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Enzyme Histochemistry of the Peripheral Blood Lymphocytes in Arabian Horses

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Abstract: The aims of this study were to determine the percentages of A-Naphthyl Acetate Esterase (ANAE) positive and Acid phosphatase (Acp)-positive Peripheral Blood Lymphocytes (PBL) and the proportion of peripheral blood lymphocytes in the Arabian race horses. Blood samples obtained from the Vena jugularis of 40 healthy Arabian race horses at four years old were analyzed. The animals were selected randomly from Sanliurfa region. Mean percentages of ANAE-positive PBL were determined as 76%, whereas mean percentages of ACP-positive PBL were determined as 28.46%. Proportion of the peripheral blood lymphocytes was also found as 46.20%. In conclusion, the enzyme histochemical techniques are simple, inexpensive and well performed for laboratory conditions. Also, these techniques may help early diagnosis of different diseases. Further studies about enzyme histochemistry in Arabian race horses should be performed.

Key words: α-Naphthyl acetate esterase, acid phosphatase, Arabian horses, PBL, blood hyphocytes

INTRODUCTION

Enzyme histochemical techniques have practical value for the study of immature blast cells and lymphocytes, because Romanowsky-stained films might be insufficient for identifying the differentiation and maturation characteristics of the cells (Catowsky, 1991). α-Naphthyl Acetate Esterase (ANAE) and Acid Phosphatase (ACP) activities have been used as cytochemical markers to identify lymphocytes (Osbaldiston and Sullivan, 1978; Sur *et al*., 2004). α-Naphthyl acetate esterase is a lymphocyte lysosomal enzyme (Knowles *et al*., 1978) that has been demonstrated in mature and immunocompetent T lymphocytes. The positivity of ANAE has widely been investigated in various species, including chickens (Asti *et al*., 1999; Maiti *et al*., 1990), ostriches (Ergun *et al*., 2004a), Turkeys (Ergun *et al*., 2004b), painted storks (Salakij *et al*., 2003), rock partridges (Donmez and Sur, 2008), pheasant (Sur *et al*., 2004), camels (Sandikci *et al*., 2005), sheep (Sur, 2004), gazelles (Altunay *et al*., 2008), cats (Yoruk *et al*., 1998), dogs (Sur *et al*., 2003) and Angora rabbits (Ozcan, 2005).

The ANAE technique is useful in differentiating granulocytic, monocytic and lymphocytic leukemias (Osbaldiston and Sullivan, 1978) and has also been used to detect these cells in malignant nonlymphoid human tumors (Svennevig, 1980). The enzyme is possibly responsible for the cytotoxic effects of T lymphocytes and the phagocytic activity of monocytes (Mueller *et al*., 1975). Acid phosphatase is a member of the acid hydrolases.

The enzymatic activity is gained at early stages of T-lymphocyte maturation in the human thymus (Basso *et al*., 1980). However, ACP is considered to be related to B-lymphocyte maturation and is accepted as a B-cell marker in the chicken (Graczyk, 1987) because the enzyme is commonly present in lymphocytes originating from the bursa of Fabricius.

The enzyme has diagnostic value in the differential diagnosis of lymphoproliferative disorders (Wehinger and Mobius, 1976) and its reaction increases significantly in most types of acute and chronic T-cell lymphoproliferation in humans (Catowsky, 1991). Some historical information declares that Arabian horse was raised in Mesopotamia and around 3000 years before Christ (Altinsaat, 2008). Sanliurfa is a city located in Mesopotomia. Arabian race horse breeding has a great economical importance in Sanliurfa region. Sanliurfa has one hippodrome for race horses, so breeding of race horses is considered as an industry in this city.

The aims of this study were to determine the percentages of ANAE- and ACP-positive PBL and proportion of the Peripheral Blood Lymphocytes (PBL) in the Arabian horses.

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MATERIALS AND METHODS

Blood samples obtained from the jugular vein of 40 healthy arabian race horses at four years old were analyzed. The animals were selected randomly from Sanliurfa region. Two blood smears for each staining procedure from each horse were prepared and air-dried. Two blood smears from each animal were stained with May-Grünwald-Giemsa stain according to a standard staining method (Konuk, 1981).

