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Research Article

Determination of Alpha Naphthyl Acetate Esterase Activity in the Peripheral Blood Leukocytes in Angora Rabbits

Ziya ÖZCAN
Department of Histology Embryology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY

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Abstract: The aim of this study was to determine the alpha naphthyl acetate esterase (ANAE) activity in the peripheral blood leukocytes in Angora rabbits.

The heparinized blood samples obtained from 10 healthy adult Angora rabbits were used. ANAE stainings were applied at different pH values for the determination of ANAE activities.

The ANAE positive reactions were observed in the T lymphocytes, monocytes, neutrophils, eosinophils and platelets. The ANAE positive lymphocyte rate was found to be 68.2%.

Key Words: Leukocyte, ANAE activity, Angora rabbit

Ankara Tavşanlarının Perifer Kan Lökositlerinde Alfa Naftil Asetat Esteraz Aktivitesinin Belirlenmesi

Özet: Bu çalışma Ankara tavşanlarının perifer kan lökositlerinde alfa naftil asetat esteraz aktivitesinin belirlenmesi amacıyla yapıldı.

Çalışmada materyal olarak 10 adet sağlıklı ve erişkin Ankara tavşanlarından alınan heparinize kan örnekleri kullanıldı. ANAE aktivitesinin belirlenmesi için değişik pH' larda ANAE boyaması uygulandı.

İncelenen preparatlarda T lenfosit, monosit, nötrofil ve eozinofil granulositler ile kan pulcuklarında ANAE'ye karşı pozitif reaksiyona rastlandı. ANAE pozitif lenfosit oranı % 68,2 olarak saptandı.

Anahtar Sözcükler: Lökosit, ANAE aktivitesi, Ankara tavşanı

Introduction

The peripheral blood leukocytes are classified as T or B lymphocytes depending on their immunological properties (1,2). Although it is possible to differentiate the T lymphocytes and B lymphocytes by E rosette and EAC rosette techniques, respectively, these techniques are expensive, time consuming and are not applicable to tissue sections (2). For these reasons, investigators have directed their studies on enzyme activities (3-6). In recent years, several studies have been reported on the presence of alpha naphthyl acetate esterase (ANAE), a nonspecific esterase, activity in T lymphocytes but not in B lymphocytes (3,5,7-9).

The staining properties of the peripheral blood lymphocytes to ANAE have been shown to vary depending on the pH of the stain in humans and various animal species (5,8,10). The optimal staining pH values have been reported to be 5.8 in humans (8), 6.4 in pigs (11), 5.0 in rats (6), 6.5 (12), 5.8 (13) in dogs, 7.2 (14), 5.8 (13) in chicken, 5.8 in sheep and goats (13), 6.2 in horses and cattle and 6.4 in cats (13).

It has been shown that ANAE positive reaction of T lymphocytes appear as 1-2 localized granules, while B lymphocytes give negative reaction with monocytes showing diffuse staining in the peripheral blood of humans and other animals (3,6,7,11,13). The rates of T lymphocytes have been found to be 60% in humans (8), 64.2% in cattle (9) and 56% in chickens in the peripheral blood by ANAE staining (14).

The neutrophil granulocytes were reported to be stained negatively by ANAE, in humans (3,5,9), pigs (12), dogs (13), cats (12,13), and horses (13), while those of rats (12,13), guinea pigs (12), sheep and goats (12,13), and cattle (13) gave positive reactions.

In eosinophil granulocytes the stainings against ANAE were negative in humans (3,15), rats, cats, sheep and goats (12), and positive in dogs (12), cattle, sheep and goats (13).

In platelets of cats, horses, cattle, sheep and goats and the thrombocytes of chicken, ANAE stainings were negative and in contrast, the strong granular positive reactions were reported in rats and dogs (13).

The aim of this study was to determine the alpha naphthyl acetate esterase activities in the peripheral blood leukocytes in Angora rabbits.

Materials and Methods

The heparinized blood samples taken from 10 healthy Angora rabbits were used as materials in this study. The smears were prepared from the heparinized blood samples from each animal and dried at room temperature. Then, they were fixed in glutaraldehyde-acetone fixative at -10 ºC for 3 minutes. Afterwards, smears were washed 3 times in distilled water and dried at room temperature.

To determine the ANAE activity, an incubation solution prepared by adding to a 40 ml portion of an 0.067 M phosphate buffer (pH 5.0) 2.4 ml of hexazotized pararosanilin solution and a 0.4 ml acetone in which 10 mg of alpha naphthyl acetate had been dissolved, was used (6). The incubation solutions were adjusted to 7 different pH values using a 2N NaOH as 5.0, 5.4, 5.8, 6.2, 6.4, 6.8, and 7.2 and for each pH value 3 hourly stainings were carried out and smears were counterstained with methylene blue for 10 minutes. The positive lymphocyte rates were determined by counting 300 lymphocytes in every smear (x 40 magnification).

