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EOSINOPHIL GRANULOCYTES AND PLASMA CELLS IN JEJUNAL MUCOSA OF DOGS NATURALLY INFECTED WITH OR WITHOUT INTESTINAL PARASITES

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Barsak parazitleri ile doğal enfekte ve enfekte olmayan köpeklerin jejunum mukozasında eozinofil granulositler ve plazma hücreleri

Özet: *Bu araştırma, barsak parazitleri ile doğal enfekte ve enfekte olmayan köpeklerin jejunum mukozasında eozinofil granulositler ve plazma hücrelerinin dağılımlarının belirlenmesi amacıyla gerçekleştirildi. Ayrıca perifer kanda total lökosit ve eozinofil granulosit sayıları da belirlendi. Araştırmada, barsak parazitleri ile doğal enfekte oldukları belirlenen 7 ve gaitasında parazit belirlenemeyen 4 adet olmak üzere toplam 11 adet, belirgin bir ırk özelliği göstermeyen köpek kullanıldı.*

Köpeklerin jejunumundan doku örnekleri operatif olarak alındı. Doku örneklerinin tespiti için %10 tamponlu nötr formalin kullanıldı. Kesitler eozinofil granulositlerin demonstrasyonu için Congo red, plazma hücrelerinin demonstrasyonu için metil green pyronin ile boyandılar. Her iki hücre de jejunal mukozada villus- kript ünitede sayıldı. Perifer kan lökosit ve eozinofil granulosit konsantrasyonları standart tekniklerle hemositometrede gerçekleştirildi.

İncelenen doku kesitlerinde eozinofil granulositlerin lamina propriya'da özellikle villuslarda, kriptlerin bazal kısımlarında ve lamina subglandularis'te yoğun olarak bulundukları görüldü. Ayrıca genellikle kriptlerde ve nadiren villusların tabanında intraepiteliyal eozinofil granulositlerin bulunduğu gözlendi. Bazı eozinofil granulositlerin de barsak lumeninde bulundukları dikkati çekti. Pironinofilik hücrelerin özellikle villuslarda yoğun olarak bulundukları, daha az olarak da kript bölgesinde yer aldıkları gözlendi.

Barsak parazitleri ile enfekte olan ve gaitasında parazit belirlenemeyen gruplar arasında total lökosit ve kan eozinofil granulosit konsantrasyonları ile doku eozinofil granulositleri ve plazma hücreleri için elde edilen veriler karşılaştırıldığında, sadece enfekte grupta doku eozinofil granulosit sayılarının fazlalığı anlamlı bulundu ($p< 0.05$).

Anahtar kelimeler: *Eozinofil granulosit, intestinal parazitozis, jejunum, köpek, plazma hücresi*

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Summary: *This study was carried out to determine the distribution of eosinophil granulocytes and plasma cells in the jejunal mucosa of the dogs naturally infected or not infected with intestinal parasites. In addition, the total leukocyte and eosinophil granulocyte concentrations were determined in peripheral blood. For this purposes, 11 mongrel dogs were used where 7 of them were determined to be infected naturally with intestinal parasites, and in 4 of them there were no evidence of an actual intestinal parasitic infection.*

The tissue specimens of the jejunum were obtained by abdominal surgery from both groups. Specimens were fixed with 10% neutral buffered formalin. The tissue sections were stained with either Congo red for eosinofil granulocytes or methyl green pyronin for plasma cells. Tissue sections were examined by light microscopically. Both cells were counted in villus-crypt units of jejunal mucosa. The determination of the leukocyte and eosinophil granulocyte concentrations in peripheral blood were carried out from blood samples with standart technics.

It was observed that eosinophil granulocytes were located in lamina propria of jejunal mucosa, especially within villus intestinalis, basal parts of crypts and lamina subglandularis. Furthermore, eosinophil granulocytes were also located intraepithelially in crypts and rarely at the base of the villus. Some eosinophil granulocytes were also seen within intestinal lumen. The pyroninophilic cells were identified in jejunum. They were intensively prominent in villus intestinales, but they were found also in crypts in a lesser extent.

