Enhancement of Gastrointestinal Absorption of Ovalbumin Caused by Spermine Induces an Increase in Plasma Histamine Levels in Mice Sensitized to Ovalbumin

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The aim of this study was to determine whether a spermine (SPM)-induced increase in gastrointestinal absorption of an allergen leads to an anaphylactic response in sensitized mice. First, we examined the enhancing effect of SPM on the gastrointestinal absorption of ovalbumin (OVA) in an *in situ* jejunum loop study in rats and an *in vivo* oral absorption study in mice. Second, we investigated whether enhancement of gastrointestinal absorption of OVA caused by SPM induces an anaphylactic response in mice sensitized to OVA. In the *in situ* jejunum loop study in rats, a significant amount of immune-reactive OVA was detected in the plasma after co-administration of OVA and SPM. Oral co-administration of OVA and SPM to mice *in vivo* also increased plasma OVA concentrations in an SPM dose-dependent manner. Furthermore, in sensitized mice, a significant increase in plasma histamine levels occurred along with the increase in plasma OVA levels after co-administration of OVA with SPM. This finding suggests that an SPM-induced increase in gastrointestinal absorption of OVA leads to an anaphylactic response. These results indicate that excess oral ingestion of SPM may have widespread health effects, including the induction of food allergies, *via* modulation of the function of the gastrointestinal epithelial barrier.

Key words spermine; intestinal absorption; anaphylactic response; ovalbumin; histamine

The polyamines spermidine and spermine (SPM) and their precursor putrescine are ubiquitous polycationic compounds found in all mammalian cells.[1—5] These compounds are essential for the maintenance of cell growth in many tissues, and intracellular levels of polyamines are maintained within narrow limits.

The polyamines are present in variable amounts in almost all food or foodstuffs. In humans, a typical diet might contribute hundreds of micromoles of polyamines per day to the gut lumen,[6,7] and ingested food may be the major source of polyamines in the lumen of the upper small bowel. Exogenous polyamines play a role in the maintenance of normal growth and function of the intestinal tract.[8,9] Oral administration of SPM induces precocious maturation of the intestine.[10—15] Oral polyamines have also been shown to have beneficial effects on healing[16,17] and prevention[18—20] of gastrointestinal damage.

The gastrointestinal tract acts as a barrier against the invasion of exogenous proteins into the body. This barrier function is due to enzymes that degrade the proteins and also due to impermeability of the proteins to the membrane due to their large molecular weight. Because most food-originated proteins are digested in the gastrointestinal tract before absorption, naturally occurring protein uptake from the intestinal epithelium should be low. In reality, however, the barrier function of the small intestinal epithelium in mammals is incomplete and, to a limited extent, macromolecules can pass from the lumen into the circulation.[21,22]

In an *in situ* loop study and in an *in vivo* oral absorption study, we showed that polyamines, especially SPM, can enhance the intestinal absorption of fluorescein isothiocyanate-labeled dextran (molecular weight (MW) 4400, FD-4), a hydrophilic model macromolecule, without causing any significant damage in the intestinal tract in rats.[23] We also suggested that the absorption-enhancing mechanism of SPM partly includes opening the tight junctions of the epithelium *via* the paracellular route by measuring FD-4 permeation and transepithelial electrical resistance in the human intestinal Caco-2 cell line.

We have speculated that excess absorption of macromolecules by the action of SPM, one of the compounds in food, has widespread health effects. Thus, modulation of the gastrointestinal epithelial barrier by ingested luminal polyamines may influence the induction of food allergies. Gastrointestinal permeability enhanced by daily ingestion of polyamines plays a part in the regulation of the induction of tolerance because oral tolerance induction is related to the gradual and continuous absorption of a food allergen.[24,25] In contrast, after sensitization to an antigen, an increase in gastrointestinal permeability by polyamines may facilitate the absorption of the allergen into the circulation and lead to an anaphylactic response as an immediate hypersensitivity reaction to the antigen.

The aim of this study was to determine whether an SPM-induced increase in gastrointestinal absorption of an allergen leads to an anaphylactic reaction in sensitized mice.

First, we examined the effect of SPM on enhancing gastrointestinal absorption of ovalbumin (OVA), as the allergen, in an *in situ* jejunum loop study in rats and an *in vivo* oral absorption study in mice. Second, we investigated whether SPM-induced enhancement of gastrointestinal absorption of OVA induced an anaphylactic response in mice sensitized to OVA.

