Decreased Apoptotic Polymorphonuclear Leukocyte Rate in Dogs with Pyometra

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(Received 8 October 2002/Accepted 9 September 2003)

ABSTRACT. Polymorphonuclear neutrophil (PMN) apoptosis was examined in three dogs with pyometra by TUNEL assay in a 24-hr incubation period and compared with that in healthy control dogs (n=5). The incidence of apoptotic PMNs in dogs with pyometra was 26.4 ± 5% and that in healthy dogs was 54.3 ± 7%. The results indicated that apoptotic PMN rates in dogs with pyometra were significantly lower than those in control dogs (p<0.05), suggesting the prolongation of PMN survival.

KEY WORDS: canine, neutrophil apoptosis, pyometra.

NOTE Internal Medicine

Polymorphonuclear neutrophil (PMN) has the shortest half-life among all kinds of circulating leukocytes and are programmed to die within 24 hr after leaving the bone marrow [6, 11, 19, 20, 28, 33]. Aging PMNs spontaneously undergo apoptosis and are phagocytized by monocytes and macrophages [12, 27]. Since modulation of the PMN life span might be related to the host defense against pathogens [21], factors related to PMN apoptosis are under examination. Recent evidence demonstrates that the in vitro life span of mature human PMNs can be extended significantly by incubation with proinflammatory mediators and cytokines, including bacterial lipopolysaccharide (LPS) [6, 13, 19, 32, 34], host-derived cytokines of granulocyte colony-stimulating factor [1, 2, 6, 7, 34], granulocyte-macrophage colony-stimulating factor [2, 6, 7, 19], interleukin-8 (IL-8) [2, 6], IL-1 [6, 25] and interferon-γ [6, 25]. Conversely, cytokines such as IL-4 [2, 10, 25], transforming growth factor-β (TGF-β) [2, 6, 8], and TNF-α [6, 13, 23, 25, 29, 31, 32] accelerate the apoptotic program in PMNs. PMN apoptosis is accelerated in human diseases such as acquired immunodeficiency syndrome (AIDS) [24], uremia [4, 5, 15], and liver cirrhosis [18], and inhibited in sepsis [17], inflammatory bowel disease [3, 14], and diabetes [30].

Since the relationship of PMN apoptosis to clinical diseases in dogs has not been well investigated, the frequency of PMN apoptosis in dogs with pyometra as a typical chronic inflammatory disease was investigated and compared with that of healthy dogs.

Three dogs with pyometra examined were a golden retriever, a shiba dog and a mongrel dog, seven to eleven years old and they were diagnosed from ultrasonography as pyometra. Whole blood samples were determined with an automatic blood cell counter (MEK-6158, Nihon Kohden, Tokyo, Japan). The peripheral blood smears were stained with Wright-Giemsa stain and the hemogram was examined by light microscopy (×1,000). PMN isolation was carried out according to the method of Oguma et al. [22]. Briefly, 6 ml of EDTA treated blood samples supplemented with an equal volume of 3% dextran (Wako, Osaka, Japan) and 0.9% NaCl solution were allowed to stand for 30 min at room temperature to prepare leukocyte-rich plasma after sedimentation of the erythrocytes. The leukocyte-rich plasma was centrifuged at 200 g for 10 min. The pellets, which contained leukocytes and erythrocytes, were mixed with 0.3% NaCl for 40 sec to destroy the erythrocytes, and then mixed with 1.5% NaCl to adjust to 0.9% NaCl. The resulting leukocyte-rich plasma was centrifuged at 200 g for 10 min again. The pellets were resuspended in 5 ml PBS and overlaid on 5 ml of Ficoll-Hypaque (Lymphoprep, Nycomed Pharma, Oslo, Norway), and then centrifuged at 400 g for 20 min. The pellets were suspended in RPMI 1640 medium added with 10% fetal calf serum. The final concentration of the cells was adjusted to 4 × 10⁶ cells/ml. The samples consisted of more than 95% PMNs and were incubated at 37°C until used for examination (for 0, 6, 12 and 24 hr). After incubation for various time periods, PMNs were washed twice with PBS and collected on poly-l-lysine-coated slides with cytospin (Shandon Scientific Limited, UK) and dried for 15 min. The samples were fixed with 10% neutral buffered formalin at room temperature for 25 min. The TUNEL assay was performed with the DeadEnd Colorimetric Apoptosis Detection System, following the manufacturer’s instructions (Promega Corporation, WI). For counterstaining, 0.5% (w/v) methyl green in 0.1 M sodium acetate was used. The bound horseradish-peroxide-labeled streptavidin was detected with 3,3′-diaminobenzidine (DAB). The TUNEL-positive and -negative cells were counted and at least 500 cells were evaluated in each preparation. Results are expressed as the mean ± standard error (SE). The statistical differences in apoptotic cell rates between the dogs with pyometra and healthy dogs were determined by the Wilcoxon–Mann–Whitney test. Dif-
Blood samples were tested in an automatic blood cell counter and blood smears were stained with Wright-Giemsa stain. Results represent the mean ± SE. PCV: packed cell volume, WBC: white blood cell, band: band form neutrophil, seg: segmented form neutrophil, lymph: lymphocyte, mono: monocyte, eos: eosinophil, baso: basophil. The blood cell count and band form rate in PMN samples would be estimated to be 55.7% (54.3% × 100/97.5%) at 24-hr incubation. When segmented PMNs from dogs with pyometra undergo apoptosis at the same rate as those from healthy dogs, the apoptotic rate of PMNs samples from dogs with pyometra would be estimated to be 38.8% (69.7% × 55.7%) at 24-hr incubation, because PMN samples from dogs with pyometra were composed of 30.3% band form neutrophils.

Therefore, PMNs from dogs with pyometra could be more resistant to apoptosis than those from healthy dogs, indicating that PMNs survival time was prolonged in these dogs with pyometra.

On the other hand, canine pyometra is reported to be a secondary infection usually due to contaminants introduced through the cervix after hormone-induced changes in the uterus [16]. Therefore, bacterial products, PMN products and a large amount of various cytokines might be induced in dogs with pyometra. These substances might modulate and inhibit PMN apoptosis [9, 17]. Inhibition of PMN apoptosis might increase the opportunity for PMN to phagocyte bacteria and would be convenient for defense against microbial infection. Nevertheless, large amounts of inflammatory cytokines [26] and PMN products might worsen the general physical condition and induce organ dysfunction in affected dogs.

Further investigation of the molecular mechanism of inhibition of PMN apoptosis is required to understand the canine innate immune response.

**REFERENCES**

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