A t-Test for independent groups showed that the eosinophil granulocyte count in jejunal mucosa was higher in parasitized dogs than in jejunal mucosa of noninfected dogs ($p< 0.05$). But there were no significant differences in concentrations of leukocytes and eosinophil granulocytes in peripheral blood, as well as in the number of plasma cells in jejunal mucosa when compared the dogs with intestinal parasite with those showing no parasite in feces.

Key words: *Dog, eosinophil granulocyte, intestinal parasitosis, jejunum, plasma cells.*

Introduction

Eosinophil granulocytes are cells with numerous membrane-bound specific granules some of them having usually electron-dense cristalloid internum, crystalloid cores, also called "central core". These specific granules contain lysosomal enzymes as well as most of the cationic proteins unique to eosinophil granulocytes. The cristalloid core of the granule, when present, composed of major basic protein (MBP), and the noncore matrix contains eosinophil cationic protein (ECP), eosinophil peroxidase and eosinophil-derived neurotoxin (12, 31).

Eosinophil granulocytes defend against large, nonphagocytable organisms, most notably the multicellular helminthic parasites (6), fungal agents (20) and foreing proteins (4). One of the components of lysosomal granules of eosinophil granulocytes, the MBP, is a potent cytotoxin for certain parasites (28). Eosinophil granulocytes react to the helmiths when a sensitivity to the protein of the parasite has developed (allergic state) or the protein or secretory product of the parasite is released in the body. Initial binding of eosinophil granulocytes to parasitic targets can be mediated by antiparasite IgG or IgE antibodies or by C3b deposited on the surfaces of parasites

(38). Although single-celled protozoan parasites can be killed by eosinophil granulocytes, eosinophilia is heightened not by infections with protozoa except Isospora belli but rather by helminthic parasites (6). Eosinophil granulocytes can kill a wide number of helminthic parasites, especially in their larval stages (6, 21, 36). Although other cells can also kill such parasites, eosinophil granulocytes are particularly toxic to helminths for several reasons. First, the cationic proteins they deposite after binding to the surface of the parasite, especially MBP and ECP are potent helminthotoxins (1). Eosinophil peroxidase generates hypohalous acids that also kill parasites. Finally, eosinophil oxydative products also mediate helminthotoxicity (6, 45).

Plasma cells are regularly found in the lamina propria of the gastro-intestinal tract according to the demand for local antibody production (33). Investigations of the canine intestinal tract have shown the immunoglobulin (Ig)-positive cells in either small or large intestine (15, 18, 43). But, there is no difference in the number of plasma cells in different intestinal regions under normal circumstances (15).

In many parasitic disease of intestine both plasma cells and eosinophil granulocytes are prominent (8, 34, 35, 46). There is an interaction between these two cell types in opsonisation procedure of parasitic agents. Eosinophil granulocytes undergo exocytosis to expell their granular constituents when they come in close contact with an opsonised (antibody- and complement-coated) parasite (38).

In an another study in which the same animals were used, we found that the number of mast cells were significantly higher in naturally infected dogs compared with uninfected dogs (13). Also, the aim of the present study was to identify the distribution of eosinophil granulocytes and plasma cells within jejunal mucosa of the dogs naturally infected with intestinal parasites. In addition, the total leukocyte and eosinophil granulocyte concentrations were also determined in peripheral blood.

Materials and Methods

Animals

In this study, 11 mongrel dogs (6 females and 5 males) were used weighing 12-24 kg and age of 1 to 2,5. The dogs had been used in the other study (13). The dogs were divided into two groups; the first group was determined as naturally infected with intestinal parasites (n = 7), and the second group showed no an actual intestinal parasitic infection (n = 4).

Surgery

Jejunal biopsies were obtained from both groups by abdominal surgery. For this purpose the dogs were anesthesized with 10 mg/kg ketamin hydrochlorur (Ketanes, Alke) given intramuscularly. Jejunal biopsies were removed and than, additional anesthesia was induced with 2 mg/kg i.m. xylazin hidrochlorur (Rompun®, Bayer). Ampisilin trihydrat (5-10 mg/kg, Alfasilin /Abfar) was used for three days as antibiotic to protect possible infections in post-operative period which was started at the day of operation.