MATERIALS AND METHODS

Materials SPM tetrahydrochloride was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). OVA (grade

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V) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of research grade.

Animals Male Sprague-Dawley (SD) rats (6 weeks) and female BALB/C mice (6 weeks) were purchased from Tokyo Experimental Animals (Tokyo, Japan) and maintained in an environmentally controlled room (25 °C) with a 12-h light/dark cycle and allowed access to standard laboratory chow (Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*. The experimental protocol and design were approved by the Institutional Animal Care and Use Committee at the Life Science Center of Josai University and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Immunization Protocols Mice were sensitized by intraperitoneal injection with 0.2 ml saline 5 μg OVA (grade V; Sigma) adsorbed to 2 mg of alum. One week after the first immunization, mice were given boosters using the same dose of antigen, and sensitization was carried out at weekly intervals for 5 weeks.

Ex Vivo* Studies in Rats (*in Situ* Closed Loop Technique)** Rats (250—300 g) were fasted for 18 h before the experiment and were anesthetized with intraperitoneal urethane (1 g/kg). Ten centimeters of a jejunal loop was prepared after the lumen was washed with saline. OVA was dissolved at a concentration of 0.1 mM in phosphate buffered saline (PBS) (pH 7.4) containing 10 mM SPM. The solution (1 ml) was introduced into the loop *via* a microsyringe, and blood was withdrawn from the jugular vein at designated times. Blood samples were centrifuged (18000×g***, 5 min), and the concentration of OVA in plasma was determined at designated times (0, 30, 60, 90, 120, 150, 180 min) over a 3-h period using a two-site enzyme immunoassay using an enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan). OVA (grade V; Sigma) was used as a standard sample.

In Vivo* Studies in Mice** Mice were fasted for 18 h before the experiment. A solution (0.2 ml) of OVA (10 mg) containing SPM (0, 0.2, 0.4, 1.2, 2.0 mg) was administered by gastric intubation. Thirty minutes after administration of OVA, blood was withdrawn from the jugular vein. Blood samples were centrifuged (18000×g***, 5 min) and the plasma concentration of OVA was determined using a two-site enzyme immunoassay using an ELISA kit.

Determination of Plasma Histamine in Anaphylactic Response Mice sensitized by OVA were fasted for 18 h before the experiment. The solution (0.2 ml) of OVA (10 mg), with or without SPM (1.2 mg), was administered by gastric intubation. Thirty minutes after administration of OVA, blood was withdrawn from the jugular vein. Blood samples were centrifuged (18000×***g***, 5 min), and plasma histamine levels were measured by the post-column high-performance liquid chromotography method, as described previously.[26] In addition, the plasma concentration of OVA was determined using a two-site enzyme immunoassay using an ELISA kit.

Statistical Analysis The statistical significance of data was determined by the student *t*-test. A *p*-value less than 0.05 was considered significant.

RESULTS

Evaluation of OVA Absorption in the *in Situ* Intestinal Loop Study in Rats The time course of OVA concentration in plasma after administration into the jejunal loop in the presence of SPM is shown in Fig. 1. When 1 ml of 10 mM SPM solution containing OVA was administered into the intestinal loop, the absorption of OVA from the jejunum was time-dependently increased by SPM compared with controls (PBS containing OVA). When OVA was administered alone, limited absorption was observed.

Evaluation of OVA Absorption *in Vivo* in Mice The absorption-enhancing effect of SPM was further evaluated by an *in vivo* study. OVA concentration in plasma after oral co-administration of OVA and SPM in BALB/c mice is shown in Fig. 2. We examined whether different doses of SPM (0, 0.2, 0.4, 1.2, 2.0 mg) enhanced the absorption of OVA. The effect of SPM administration on the increase in OVA concentration in plasma appeared to be dose-dependent, and co-administration of 1.2 mg SPM led to the largest increase in the absorption of OVA.

Figure 3 shows OVA concentration in plasma after oral administration of 0.1 and 1.0 mg OVA with or without co-administration of 1.2 mg SPM. Oral administration of 0.1 and 1.0 mg OVA in the presence of SPM increased plasma OVA concentration compared with controls. OVA concentrations in plasma when 0.1 mg of OVA and SPM were orally co-administered were comparable to when 1.0 mg of OVA was administered alone.

