Activation of Canine Monocytes and Polymorphonuclear Cells by Serum Thymic Factor (FTS) in vivo

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ABSTRACT. The effect of serum thymic factor (FTS) was evaluated from the immunoresponse augmented in canine monocytes and polymorphonuclear cells (PMN) using the chemiluminescence technique. FTS did not affect the number of leukocytes and differential count of leukocytes. CL activity of the whole blood was significantly elevated by FTS from 72 hr to 120 hr after administration (p<0.05), and that at 96 hr after administration was about 3-fold higher than that before the administration. The CL response of PMN was significantly elevated by FTS administration from 24 hr to 96 hr after administration (p<0.05), and that at 48 hr after administration was about 7-fold higher than prior treatment. FTS also significantly elevated the CL response of monocyte from 24 hr to 96 hr after administration (p<0.01), and the CL count of monocyte in 24 hr and 48 hr was about 100-fold higher than that before FTS administration. These findings suggested that FTS may be efficacious and useful immuno-potentiator for canine monocytes and PMN. — KEY WORDS: canine, FTS, monocyte, polymorphonuclear cell.


Thymic hormones (e.g., thymosin, thymopoietin, thymic humoral factor, facteur serum thymic factor (FTS)) [26] are secreted from the thymus, and these hormones induce proliferation, differentiation and maturation of the T cell precursor in the thymus grand. FTS, purified from swine serum by Bach et al. in 1975 [2], is a polypeptide composed of 9 amino acids (pGln-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn) and with a molecular weight of 857 [6]. FTS is secreted from the epithelial cells of the thymus grand, and circulates through the blood binding with carrier protein [13]. The expression of FTS function requires a metal ion (Zn²⁺) [7] and five amino acids (Lys-Ser-Gln-Gly-Gly) [12].

FTS binds to the receptor on the T cell membrane as the affinity ranges from 10⁷ to 10⁹ M, and affects on the function of various T cell subsets [2, 23]. In humans and mice, expression of the T cell marker such as Thy-1, CD3, CD8, mixed lymphocyte reaction and reaction for mitogen in lymphocytes was raised by FTS [5, 9, 11, 14, 27], and the natural killer (NK) cell activity was regulated by FTS both in vivo and in vitro [4, 8]. Thymic hormones also induce the production of lymphokines, such as interferon-γ (IFN-γ) [26], interleukin 2 (IL-2) [21], monocyte migration inhibitory factor (MIF) [25]. A previous study demonstrated that FTS significantly increased the resistance to lethal doses of Salmonella typhimurium in mice in vivo [19]. These findings suggested that FTS inhibited the infection by affecting other cells that play a role in the cellular immunity mediated by the lymphocytes. However, little is known about the effect of FTS on activation of monocytes and polymorphonuclear cells (PMN) in vivo.

Herein, we examined changes of monocyte activity and PMN activity after the administration of FTS to dogs.

MATERIALS AND METHODS

Animals: All experiments were performed using male and female healthy normal beagles each weighing from 7 to 9 kg. Eleven beagles were divided into two groups; the FTS group consisting of 8 beagles administered 6 µg/kg of FTS subcutaneously (s.c.) and the control group consisting of 3 beagles administered 1 ml of physiological saline in s.c. FTS (zinc free form) kindly provided from Mitsui Pharmaceuticals Co. Ltd., was dissolved in physiological saline at a concentration of 50 µg/ml. One hundred mg of limulinol and 50 µl of triethylamine (Wako Pure Chemicals, Japan) were added to 50 ml of RPMI 1640 and then allowed to dissolve. After complete dissolution with sufficient agitation, the solution was passed through a 0.45 µm membrane filter (Millipore Co., U.S.A.) and stored in the dark at -20°C until use.

Preparation of PMN: The blood was collected every 24 hr for 120 hr via a heparinized catheter inserted into the cephalic vein. The PMN were isolated from heparinized blood by discontinues centrifugation (1.0693 and 1.0783; Percoll, Sigma Co., Ltd., U.S.A.) at 400 × g for 40 min. Isolated PMN were washed with Dulbecco's phosphate-balanced salt solution (PBS) prior to centrifugation at 100-150 × g for 5 min. The PMN were contained more than 95% in the preparation by the detection under the microscope.

Preparation of monocytes: The peripheral blood mononuclear cells (PBM) were isolated from heparinized
blood by Lymphoprep (Nycomed Pharma As., Norway) centrifugation at 400 x g for 40 min. Isolated PMN were washed with PBS prior to centrifugation at 100-150 x g for 5 min. For the preparation of monocytes, these PMN were resuspended in 2 ml of RPMI1640 (Nissui Co., Ltd., Japan) supplemented with 10% fetal bovine serum and incubated for 1 hr at 37°C in a 35-mm plastic petri dish (Falcon Co., #3003, U.S.A.), and monocytes were collected by trypsinization and resuspended in RPMI1640.