Results

ANAE positive stainings were observed in the lymphocytes at different pH values, however, the best reaction was noted at pH 5.8. A greater proportion of the ANAE positive lymphocytes was observed to contain 1-2 specific granules (Figure 1, arrows). In some lymphocytes, the granule counts were noticeably higher (Figure 2, arrows).

The results of the ANAE stainings in lymphocytes are shown in Table. The ratio of the ANAE positive lymphocytes was 68.2% while that of negatively staining ones were found to be 31.8%.

The monocytes were observed to exhibit diffuse staining at all pH values, with the strongest reaction recorded at pH 6.2 (Figure 3, arrow).

The granular positivity was observed in the neutrophil (Figure 4, arrows) and eosinophil granulocytes at all pH values applied. The best reaction, however, was at pH 6.2.

Also, in the platelets, the granular positivity was observed at all pH values (Figure 5, arrows). The strongest reaction was noted at pH 6.2.

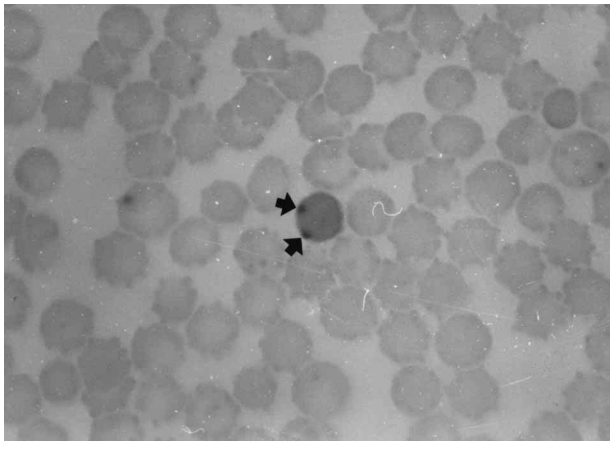

Figure 1. ANAE staining (pH 5.8) in Angora rabbit. Arrows: ANAE positive granules in T lymphocyte. ANAE x 1280.

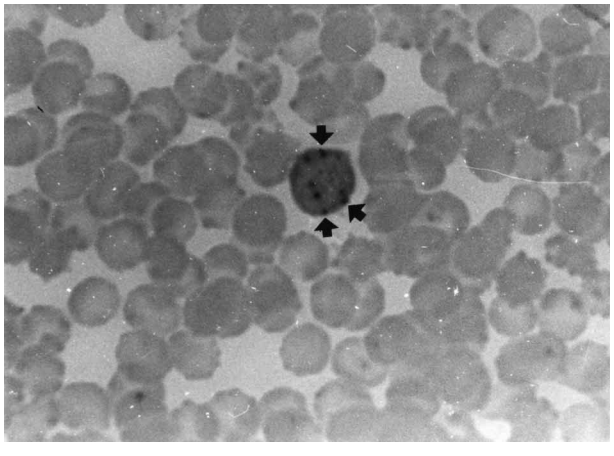

Figure 2. ANAE staining (pH 5.8) in Angora rabbit. Arrows: ANAE positive granules in T lymphocyte. ANAE x 1150.

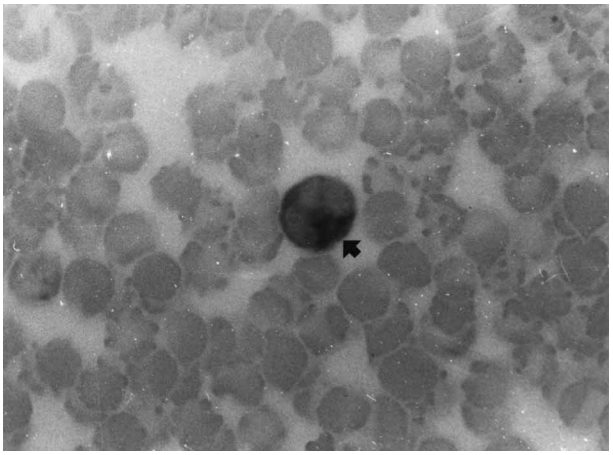

Figure 3. ANAE staining (pH 6.2) in Angora rabbit. M: diffuse ANAE positive reaction in monocyte. ANAE x 1550.

