocyte, but the incubation period used was two minutes instead of one minute (AL IZZI et al., 1982). Erythrocytic, lymphocytic and megakaryocytic series cells of Libyan Barbary sheep were peroxidase negative. Similar findings were recorded in human bone marrow (DACIE and LEWIS,1975; SONNENWIRTH and JARETT, 1980).

Sudan black B is used to stain the granules of leukocytes, many of which appear to contain phospholipids. There is a close parallelism between sudanophilia and a positive peroxidase reaction (DACIE and LEWIS, 1975). In the present study, granulocytic series cells, except myeloblasts, were Sudan black B positive. Similar findings were observed in human bone marrow granulocytes (SONNENWIRTH and JARETT, 1980). In animals, peripheral blood neutrophils and eosinophils were Sudan black B positive, while basophils showed weak activity in cattle. In dog, cat and horse basophils were Sudan black B negative (RASKIN and VALENCIANO, 2000). Some sheep monocytes were Sudan black B positive while others were negative. These results were in agreement with those recorded in humans (SONNENWIRH and JARETT, 1980) and animals (RASKIN and VALENCIANO, 2000). The erythrocytic, lymphocytic and megakaryocytic series cells of sheep examined in this study were Sudan black B negative. These cells in humans were also Sudan black B negative (SONNENWIRTH and JARETT, 1980).

In most instances of acute myeloblastic leukemia the leukemic myeloblasts show the granular type of sudanophilia normally seen in the more mature forms of the granulocytic series. This sudanophilia, which probably represents a form of nuclear-cytoplasmic dissociation in leukemic cells, may be helpful in the identification of myeloblasts, which may not be clearly identifiable by Romanowsky stains. Auer bodies are Sudan black B positive (SONNENWIRTH and JARETT, 1980).

In blood cells, positive PAS reaction usually indicates the presence of glycogen (DACIE and LEWIS, 1975). In this study, bone marrow granulocytic series cells, except myeloblasts, reacted positively to PAS. A similar reaction was observed in human bone marrow granulocytes (SONNENWIRTH and JARETT, 1980). Peripheral blood neutrophils in dog, cat, cow and horse were PAS positive, whereas eosinophils and basophils showed weak activity (RASKIN and VALENCIANO, 2000). Some lymphocytes and monocytes of sheep examined here were PAS positive. These findings were in agreement with those reported for human bone marrow and animal peripheral blood leukocytes (DACIE and LEWIS, 1975; SONNENWIRTH and JARETT, 1980; RASKIN and VALENCIANO, 2000). Megakaryocytes and thrombocytes were PAS positive. A similar observation was

recorded in humans (DACIE and LEWIS, 1975; SONNENWIRTH and JARETT, 1980) and animals (RASKIN and VALENCIANO, 2000).

The sensitivity of bone marrow cells peroxidase, Sudan black B and PAS to storage was monitored. It appeared that peroxidase activity of bone marrow granulocytes diminished significantly, while Sudan black B and PAS reactions were not affected after one month of storage at - 4 ^{0}C. Similarly, RASKIN and VALENCIANO (2000) reported that peroxidase activity was sensitive to storage, while sudanophilic materials within cells and PAS stain were stable in storage.

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SAŽETAK

Istraživanje je provedeno na uzorcima koštane srži 15 klinički zdravih libijskih berberskih ovaca. Hemopoetske stanice, uključujući one mijeloične, eritrocitne i megakariocitne linije, limfociti, plazma stanice, monociti i mitotičke stanice bile su identificirane na osnovi njihovih morfoloških značajki u razmascima koštane srži obojenima po May-Grünwald-Giemsi. Citokemijske reakcije stanica koštane srži pokazale su da su svi granulociti, osim mijeloblasta i bazofila, bili pozitivni na peroksidazu. Monociti su bili negativni na peroksidazu, iako je vrlo malo njih pokazivalo slabu aktivnost. Stanice eritrocitne, megakariocitne i limfocitne linije bile su peroksidaza negativne. Stanice granulocitne linije bile su pozitivne na Sudan crnilo B, osim mijeloblasta. Neki monociti bili su pozitivni s nekoliko diseminiranih crnih granula, dok su drugi bili negativni. Stanice eritrocitne, megakariocitne i limfocitne linije bile su Sudan crnilom B negativne. Svi razvojni oblici granulocita reagirali su pozitivno na oksidaciju perjodnom kiselinom i bojenjem Shiffovim reagensom (PAS), osim mijeloblasta. Monociti su bili, ili negativni ili pozitivni. Neki limfociti pokazivali su nekoliko sitnih ili čak krupnih granula pozitivnih na PAS. Megakariociti su bili pozitivni na PAS, dok su sve stanice eritrocitne linije bile tim bojenjem negativne. Ustanovljena je sukladnost srednjih vrijednosti postotaka pozitivnih stanica obojenih trima citokemijskim bojenjima i broja granulocita koštane srži obojenih po May-Grünwald-Giemsi.

Ključne riječi: ovca, koštana srž, citokemija, peroksidaza, Sudan B crnilo, oksidacija perjodnom kiselinom, Shiffovo bojenje