ANAE Histochemistry: To determine ANAE activity, blood smears were fixed in a glutaraldehyde-acetone solution at -10°C for 3 min, rinsed in distilled water and then air-dried. An incubation solution was prepared by mixing 20 mg of substrate, α-naphthyl-acetate (N-8505, Sigma, Steinheim, Germany) dissolved in 0.8 mL of acetone (Merck, Darmstadt, Germany), 4.8 mL of hexazotized pararosaniline [hexazotization was performed by mixing equal volumes (2.4 mL each) of 4% sodium nitrite (Merck) and 2% pararosaniline (Merck)] and 80 mL of PBS (pH 5). Final pH of the incubation solution was adjusted to 5.8 with 1 N NaOH and the solution was filtered. After a 2 h incubation at 37°C, the smears were rinsed 3 times in distilled water and nuclei were stained for 20 min in 1% methyl green prepared in acetate buffer (pH 4.2). Control specimens were prepared by incubating the smears in incubation solution without α-naphthyl acetate (Celik *et al*., 1991).

ACP Histochemistry: Acid phosphatase was demonstrated by the method of Sur (2004) with minor modifications. In this technique, blood smears were fixed in formal-calcium at +4°C for 10 min and the smears were rinsed 3 times in distilled water. An incubation solution was prepared by mixing 10 mg of naphthol AS-BI phosphate (N-2125, Sigma) dissolved in 1 mL of N,N dimethyl formamide (Sigma), 13 mL of distilled water and 1.6 mL of hexazotized pararosaniline (prepared as in the ANAE incubation solution) and 5 mL of Michaelin's veronal acetate buffer (pH 5). Final pH of the solution was adjusted to 5.0 with 1 N NaOH and the solution was filtered. After a 2 h incubation at 37°C, the slides were rinsed 3 times in distilled water and the nuclei were stained for 20 min with 1% methyl green prepared in acetate buffer (pH 4.2). The incubation solution for the control smears did not contain naphthol AS-BI phosphate.

All specimens were examined under a light microscope (Olympus BX51 system microscope, Olympus DP71 microscope digital camera). The May Grünwald Giemsa-stained smears were used to determine lym phocytes proportions. In each of the specimens demonstrating ANAE and ACP, 200 lymphocytes were counted and positivity rates were expressed as the percentage of counted cells.

RESULTS AND DISCUSSION

In the blood smears examined, most of the Peripheral Blood Lymphocytes (PBL) staining ANAE positive showed 1-3 dot-like brown granules, situated in the cytoplasm (Fig. 1). Mean ANAE-positive of the PBL was found as 76%. Enzymatic reaction has not been displayed in control specimens.

The PBL having 1-2 pinkish cytoplasmic granules were considered as ACP positive. Significant number of PBL has shown ACP positivity (Fig. 2). The percentage

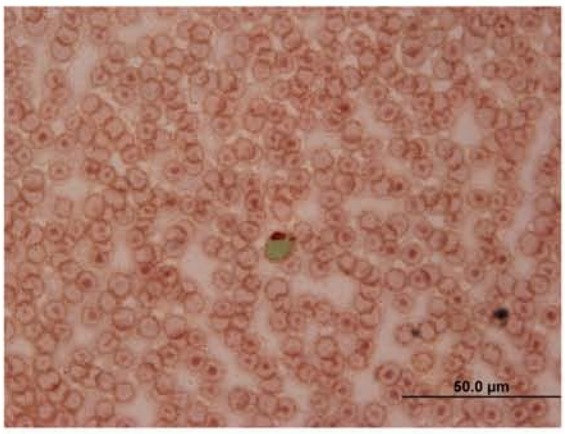

Fig. 1: An ANAE positive peripheral blood lymphocyte with brown granules, situated in the cytoplasm