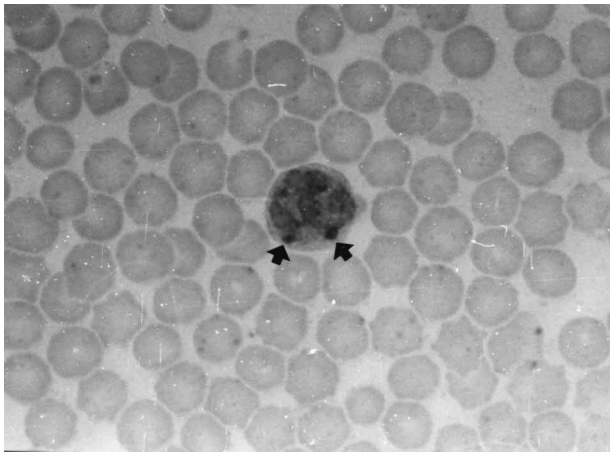

Figure 4. ANAE staining (pH 6.2) in Angora rabbit. N: ANAE positive granules in neutrophil granulocyte (arrows). ANAE x 980.

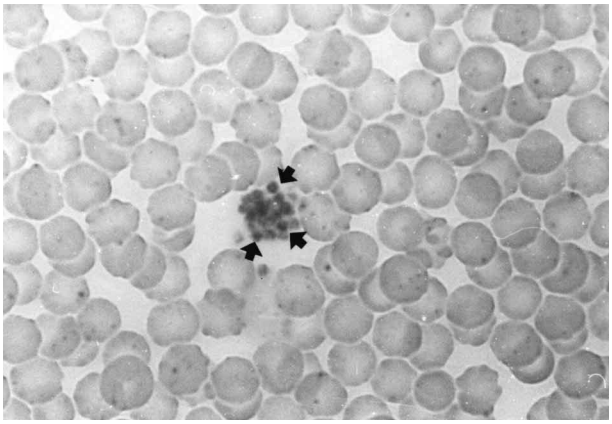

Figure 5. ANAE staining (pH 6.2) in Angora rabbit. Arrows: ANAE positive reaction in platelets. ANAE x 1150.

Table. ANAE stainings in the peripheral blood lymphocytes in Angora rabbits.

Number of animals	Lymphocyte (%)	
	ANAE positive	ANAE negative
10	68.2	31.8

Discussion

Several investigators have worked on the lysosomal enzymes to identify the peripheral blood lymphocytes (3,4,6,13). In recent years, alpha naphthyl acetate esterase has been reported to be present in T lymphocytes but not in B lymphocytes (3,5,8,16).

In the studies conducted on the peripheral blood T lymphocytes of humans (8,10,15), positive reactions in the T lymphocytes have been reported to present as single red granules with ANAE staining. In this study, also, as mentioned above by other workers, ANAE positive reaction was encountered as 1-2 granules, however, in some lymphocytes the number of granules demonstrating positive reaction was noted to be more than two.

In some studies, the best reaction for ANAE positivity has been reported to be at pH 5.8 in the peripheral blood of humans (5,8,10), pH 8 (3), at pH 6.4 in swine (11), at pH 6.5 (12), pH 5.8 (13) in dogs, at pH 7.2 (14), pH 5.8 (13) in chicken, at pH 5.8 in sheep and goats, at pH 6.2 in cattle and horse, at pH 6.4 in cats (13). In this study the best reaction of the lymphocytes against ANAE staining was obtained at pH 5.8. These findings are in agreement with those reported by other workers in humans (5,8,10), chicken, dogs, sheep and goats (13). However, it does not seem to agree with the findings reported by other authors in humans (3), dogs (12), chicken (14), and horses and cattle (13).

In studies conducted in humans, the ratio of ANAE positive T lymphopcytes in the peripheral blood has been reported to be 85% (8), 70% (5), and 63% (10). This ratio has been reported as 68% in cattle (16), and 56% in chicken (14). In this study the ANAE positive lymphocyte ratio was found to be 68.2%. The ratio is in accordance with those of humans and cattle, but considerably higher than chicken.

Also, in monocytes, staining was observed to be diffuse at all pH values. The findings obtained from monocytes are also in accordance with those of other authors (3-5,7,13).

Against ANAE staining the neutrophil granulocytes have been reported to give negative reactions in humans (3,9,12,15), swine (12), cattle (9), cats (12,13), dogs and horses (13) but positive in rats, guinea pigs (12), sheep and goats (12,13), while others have been reported to give positive staining in cattle (13). In this study, a positive reaction to the ANAE stain was observed in the neutrophil granulocytes. These findings supports the findings by other investigators in rats, guinea pigs, sheep, goats, and cattle as mentioned above, while it contrasts with those obtained from humans, swine, cats and dogs.

