Accelerated Neutrophil Apoptosis in 2 Canine Cases of Hepatic Disorder

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ABSTRACT. Accelerated neutrophil apoptosis was confirmed by TUNEL assay in two canine cases of hepatic disorder. One dog was diagnosed as having lymphocytic hepatitis and the other lymphocytic cholangitis by histopathology of liver biopsy specimen.

KEY WORDS: apoptosis, inflammatory hepatic disorder, neutrophil.

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The neutrophil (PMN: polymorphonuclear leukocyte) plays a major role in biophylaxis against many infectious agents. PMNs have the shortest life span of all leukocytes, surviving in peripheral blood for hours to days before being apoptotic. Neutropenia is frequently detected in human cirrhotic patients, predisposing to bacterial infections, and it appears to be related to the increased PMN apoptosis [5]. However, the relations between PMN apoptosis and diseases have not been well investigated, and few discussions have been reported on PMN apoptosis in dogs. Previously, rates of PMN apoptosis in healthy dogs have been determined by the TUNEL assay [11], however those in diseased dogs have not been reported. In the present study, the accelerated apoptosis of PMNs was confirmed in two patient dogs diagnosed as having inflammatory hepatic disease (Cases 1 and 2).

Case 1 was a four-year and six-month-old, spayed female Labrador retriever and referred to us with chronic vomiting and diarrhea. In blood analyses of plasma enzymes associated with liver disorders, total bile acid and total bilirubin levels were conspicuously high. Lymphocyte count was decreased and eosinophils were not detected in complete blood count (CBC) (Table 1). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were 10.2 and 14 seconds, respectively. The histopathological diagnosis of a liver biopsy specimen was lymphocytic hepatitis. The dog was treated with prednisolone, ursodeoxycholic acid, amoxicillin, metronidazole, vitamin K and amino acid solution. The dog's condition gradually improved and it was discharged from hospital. PMN isolation was carried out on the second day of hospitalization.

Case 2 was a female, two-year and eight-month-old Borzoi and admitted with anorexia and jaundice. Total bile acid and total bilirubin levels were conspicuously high in blood chemistry. Lymphocytes were decreased in number and eosinophils were not detected (Table 1). PT and APTT were 8.2 and 12 seconds, respectively. Lymphocytic cholangitis was diagnosed by histopathological examination of a liver biopsy specimen. The dog was initially treated with ursode-

oxycholic acid, ampicillin, glutathione and glycyrrhizin, and late additional medications included prednisolone, azathioprine, metronidazole, misoprostol and carbazochrome. Despite treatment, complications with disseminated intravascular coagulation appeared and the dog died. PMN isolation was achieved on the third day of hospitalization.

The procedure of PMN isolation was reported previously [11]. PMNs separated from peripheral blood were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum. Final concentration of the cells was 4×10^6 cells/ml. These cell suspensions were incubated at 37°C for various time periods. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay was performed using DeadEnd Colorimetric Apoptosis Detection System (Promega, Madison, U.S.A.). After incubation for 0, 4, 8, 12 and 24 hr, PMNs from patient and control dogs were stained according to the manufacturer's instructions. At least 500 cells were evaluated in each preparation. The TUNEL-positive and negative cells were counted and apoptotic cell rates were calculated.

The apoptotic PMN rates of 2 patient dogs and 3 healthy control dogs (A, B, C) are given in Table 2 and Fig. 1.

Apoptotic PMNs of both patient dogs were markedly higher than those of the healthy dogs. The result was consistent with the results of human cirrhotic patients, and the correlation between PMN apoptosis and diseases should be further investigated in a large number of cases. Not only in dog but also in human, the concrete mechanisms of accelerated PMN apoptosis in liver disorders remain unknown.