Tissue processing and staining

Tissue samples were fixed in 10% neutral buffered formaline (NBF) for 24 hours, and than embedded in parafin. Serial 5 mm thick sections were cut with 30 mm intervals. The tissue sections were stained with either Congo red for eosinophil granulocytes (17) or with methyl green pyronin for plasma cells (10).

The counting of cells in tissue sections

For counting the cells, the mucosa was diveded into "villus-cript" (VC) units. The number of eosinophils and plasma cells lying between two gland crypts and in the lamina propria of the villus above were counted. The

whole area comprised a VC unit and was delimited basally by the muscularis mucosa (27). The eosinophils and plasma cells at three VC units (x400 magnification) were counted for each section and five seperate slides were counted for each tissue block.

Blood sampling and determination of concentrations of leukocytes and eosinophil granulocytes

The blood samples were withdrawn from each dog into the test tube with EDTA before anaesthesia, and eosinophil granulocytes and leukocyte counts were carried out within following 2 hours. Leukocyte counts were determined in improved Neubauer hemocytometry with the standard technique using a solution *ad modum* Türk (44), and eosinophil granulocyte concentration was determined in Fuchs-Rosenthal hemocytometry with a solution ad modum Pilot (32).

Statistical evaluation

The data were statistically evaluated by t-Test for unpaired groups using SPSS computer program (37).

Results

In grup I, the dogs were naturally infected with intestinal parasites (Table 1).

The total white blood cell and eosinophil granulocyte consentrations are seen in Table 2.

The tissue sections were examined light microscopically. It was seen that eosinophil granulocytes and precursors were located intensively in lamina propria of jejunal mucosa, especially within villus intestinales (Figure 1), in basal parts of crypts and in lamina subglandularis (Figure 2). Furthermore, eosinophil granulocytes were located also intraepithelially, especially in crypts and rarely

Table 1. The intestinal parasites of naturally infected dogs.
Tablo 1. Doğal enfekte köpeklerin barsak parazitleri.

Animal No	Helmint (number/ oocyte count)				Protozoon (oocyst count)	
	Nematod		Cestod			
1	*Uncinaria stenocephala* *Toxocara canis*	3/1100 2/	*Dipylidium caninum*	5/1150	*Isospora spp.*	500
2	*Toxocara canis*	8/200000	-		-	
3	-		*Dipylidium caninum*	/500	*Isospora spp.*	350
4	-		*Cestod*	In tissue sections	-	
5	*Toxocara canis*	1/	*Dipylidium caninum*	23/ 250	*Isospora spp.*	200
6	-		*Taenia spp.*	/400	-	
7	*Toxocara canis*	2/500	-		*Isospora spp.*	100

Table 2. Total leukocyte and eosinophil granulocyte concentrations in peripheral blood of dogs (per ml of blood).
Tablo 2. Köpeklerde perifer kanda, ml'de total lökosit ve eozinofil granulosit konsantrasyonları

Groups	Leukocytes			Eosinophil granulocytes		
	X	±	SEM	X	±	SEM
Group I (n = 7)	11226		2486	84.00		17.40
Group II (n = 4)	12219		3778	63.86		43.3