Evaluation of OVA Absorption and Plasma Histamine Levels in Sensitized Mice To investigate the influence of

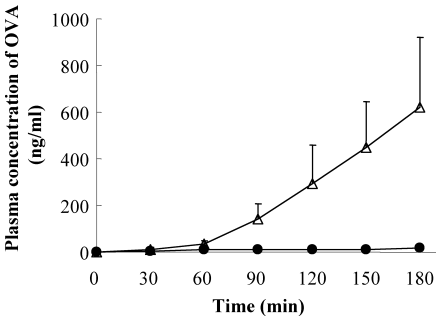

Fig. 1. Effect of SPM on OVA Absorption in the *in Situ* Rat Jejunum Loop Study

The lumen was washed with saline, and the jejunum was treated with 1 ml of OVA solution in the presence (△) or absence of SPM (●). Data are expressed as mean±S.D. (n=7).

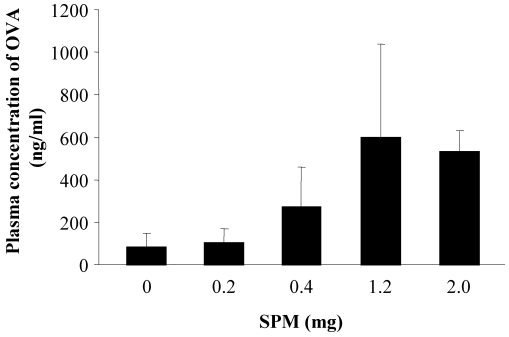

Fig. 2. Effect of SPM on OVA Absorption after Oral Administration to Normal BALB/C Mice

OVA (10 mg) was co-administered orally with various amounts of SPM. Plasma concentrations of OVA were determined 30 min after administration of OVA with SPM. Data are expressed as mean±S.D. (n=5).

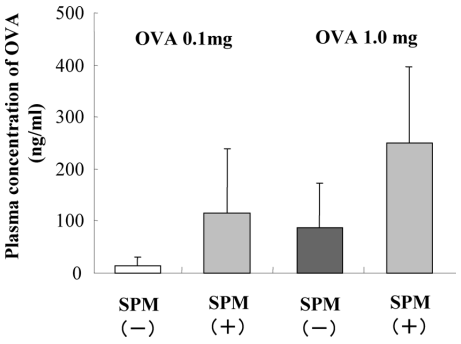

Fig. 3. Evaluation of the Effect of SPM (1.2 mg) on OVA Absorption after Oral Administration to Normal BALB/C Mice

OVA (0.1 or 1.0 mg) was administered orally with or without SPM (1.2 mg) to BALB/C mice. Plasma concentrations of OVA were determined 30 min after administration of OVA. Data are expressed as mean±S.D. (n=3—5).

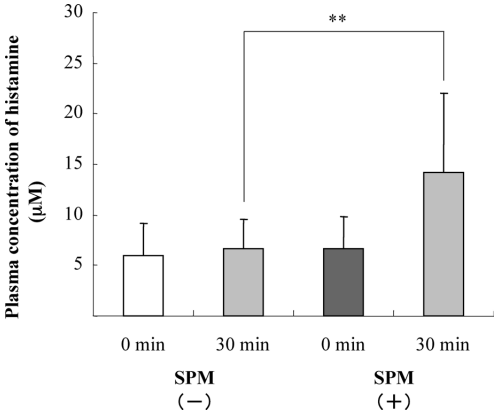

Fig. 4. Determination of Plasma Histamine Levels after Oral Co-administration of OVA and SPM in BALB/C Mice Sensitized by OVA

Plasma histamine levels were determined 30 min after administration of OVA (10 mg) with or without SPM (1.2 mg). Data are expressed as mean±S.D. (n=9—10). ** $p<0.01$ compared with controls.

