The effects of spermine (SPM) on the production of Th1 and Th2 cytokines using peripheral blood mononuclear cells (PBMCs) derived from cedar pollinosis subjects were investigated. PBMCs produced by PBMCs stimulated by 10 μM SPM was significantly lower than that in the controls. These findings indicate that blood SPM is an important factor in the Th1/Th2 balance.

Key words: polyamine; spermine; Th1/Th2; cytokine; polyamine, spermine, polyamine, spermine, polyamine, spermine

Polyamines (PAs), viz., putrescine, spermidine, and spermine (SPM), are organic cations required for animal cell growth and differentiation and at various steps of DNA, RNA, and protein synthesis. Although PAs are synthesized in cells, recent studies have demonstrated the importance of PAs generated from extracellular sources. Immunocytes, including monocytes and lymphocytes, absorb PAs from their surroundings and influence cellular functions. It is well known that PAs, particularly SPM, which is more effective than putrescine or spermidine, suppresses the production of proinflammatory cytokines from macrophages in vitro and in vivo. Recent studies have revealed that SPM decreases the expression of lymphocyte function-associated antigen type 1 (LFA-1), which mediates the adhesion and migration of leukocytes in immune and inflammatory processes, on human lymphocytes, but no researcher has investigated the relationship between PAs and the Th1/Th2 balance in type I allergic patients. Our previous study indicated the possibility that a low concentration of intestinal PAs, putrescine and spermidine, a resource of blood PAs produced by intestinal microbiota, is an important factor in the onset of adult-type atopic dermatitis. In this study, we investigated the effects of SPM on the production of Th1 and Th2 cytokines using peripheral blood mononuclear cells (PBMCs) derived from subjects with cedar pollinosis, a typical type I allergy.

PBMCs were obtained from nine cedar pollinosis subjects (three males and six females; average age, 29.4 years), and were isolated by density gradient centrifugation. Isolated PBMCs were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated dialyzed fetal bovine serum (Invitrogen, Carlsbad, CA), 0.1% L-glutamine, 50 μM 2-mercaptoethanol, and 0.5% penicillin-streptomycin (Invitrogen) at a concentration of 2.0 × 10^6 cells/ml. One ml of this cell suspension was plated into a Falcon 24-well culture plate. SPM (Wako Pure Chemicals, Osaka, Japan) was added to the PBMCs at concentrations of 0 (control), 1, 10, and 100 μM, and Concanavalin A (ConA) was added at a concentration of 5 μg/well. After 48 h of incubation, the concentrations of interferon-γ (IFN-γ), interleukin (IL)-4, IL-10, and IL-12 in the supernatant were analyzed using commercial cytokine ELISA kits (BioSource International, Camarillo, CA). Cell survival was analyzed with a Colorimetric (MTT) assay kit (BioSource International, Temecula, CA) and by trypan blue staining. The change in each cytokine was analyzed by paired t-test. Calculations were performed with computer software STATISTICA (Design Technologies, Tokyo).

Although MTT assay and trypan blue staining using cultured cells confirmed that 1 and 10 μM SPM were not toxic to human PBMCs during a 48 h treatment, 100 μM SPM had weak toxicity to PBMCs. Hence, we omitted data obtained by 100 μM SPM stimulation from this study.

The effects of SPM on cytokine production of PBMCs derived from the subjects are shown in Fig. 1. The concentrations of INF-γ and IL-12 did not change on SPM stimulation. However, the IL-4 concentration in PBMCs stimulated by 10 μM SPM was significantly lower than that on the controls (p = 0.003). The IL-10 concentration due to PBMCs stimulated by 10 μM SPM (p = 0.057) also tended to be lower than that in the controls.
These results indicate that Th2 cytokine production of PBMCs stimulated by ConA is downregulated when the SPM concentration is 10 μM. This is a very interesting finding, because human blood SPM concentration ranges from 4 to 14 μM.7) Zhang et al.6) reported that SPM uptake is required for suppression of cytokine synthesis in monocytes by SPM, and that an increased SPM uptake in activated monocytes provides a mechanism preventing excessive monocyte activation. We assume that the downregulation of Th2 cytokine of PBMCs stimulated by ConA due to SPM stimulation is dependent on the incorporation and accumulation of SPM in activated Th2 cells as well as monocytes, because lymphocytes absorb PAs from their surroundings.3,4) The concentration of Th1 cytokines did not change under SPM stimulation, indicating that the downregulation of Th2 cytokines is influenced by direct SPM stimulation rather than by Th1 cytokines.

The fact that Th1 (proinflammatory) cytokines were not suppressed by SPM stimulation is not in agreement with the results of previous studies in which PBMCs were used.5,6) This difference is probably due to the mitogens used. That is, while in the present study PBMCs were stimulated by ConA to activate the T-cells, the previous studies used lipopolysaccharide for macrophage activation. Hence, it is likely that these supernatants contained small amounts of Th1 cytokines derived from macrophages.

Blood cells can take up PAs from their surroundings, and the largest source of PAs in the body is the intestinal lumen.2) Therefore, in our previous study, the concentration of intestinal PAs, putrescine and spermidine, in adult patients with atopic dermatitis was lower than that in healthy adults; this reduction leads to decreases in the blood PA concentration and the induction of Th2 cytokine production in adult patients with atopic dermatitis. In other words, the Th1/Th2 balance of type I allergic patient is probably improved by the elevation in intestinal PA concentration due to PA-rich meals or probiotics.9,10) In fact, when we administered probiotic yogurt to adult patients with atopic dermatitis, an improvement in the Th1/Th2 balance followed the increase in intestinal spermidine concentration due to the consumption of probiotic strain LKM512.10) However, the fact that serum INF-γ was increased and IL-4 and IL-10 were not suppressed by this probiotic consumption is not in agreement with results of the present study. Further study is required to clarify the difference between the previous results (in vivo) and the present results (in vitro).
In the present study, we found that blood SPM is an important factor in the Th1/Th2 balance of cedar pollinosis subjects.

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