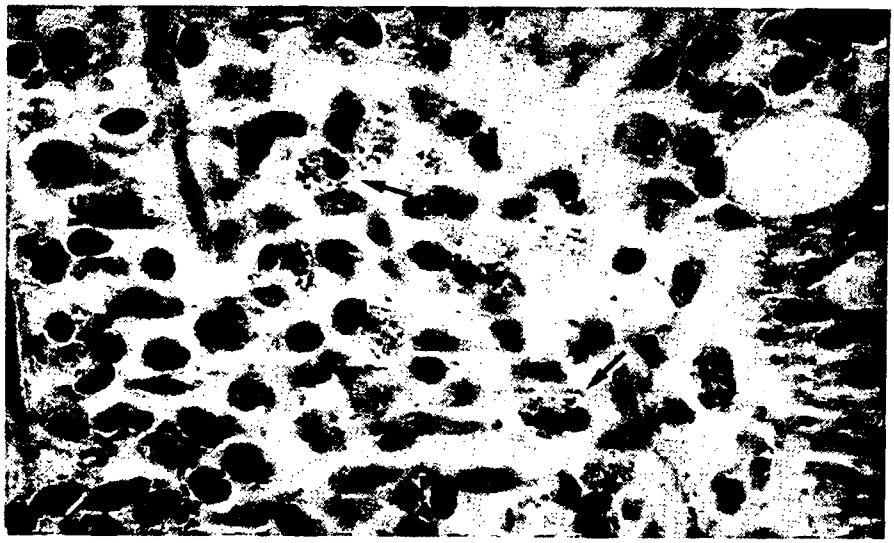

Figure 1. Eosinophil granulocytes observed in villus intestinales (arrows). Congo red. x1240.
Şekil 1. Villus intestinalis'de gözlenen eozinofil granulositler (oklar) Congo red. x1240.

Figure 2. Eosinophil granulocytes in lamina subglandularis (arrows). Congo red. x1180.
Şekil 2. Lamina subglandularis'de eozinofil granulositler (oklar) Congo red. x1180.

at the base of the villus (Figure 3A). It was also observed that some eosinophil granulocytes were present within intestinal lumen (Figure 3B).

The pyroninophilic cells were identified in jejunum, especially in villus intestinalis intensively. But they were found also in crypts in a lesser extent (Figure 4).

Eosinophil granulocytes and plasma cell counts per VC unit is given in Table 3.

The statistical evaluation of the data showed that the difference of the mean values for eosinophil granulocyte and leukocyte concentrations found in peripheral blood were not statistically significant. Also, the difference of plasma cell counts in tissues were not statistically significant. But the difference between mean tissue eosinophil granulocyte counts of dogs with parazites in feces and those showing no parazites were significant ($p< 0.05$)(Table 3).

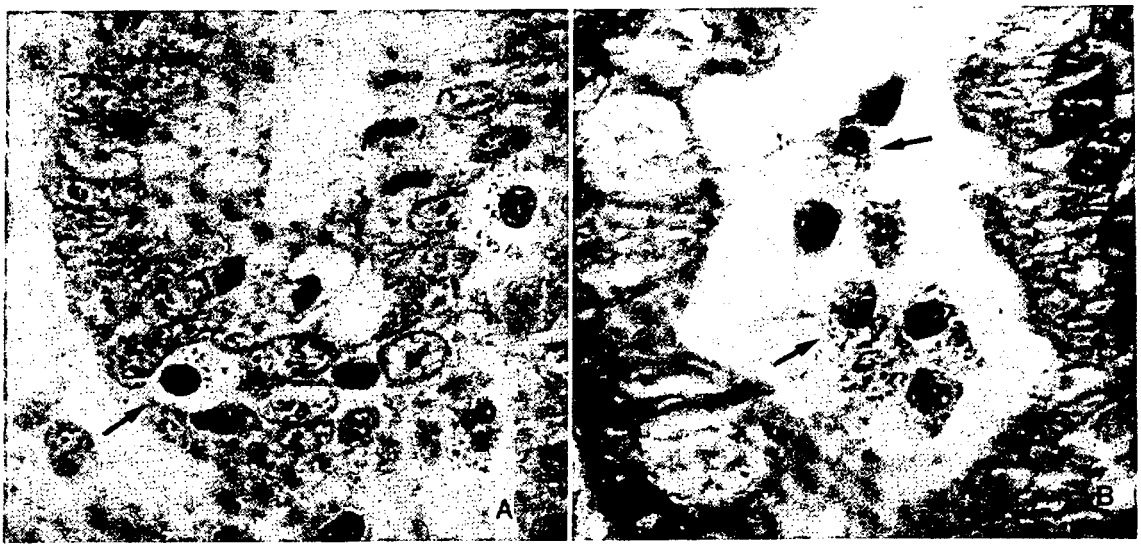

Figure 3. A. Intraepithelial eosinophil granulocytes (arrow). Congo red. x1200. B. Eosinophil granulocytes in intestinal lumen (arrows). Congo red. x1180.
Şekil 3. A. İntraepiteliyal eozinofil granulositler (ok). Congo red. x1200. B. Barsak lumeninde eozinofil granulositler (oklar) Congo red. x1180.