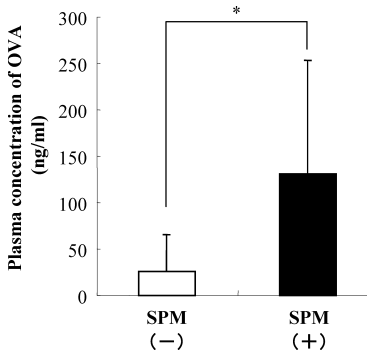

Fig. 5. Effect of SPM on OVA Absorption after Oral Administration to BALB/C Mice Sensitized by OVA

OVA (10 mg) was administered orally with or without SPM (1.2 mg) to BALB/C mice sensitized by OVA. Plasma concentrations of OVA were determined 30 min after administration of OVA. Data are expressed as mean±S.D. (n=9-10). * $p<0.05$ compared with controls.

the absorption-enhancing effect of SPM on the OVA-induced hypersensitivity reaction, plasma histamine levels in sensitized mice were measured. As shown in Fig. 4, oral co-administration of OVA and SPM produced an increase in plasma histamine levels compared with oral administration of OVA alone.

Plasma concentrations of OVA were also significantly increased after oral co-administration of OVA and SPM compared with oral administration of OVA alone (Fig. 5). These results suggest that an increase in gastrointestinal absorption of OVA by SPM may lead to an anaphylactic response. The absorption of OVA in sensitized mice decreased to about 20% compared with that of normal mice after oral administration of OVA. About 600 ng/ml of OVA was detected in the plasma after co-administration of OVA (10 mg) and SPM (1.2 mg) in normal mice; however, only about 130 ng/ml was detected when OVA and SPM were co-administered to sensitized mice (Figs. 2 and 5) at the same dosage.

DISCUSSION

In the present study, we first investigated the enhancing effect of a polyamine, SPM, on the gastrointestinal absorption of a natural protein, OVA, in an *in situ* closed loop study and an *in vivo* oral absorption study.

In the *in situ* jejunum loop study, a significant amount of immune-reactive OVA was detected in the plasma after co-administration of OVA and SPM. The increase in OVA absorption was gradual 1 h after administration. In previous studies, the same tendency was found to occur in terms of jejunal absorption of FD-4 in the presence of SPM.[23] Oral co-administration of OVA and SPM to unanesthetized mice *in vivo* also increased plasma OVA concentrations in an SPM dose-dependent manner. Although the exact mechanisms of the absorption-enhancing effect of polyamines are unclear, this result could be considered to represent the absorption of OVA under physiological conditions. It has been reported that a significant amount of intact OVA was detected in both the plasma and the lymph fluid after oral administration in rats.[22]

The mechanisms of the absorption of macromolecules include paracellular passage and transcellular endocytosis.[27—31] Intestinal epithelial cells possess a negatively charged surface like other cells. The electrostatic interaction of protein with the intestinal epithelial cells may be a factor determining its intestinal absorption. It has been reported that the intestinal absorption of a protein is, at least partially, determined by its electrical charge.[32] At physiological pH levels, SPM is positively charged and can interact with many anionic structures, including protein.[33] Therefore, SPM may interact with OVA, and interaction of the positively charged amino groups of SPM with the negative membrane components may influence intestinal permeability.

We next investigated whether SPM-enhanced gastrointestinal absorption of OVA induced an anaphylactic response in mice sensitized to OVA. Several studies have reported an anaphylactic response with an increase in concentrations of serum or plasma histamine representing an immediate hypersensitivity response to antigen stimulation after sensitization.[25,34,35] Results showed that a significant increase in plasma histamine levels occurred as the immediate hypersensitivity response to antigen stimulation, along with an increase in plasma OVA level after co-administration of OVA with SPM in sensitized mice. In contrast, no increase in either plasma histamine or OVA levels was seen when OVA was administered alone. These results suggest that an increase in gastrointestinal permeability by SPM may facilitate the absorption of OVA into the circulation and may lead to an

anaphylactic response, as an immediate hypersensitivity response to OVA, in sensitized mice.

We also found that plasma concentrations of OVA in sensitized mice were significantly lower than levels in normal mice after oral administration of OVA, that is, the absorption of OVA in sensitized mice decreased to about 20% compared with that in normal mice after oral co-administration of OVA (10 mg) and SPM (1.2 mg). These results suggest that gastrointestinal absorption of a specific antigen is influenced by sensitization. It is known that immune responses at mucosal surfaces, including the intestinal epithelium, are characterized by production of immunoglobulin A (IgA), and Peyer's patches are considered a major inductive site for initiation of high-affinity secretory IgA in the gastrointestinal tract.[36] It has been reported that oral OVA-immunization in mice results in the generation of OVA-specific IgA Ab responses in the gastrointestinal tract.[37] Based on the low OVA absorption in sensitized mice compared with normal mice after oral administration of OVA, we speculate that OVA-specific IgA secreted into the gastrointestinal lumen in mice sensitized to OVA might block OVA access to the gastrointestinal epithelium.