In eosinophil granulocytes negative reactions against ANAE have been reported for humans (3,5,9,15), cattle (9), rats, cats, sheep and goats (12) and positive reactions for dogs (12,13), rats, horses, cattle, sheep and goats (13). Since positive reactions are observed in this study, our findings are not in accordance with those reporting negative reactions in humans (3,5,9,15) rats, sheep and goats (12), and cattle (9).

In contrast to the negative staining of the platelets in the cats, horses, cattle, sheep and goats to ANAE, positive reactions have been reported in rats and dogs (13). The results obtained from this study seems similar to those of obtained by other workers studied rats and dogs.

According to the results obtained from this study, it was concluded that the peripheral blood leukocytes of the Angora rabbits gave the best reaction against the ANAE stain at pH 5.8 for lymphocytes, and pH 6.2 for monocytes, neutrophils and eosinophil granulocytes and platelets, and the peripheral T lymphocytes could be determined specifically by ANAE demonstration.

References

1. Barret, T.J.: Basic immunology and its medical application. The C.V. Mosby Company, London. 1980; 48-65.
2. Fudenberg, H., Stites, D., Caldwell, J., Wells, V.: Basic clinical immunology. Lang Medical Publication, California. 1978; 78-95.
3. Higgy, K.E., Burns, G.F., Hayhoe, F.G.: Discrimination of B, T and Null lymphocytes by esterase cytochemistry. Scand. J. Haematol., 1977; 18: 437-448.
4. Kajikawa, O., Koyama, H., Yoshikawa, T., Tsubaki, S., Saito, H.: Use of Alpha-naphthyl acetate esterase staining to identify T lymphocytes in cattle. Am. J. Vet. Res., 1983; 44: 1549-1552.
5. Knowles, D.M., Hoffman, T., Ferrarini, M., Kunkel, H.G.: The demonstration of acid alpha naphthyl acetate esterase activity in human lymphocytes usefulness as a T cell marker. Cell. Immun., 1978; 35: 112-123.
6. Mueller, J., Brundel, Re, G., Buerki, H., Keller, H.U., Hess, M.W., Cottier, H.: Nonspecific acid esterase activity: A criterion for differentiation of T and B lymphocytes in mouse lymph nodes. Eur. J. Immunol., 1975; 5: 270-274.
7. Basso, G., Cocito, M.G., Samenzato, G., Pezzutto, A., Zanesco, L.: Cytochemical study of thymocytes and T lymphocytes. Br. J. Haematol., 1980; 44: 577-582.
8. Mueller, J., Keller, H.U., Hagmann, J.D., Cornidey, R.J., Ruchti, C., Cottier, H.: Nonspecific esterase in human lymphocytes. Int. Arch. Allergy. Appl., 1981; 64: 410-421.
9. Yang, T.J., Jantzen, P.A., Williams, L.F.: Acid alpha naphthyl acetate esterase: presence of activity in bovine and human T and B lymphocytes. Immunology, 1979; 38: 85-92.
10. Kulenkampff, J., Janossy, G., Greaves, M.F.: Acid esterase in human lymphoid cells and leukaemic blasts: A marker for T lymphoctyes. Br. J. Haematol. 1977; 36: 231-240.
11. Ramos, J.A., Ramis, A.J., Marco, A., Domingo, M., Rabanal, R., Ferrer, L.: Histochemical and immunohistochemical study of the mucosal lymphoid system in swine. Am. J. Vet. Res., 1992; 53: 1418-1426.
12. Osbaldiston, G.W., Sullivan, R.J.: Cytochemical demonstration of esterases in peripheral blood leucocytes. Am. J. Vet. Res., 1978; 39: 683-685.
13. Aştı, R.N., Alabay, B., Kurtdede, N., Altunay, H., Ergün, L.: Farklı hayvan türlerinin perifer kan lökositlerinde alfa naftil asetat esteraz aktivitesinin belirlenmesi. Ankara Üniv. Vet. Fak. Derg., 1996; 43: 129-133.
14. Maiti, N.K., Saini, S.S., Sharma, S.N.: Histochemical studies on chicken peripheral blood lymphocytes. Vet. Res. Commun., 1990; 14: 207-210.
15. Knowles, D.M., Holck, S.: Tissue localization of T- lymphocytes by the histochemical demonstration of acid alpha-naphthyl acetate esterase. Lab. Invest., 1978; 39: 70-76.
16. Paul, P.S., Senogles, D.R., Muscoplat, C.C., Johnson, D.W.: Enumeration of T cells, B cells and monocytes in the peripheral blood of normal and lymphocytotic cattle. Clin. Exp. Immunol., 1979; 35: 306-316.