Many factors have been reported to affect PMN apoptosis [1]. For instance, tumor necrosis factor (TNF)- α and Fas ligand could promote PMN apoptosis, while interleukin (IL)-1 β , IL-2, IL-8, granulocyte-macrophage colony stimulating factor, glucocorticoids and leukotriene B₄ would suppress it [4, 6, 7, 13]. It is interesting that only a few factors have been reported as pro-apoptotic extracellular factors for PMN, while many factors have been reported as anti-apoptotic factors. It was shown that TNF- α and IL-8 levels were elevated in cases of child autoimmune hepatitis [10]. Some reports also note that nitric oxide (NO) was produced from neutrophils or monocytes in human liver cirrhosis or dys-

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Table 1. CBC and blood chemistry of dogs with hepatitis and control dogs

	unit	Case 1	Case 2	Control A	Control B	Control C
RBC	$\times 10^6/\mu\text{l}$	6.71	5.58	8.36	6.59	8.19
Hb	g/dl	16.1	13.7	18.8	14.6	17.4
Hct	%	50.3	45.1	58.3	46.0	53.4
MCV	fl	75	81	70	70	65
MCHC	g/dl	32.0	30.4	32.2	31.7	32.6
WBC	$/\mu\text{l}$	9900	9600	10600	10200	9300
band	$/\mu\text{l}$	99	288	106	51	186
seg	$/\mu\text{l}$	8811	8160	7208	6987	5022
lym	$/\mu\text{l}$	792	768	2544	2132	3441
mono	$/\mu\text{l}$	198	384	530	714	279
eosino	$/\mu\text{l}$	0	0	212	316	372
baso	$/\mu\text{l}$	0	0	0	0	0
PLT	$\times 10^3/\mu\text{l}$	281	335	239	344	215
ALP	U/l	303	2449	120	95	91
ALT	U/l	681	758	67	81	54
AST	U/l	119	191	32	25	21
GGT	U/l	52	51	4	0	3
T-Bil	mg/dl	16.67	12.44	5.47	7.18	3.76
T-BA	$\mu\text{mol/l}$	27	285	—	—	—
ALB	g/dl	2.83	2.70	3.23	3.56	2.91
TP	g/dl	6.8	7.2	6.8	7.0	6.4

RBC : red blood cell

Hb : hemoglobin

Hct : hematocrit

MCV : mean corpuscular volume

MCHC : mean corpuscular hemoglobin concentration

WBC : white blood cell

band : band nuclear neutrophil

seg : segmented nuclear neutrophil

lym : lymphocyte

mono : monocyte

eosino : eosinophil

baso : basophil

PLT : platelet

ALB : albumin

ALP : alkaline phosphatase

ALT : alanine aminotransferase

AST : aspartate aminotransferase

GGT : γ -glutamyl transpeptidase

T-Bil : total bilirubin

T-BA : total bile acid

TP : total protein

— : not determined

Table 2. Apoptotic PMN rate (%) in each dog

	Case 1	Case 2	Control A	Control B	Control C
0hr	3.1	0.0	0.0	0.0	0.5
4hr	6.3	8.2	0.0	0.8	0.4
8hr	20.5	42.7	0.5	2.0	0.4
12hr	51.2	71.7	5.4	17.8	3.2
24hr	68.8	88.0	25.9	35.2	20.9

function, and that it would accelerate PMN apoptosis [3, 8, 12]. However, cytokine and NO levels have not been evaluated in dogs with hepatic disorders. The level of plasma diverse cytokines and other mediators would be high in canine cases of inflammatory hepatic disorders, and they might induce PMN apoptosis.

Many genes, such as *bcl-2* family, were also reported as regulating PMN apoptosis, but they are only a few reports of the relation between cytokines and *bcl-2* family genes [2, 9].

Henceforth, further investigations are required into PMN apoptosis in the diseased condition and they should include cytokines, other mediators and molecular analyses. Measurement of the apoptotic rate of PMNs might be indicative

Fig. 1. PMN apoptotic rate in controls and patient dogs. Vertical bars represent mean $\pm$ standard deviation of control dogs.

of the pathophysiological state of liver disorders.

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