Figure 4. Plasma cells in villus intestinales (arrows).
Methyl green pyronin x1220.
Şekil 4. Villus intestinalis'de plazma hücreleri (oklar). Methyl green pyronin x1220.

Table 3. The eosinophil granulocytes and pyroninophilic cell counts in VC unit in the jejunal mucosa of dogs (means with SEM).
Tablo 3. Köpeklerde jejunum mukozasında, villus-kript ünitede plazma hücresi ve eozinofil granulosit sayıları.

Groups	Eosinophil granulocytes			Plasma Cells		
	X	±	SEM	X	±	SEM
Group I (n=7)	133.26*		11.90	90.91		13.50
Group II (n=4)	72.07*		18.90	72.15		24.40

* $p<0.05$

Discussion

The present study provides information about distribution of eosinophil granulocyte and plasma cells within the jejunal mucosa of dogs with or without intestinal parasites.

There are relative little studies about the distribution of eosinophil granulocytes and plasma cells in the body under physiological or physiopathological conditions, and only a small part of these studies were concerned with the distribution of these cell types within intestinal wall (15, 22, 23, 40, 43).

Experiments in the mice (7, 11, 14, 41, 46), rats (24, 25, 27, 29, 39), guinea pigs (16), turkeys (8), sheep (19) and human (9) have shown that infections with helmints and protozoan parasites are associated with pronounced intestinal mastocytosis, eosinophilia, plasmacytosis and increased antibody production.

In this study a significantly higher intestinal eosinophil granulocyte population in dogs naturally infected with intestinal parasites were determined. The eosinophil granulocytes were observed intensively in lamina propria of jejunal mucosa, especially within villus intestinales, in basal parts of crypts and in lamina subglandularis. Furthermore, eosinophil granulocytes were located intraepithelially in crypts and rarely at the base of the villus. It was also observed that some eosinophil granulocytes had been passed in intestinal lumen. These findings are in accordance with earlier studies stating that eosinophil granulocytes in intestinal tract are located mainly in lamina propria (3, 5, 21). Also, the increase of the numbers of eosinophil granulocytes in jejunum of parasitized dogs confirms the previous findings about response to parasitic agents of intestinal mucosa (14, 16, 34, 41, 42).

In sheep infected with Trichostrongylus colubriformis, increases in the populations of IgA- and IgG1-containing plasma cells in the lamina propria was identified (2). Morever, the distribution of plasma cells in intestinal mucosa with Toxacara canis and Ancylostoma caninum infected dogs were also described (26, 35). Soh and Kim (35) suggested that more plasma cells occurred only in the villi intestinales. In this study, the plasma cells were seen especially in villus intestinales intensively, but they were found also in crypts in a lesser extent. However, the increase of plasma cells in intestinal tract in respons to the intestinal parasites described by different authors (2, 35) was not supported by this study, than the plasma cells in jejunal mucosa of dogs with intestinal parasites did not differe significantly from those without parasites. But it is in good agreements with the results of Lloyd et al (26) who observed no changes in plasma cell numbers during infection with Toxacara canis in puppies.

On the other site, the statistical evaluation of the data showed that the difference of the mean values for eosinophil granulocytes and leukocytes of peripheral blood are not statistically significant. These results also confirm the previous study results about leucocyte formula and gastrointestinal parasites in goats (30).

In summary, we have identified distribution of eosinophil granulocyte and plasma cells within jejunal mucosa of dogs with or without intestinal parasites.

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