Polyamines provided by food have a potential role in the growth and development of the digestive system in neonatal mammals. SPM administered orally to suckling rats causes structural and biochemical changes in the intestinal mucosa comparable to those observed at weaning.[38] As factors that induce maturation of the digestive system, that is, precocious intestinal and pancreatic maturation,[39] dietary polyamines could play a role in the prevention of food allergies. Indeed, oral administration of SPM induces precocious maturation of the intestine resulting in the cessation of macromolecular transport in suckling rats.[10]

Dietary polyamines also seem necessary for the maintenance of normal growth and general properties in the adult digestive tract. In addition, cellular polyamines also play a critical role in maintenance of the intestinal epithelial integrity—that is, polyamines are implicated in the regulation of the intestinal epithelial barrier function.[40] Namely, polyamines are also required for normal function of tight junctions.

In the present study, we demonstrated one possibility that modulation of the gastrointestinal epithelial barrier function by excess ingestion of a luminal food component with an absorption-enhancing effect may increase the gastrointestinal absorption of a food allergen and lead to an anaphylactic response in sensitized mammals.

In adult humans, the daily intake of polyamines from a typical diet is estimated at hundreds of micromoles, and significant differences in polyamine concentrations and distribution patterns are observed between food groups.[6,7,41] For example, beans show high concentrations of spermidine and SPM, vegetables have higher levels of putrescine and spermidine, and fish, shellfish, meat, and nuts have high levels of SPM.[7] Thus, different dietary habits might lead to variations in polyamine intake. The dose and concentration of SPM selected for the present experiments approximated those used by previous investigators to induce the maturation of the intestine in animals.[10—15] The dose of SPM used in the present study is well within normal limits.

In summary, we demonstrated that SPM, which is present in variable amounts in almost all kinds of food, can enhance the gastrointestinal absorption of OVA in both normal and sensitized mice, although the absorption of OVA in mice sensitized mice to OVA was lower than that in normal mice. In sensitized mice, a significant increase in the plasma histamine levels occurred along with an increase in plasma OVA levels after co-administration of OVA with SPM. These results indicate that excess oral ingestion of SPM may have widespread health effects, including the induction of food allergies, *via* modulation of the gastrointestinal epithelial barrier.