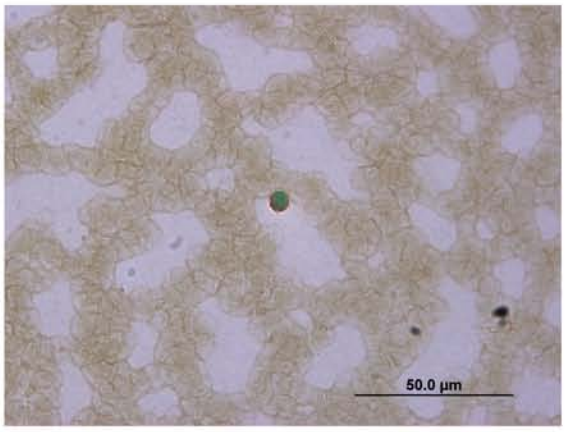

Fig. 2: An ACP positive lymphocyte with pinkish cytoplasmic granules

of ACP cells was smaller thanthat of ANAE positive cells. Mean percentage of the ACP-positive PBL was determined as 28.46%. Enzymatic reaction has not been displayed in control specimens. Proportion of the peripheral blood lymphocytes was also found as 46.20%. Enzyme histochemical tests have largely been used to identify mature T lymphocytes to differentiate types of leukocytes and leukemia cells (Inoue *et al*., 1991) and some hematological diseases (Kulenkampff *et al*., 1977; Sur *et al*., 2003). In the lymphoid leucosis of cattle (Kajikawa *et al*., 1983), in Marek's disease in chickens (Jeurissen *et al*., 1989) and in distemper in dogs (Sen *et al*., 2002), significant changes have been reported either in PBL percentages or in their enzymatic positivity rates. Sen *et al*. (2002) determined changes in the enzymatic positivity profile of PBL through the development of experimentally induced canine distemper virus infections, in which a prominent decrease was observed in the percentages of ANAE and ACP.

Izci *et al*. (2002) have monitored the effects of ophthalmically administered 2% cyclosporine on the total percentage and ANAE positivity rates of PBL of the dogs with keratoconjunctivitis sicca. Baseline levels of the dogs were 56.17% for total lymphocytes and 71.67% for ANAE positive lymphocytes. The researchers (Izci *et al*., 2002) have revealed that the total percentage and ANAE positivity of PBL gradually decreased (22.67% and 36%, respectively) in 2% cyclosporine treated dogs at 60th day of the experiment.

Donmez *et al*. (2002) reported that 54.4% of the PBL were positive for ANAE in healthy chickens. Sur *et al*. (2004) reported 36.82 and 33.73% ANAE-positive PBL for young and adult pheasants, respectively. α-Naphthyl acetate esterase-positive PBL values of 51.8% in Turkeys (Ergun *et al*., 2004b) and 59.3% in ostriches (Ergun *et al*., 2004a) have also been reported. The mean percentage of ANAE cells in the horse blood was 68% (Krumrych *et al*., 1995). In the current study, we have found a higher percentage of ANAE-positive lymphocytes than that of Krumrych *et al*. (1995)'s findings (76 and 68%, respectively). The difference can be originated from the differences of the ages, breeding and environmental conditions of the animals.

ACP-ase is of diagnostic value in the differential diagnosis of lymphoproliferative disorders (Wehinger and Mobius, 1976). Strong ACP-ase activity in B-cell prolymphocytic leukaemias is also found and one-third of the cells show a positive ACP-ase reaction (Catowsky *et al*., 1974). ACP-ase activity also increases after lymphocyte transformation (Catowsky, 1991; Goldberg and Barka, 1962) with Phytohemagglutinin (PHA) and during the transition from monocyte to tissue macrophage (Kaplow and Burstone, 1964). Studies of ACP in equine species are very few. Several studies have been performed at several animal species. The proportion of ACP-positive lymphocytes was approximately 32% in sheep (Sur, 2004) and 39% in Kangal dogs (Sur *et al*., 2003). In this study, ACP-ase positivity rates of PBL in the horses were determined as 28.46%. Similarly, Krumrych *et al*. (1995) have reported 25% positivity rate for ACP-ase in 15 healthy horses.

The proportions of lymphocytes in rock partridges were approximately 47, 49 and 46% at hatching, 5 and 12 weeks of age, respectively (Donmez and Sur, 2008), 45.2% in painted storks (Salakij *et al*., 2003); 56 and 52.9% in young and adult pheasants, respectively (Sur *et al*., 2004). In this study, we determined that the proportion of lymphocytes in the Arabian race horses was 46.20%. Knill *et al*. (1969) have also reported that the percentage of the Peripheral Blood Lymphocytes (PBL) in the Arabian horses were 45.14%.

CONCLUSION

The enzyme histochemical techniques are simple, inexpensive and well performed for laboratory conditions. Also, these techniques may help early diagnosis of different diseases. Further studies about enzyme histochemistry in Arabian race horses should be performed.

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