Chemiluminescence (CL) assay: Oxidative burst activity was used as an indicator of PMN and monocytes in peripheral blood [15, 24] and was measured by chemiluminescence [1, 17]. For the adaptation to the dark, a half ml of whole blood or 1 x 10⁶/ml of PMN or 6 x 10⁵/ml of monocytes were incubated in a plastic vial (12 x 47 mm) at 37°C for 10 min. After dark adaptation, 50 µl of luminol solution was added to each vial and the background CL was measured for 10 min. Two hundred µl of the carbonic iron solution (KAC-2; Nihon Antibody Laboratory, Japan) as a stimulant was added to a plastic vial. The measurement of CL counts was carried out at 60 min for the whole blood and at 90 min for the monocytes after luminol addition. Transitional CL activity was calculated by means of the following equation and compared thereafter:

\[ CL \text{ count} = \text{total count after stimulation} - \text{count of background} \]

where total count after stimulation was the total CL from the time of injecting the stimulant to the time when the whole leukocytes and monocytes attained the maximum count. Percent control (PC) was calculated by the equation of PC=(CL count in time after FTS administration)/(CL count before FTS administration) x 100.

RESULTS

Effect of FTS on the number of leukocytes: The changes of number of leukocytes in the FTS group were not significant until 120 hr after administration. The differential count also did not change during the same period (Fig. 1).

Changes of CL responses of whole blood: Figure 2 shows the changes of CL activity in the whole blood. The CL response of whole blood in the control group was stationary until 120 hr after administration with balanced salt-solution, but that in the FTS group significantly maintained higher activity until 120 hr after administration (p<0.05). The response curve of CL activity in the FTS group had two peaks, which appeared 24 hr and 96 hr after administration, and CL response at 24 hr and 96 hr after administration was 1.8- and 2.7-fold higher than that at pretreatment, respectively.

Changes of CL responses of PMN: The changes of CL responses of PMN after administration with FTS were shown in Fig. 3. The CL responses of PMN for control group seemed to be almost stationary until 120 hr after administration. On the other hand, those for FTS group was significantly increased with time after the administration and the peak of CL response of PMN was 48 hr after FTS administration (p<0.05). These findings indicated that the CL response of PMN was enhanced by FTS.

Effect of FTS on CL responses of monocyte: The monocyte activity measured by CL assay was significantly increased by FTS from 24 hr to 48 hr after administration (p<0.01), and the CL response of monocyte in this period for the FTS group was about 100-fold higher than that for the control group as shown in Fig. 4. This high level of CL response of monocyte for FTS still remained until 96 hr after administration, and the CL response of monocyte was reduced 120 hr after administration.

DISCUSSION

FTS did not affect the number of leukocytes and differential count of leukocytes until 120 hr after administration. FTS has various functions (e.g.: differentiation, maturation, activation of cytotoxic T cells and induction of cytokine) for T cells as a result of binding to the receptor on the T cell membrane [5, 9, 11, 14, 27]. However, the promotion of T cell growth by FTS in vitro has not been reported.

The CL technique is a simple and sensitive method for

![Graph showing CL activity of neutrophils, lymphocytes, and monocytes](image-url)
measurement of free radical content produced by phagocytic or cytotoxic cells as based on oxidative burst activity [1, 15, 17, 24]. This method also allows measurement of the activation of PMN and monocytes. The CL response of peripheral blood in the FTS group was kept at a higher level until 120 hr after administration and seemed to have two peaks, one is 24 hr after administration and the other is 96 hr. These findings were also dependent on the number of phagocytic cells in the peripheral blood, and PMN accounted for 70–80% of the leukocytes in dogs. Therefore, we examined the changes of PMN activity after administration with FTS. The PMN activity was kept at a higher level until 96 hr after FTS administration, and the peak of that was 48 hr after FTS administration. The CL response of monocyte was also strongly activated by FTS. The peak of monocyte activation was 24 hr after FTS administration and this activation was maintained until 120 hr. Matsumoto and Nakano [19] showed the antibacterial effect of FTS which was related to the increase of macrophage activity. The phagocytic cells, such as PMN and monocytes, play important role for initial host defense system against infection with microbe. Our findings suggested that FTS was increased the PMN activity same as increased the monocyte activity in dogs. Therefore, antibacterial effect of FTS might be related to the increase of both monocyte and PMN activity. FTS raised expression of the T cell marker such as Thy-1, CD3, CD8, mixed lymphocyte reaction and reaction for mitogen in lymphocytes [5, 9, 11, 14, 27]. FTS also led to the production of IL-1 from dendritic-shaped phagocytic cells of the thymic reticulum mediated by IL-2 [22]. However, the monocytes were not activated by FTS in vitro (data not shown). The macrophages were activated by IFN-γ [18], and the activating macrophages also secreted IL-1, G-CSF, TNFs [10, 16, 20], some of which activated the PMN. These findings suggested that the activation of monocytes by FTS is caused by the stimulation with IFN-γ production from T cells, and activation of PMN might be due to the production of cytokines from activating monocytes and T cells by FTS.

Moreover, these findings indicated that FTS may be an efficacious and useful immunopotentiator for initial host defense system against infection with pathogenic microbes.

REFERENCES

Fig. 4. Changes in monocytes activity after administration with or without FTS. The means of the count are shown as the percentage of the pre-treatment value (100%). Refer to Fig. 1 for explanation of each point and symbol. *1 Significantly different from the value for prior treatment, p<0.01 (Student’s t-test). *2 Significantly different from the value for control group, p<0.01 (Student’s t-test).