REFERENCES

1) Tabor C. W., Tabor H., *Annu. Rev. Biochem.*, **53**, 749—790 (1984).
2) Pegg A. E., *Biochem. J.*, **234**, 249—262 (1986).
3) Igarashi K., Kashiwagi K., *Biochem. Biophys. Res. Commun.*, **271**, 559—564 (2000).
4) Wallace H. M., *Essays Biochem.*, **46**, 1—9 (2009).
5) Agostinelli E., Marques M. P., Calheiros R., Gil F. P., Tempera G., Viceconte N., Battaglia V., Grancara S., Toninello A., *Amino Acids*, **38**, 393—403 (2010).
6) Bardocz S., Duguid T. J., Brown D. S., Grant G., Pusztai A., White A., Ralph A., *Br. J. Nutr.*, **73**, 819—828 (1995).
7) Nishibori N., Fujihara S., Akatuki T., *Food Chem.*, **100**, 491—497 (2007).
8) McCormack S. A., Johnson L. R., *Am. J. Physiol.*, **260**, G795—G806 (1991).
9) Deloyer P., Peulen O., Dandrifosse G., *Eur. J. Gastroenterol. Hepatol.*, **13**, 1027—1032 (2001).
10) Harada E., Hashimoto Y., Syuto B., *Comp. Biochem. Physiol. A Physiol.*, **109**, 667—673 (1994).
11) Kaouass M., Deloyer P., Dandrifosse G., *Digestion*, **55**, 160—167 (1994).
12) ter Steege J. C. A., Buurman W. A., Forget P. P., *J. Pediatr. Gastroenterol. Nutr.*, **25**, 332—340 (1997).
13) Greco S., Hugueny I., George P., Perrin P., Louisot P., Biol M.-C., *Biochem. J.*, **345**, 69—75 (2000).
14) Peulen O., Gharbi M., Powroznik B., Dandrifosse G., *Biochimie*, **86**, 487—493 (2004).
15) Deloyer P., Peulen O., Dandrifosse G., *Exp. Physiol.*, **90**, 901—908 (2005).
16) Wang J. Y., Johnson L. R., *Am. J. Physiol.*, **259**, G584—G592 (1990).
17) Wang J. Y., Johnson L. R., *Gastroenterology*, **102**, 1109—1117 (1992).
18) Mizui T., Shimono N., Doteuchi M., *Jpn. J. Pharmacol.*, **44**, 43—50 (1987).
19) Buzas G., Demel Z., Jozan J., Hamar J., *Acta Physiol. Hung.*, **84**, 251—258 (1996).
20) ter Steege J. C. A., Forget P. P., Buurman W. A., *Shock*, **11**, 115—119 (1999).
21) Sanderson I. R., Walker W. A., *Gastroenterology*, **104**, 622—639 (1993).
22) Tsume Y., Taki Y., Sakane T., Nadai T., Sezaki H., Watabe K., Kohno T., Yamashita S., *Biol. Pharm. Bull.*, **19**, 1332—1337 (1996).
23) Sugita Y., Takao K., Toyama Y., Shirahata A., *Amino Acids*, **33**, 253—260 (2007).
24) Stokes C. R., Swarbrick E. T., Soothill J. F., *Clin. Exp. Immunol.*, **52**, 678—684 (1983).
25) Akiyama H., Teshima R., Sakushima J., Okunuki H., Goda Y., Sawada J., Toyoda M., *Immunol. Lett.*, **78**, 1—5 (2001).
26) Kawasaki M., Toyoda M., Teshima R., Sawada J., Saito Y., *Biol. Pharm. Bull.*, **17**, 1321—1325 (1994).
27) Heyman M., Ducroc R., Desjeux J. F., Morgat J. L., *Am. J. Physiol.*, **242**, G558—G564 (1982).
28) Marcon G. D., Tome D., Kheroua O., Dumontier A. M., Heyman M., Desjeux J. F., *Am. J. Physiol.*, **256**, G943—G948 (1989).
29) Atisook K., Madara J. L., *Gastroenterology*, **100**, 719—724 (1991).
30) Yan X., Wang X., Zhang X., Zhang Q., *J. Pharmacol. Exp. Ther.*, **308**, 774—779 (2004).
31) Takano M., Koyama Y., Nishikawa H., Murakami T., Yumoto R., *Eur. J. Pharmacol.*, **502**, 149—155 (2004).

32) Nishikawa M., Hasegawa S., Yamashita F., Takakura Y., Hashida M., *Am. J. Physiol. Gastrointest. Liver Physiol.*, **282**, G711—G719 (2002).
33) Medina M. A., Urdiales J. L., Rodriguez-caso C., Ramirez F. J., Sanchez-Jimenez F., *Crit. Rev. Biochem. Mol. Biol.*, **38**, 23—59 (2003).
34) Hsieh K. Y., Hsu C. I., Lin J. Y., Tsai C. C., Lin R. H., *Clin. Exp. Allergy*, **33**, 1595—1602 (2003).
35) Akiyama H., Sato Y., Watanabe T., Nagaoka M. H., Yoshioka Y., Shoji T., Kanda T., Yamada K., Totsuka M., Teshima R., Sawada J., Goda Y., Maitani T., *FEBS Lett.*, **579**, 4485—4491 (2005).
36) Craig S. W., Cebra J. J., *J. Exp. Med.*, **134**, 188—200 (1971).
37) Yamamoto M., Rennert P., McGhee J. R., Kweon M. N., Yamamoto S., Dohi T., Otake S., Bluethmann H., Fujihashi K., Kiyono H., *J. Immunol.*, **164**, 5184—5191 (2000).
38) Dufour C., Dandrifosse G., Forget P., Vermesse F., Romain N., Lepoint P., *Gastroenterology*, **95**, 112—116 (1988).
39) Romain N., Gesell M. S., Leroy O., Forget P., Dandrifosse G., Luk G. D., *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **120**, 379—384 (1998).
40) Guo X., Rao J. N., Liu L., Zou T., Keledjian K. M., Boneva D., Marasa B. S., Wang J.-Y., *Am. J. Physiol. Gastrointest. Liver. Physiol.*, **288**, G1159—G1169 (2005).
41) Nishimura K., Shiina R., Kashiwagi K., Igarashi K., *J. Biochem.*, **139**, 81—90 (2006).