

The Role of RANTES in a Murine Model of Food Allergy

Jeong-Beom Lee, M.D., Ph.D.,^{1,2,*} Takaaki Matsumoto, M.D., Ph.D.,¹
Young-Oh Shin,¹ Hun-Mo Yang, M.D., Ph.D.,²
Young-Ki Min, M.D., Ph.D.,² Othman Timothy, M.D., Ph.D.,³
Sang-Jae Bae, M.D., Ph.D.,⁴ and Fu-Shi Quan, M.D., Ph.D.⁵

¹The 2nd Department of Physiology, Aichi Medical University
School of Medicine, Aichi, Japan

²Department of Physiology, College of Medicine,
Soonchunhyang University, Cheonan, Korea

³Yale Child Health Research Center, Yale University School of Medicine,
New Haven, Connecticut, USA

⁴Department of Dermatology, Nagasaki University School of
Medicine, Nagasaki, Japan

⁵Institute of Medicine Science, Korea University College of
Medicine, Seoul, Korea

ABSTRACT

Food allergy is an important and common health issue, and there is a need to identify and characterize the sensitizing mechanisms. One of the common causes of food allergy is ovalbumin (OVA), a dietary antigen from eggs. We hypothesized that OVA-induced food allergy in the gut involves the activation of the chemokine regulated on activation, normal T cell expressed and secreted (RANTES), which then recruits eosinophils to lesioned tissue. The purpose of this study was to clarify whether RANTES expression correlates with eosinophil infiltration in the gut of

*Correspondence: Jeong-Beom Lee, M.D., Ph.D., The 2nd Department of Physiology, Aichi Medical University School of Medicine, Nagakute, Aichi 480-1195, Japan, or Department of Physiology, College of Medicine, Soonchunhyang University, Chonan 330-090, Korea; Fax: +81-561-63-9809; E-mail: leejb@sch.ac.kr.

OVA-sensitized BALB/c mice in response to oral OVA challenge. BALB/c mice were immunized with OVA 1 μ g and sensitized after 2 weeks by intragastric administration of OVA. Sensitization to the oral OVA challenge was analyzed by examining eosinophil infiltration into the gut tissue (immunohistochemistry), mucosal eosinophil cationic protein (ECP) concentration, and RANTES mRNA expression (reverse-transcriptase polymerase chain reaction and Southern blotting) at 3, 6, 12, and 24 h after the challenge. There was marked edema of the intestinal villi, and eosinophil infiltration to the lamina propria peaked at 6 h in OVA-sensitized mice. RANTES mRNA expression peaked at 3 h and 6 h and declined thereafter. The expression of RANTES mRNA in the allergic mice was much higher than in the nonallergic, normal, or unsensitized control mice. Tissue eosinophilia and intestinal ECP levels were significantly correlated with the RANTES mRNA level. We conclude that RANTES may play a central role in the pathogenesis of food-mediated gastrointestinal allergy.

Key Words: RANTES; Eosinophils; Food allergy; Murine model; RT-PCR; Southern blotting.

INTRODUCTION

Food allergy is an important and common health issue. Allergens from fish and eggs are some of the most frequent causes of food allergic reactions reported in the literature (Poulsen et al., 2001). Egg allergens have been found in both the white and the yolk, and the egg white proteins ovomucoid, ovalbumin (OVA), ovotransferrin, and lysozyme have been described (Poulsen et al., 2001). OVA is a robust allergen and has been widely used to create animal models of food allergy for several years (Dearman et al., 2002; Knippels et al., 2000; Ohtsuka et al., 1996; Satter et al., 2002; van Halteren et al., 1997; Yamashiro et al., 1994). It is therefore necessary to identify and characterize the immunopathological mechanisms of OVA-mediated food allergy.

Chemokine is the general term for the cytokines that possess chemotactic activity for leukocytes and play important roles in allergy. Chemokines, which are 8–10-kD proteins released through secretion, activate and attract leukocytes to inflammatory foci by binding to specific G-protein-coupled seven transmembrane-spanning cell surface receptors (Saito and Chihara, 2001). For eosinophils in particular, migration from the circulation is controlled in part by chemokines through a family of G-protein-coupled chemokine receptors (CCRs). Two subfamilies, CXC and CC chemokines, are distinguished based on the position of the first two cysteines. Some of the chemokines that have chemotactic activity for eosinophils include interleukin IL-5 (Yamaguchi et al., 1988), IL-8 (Warringa et al., 1993), IL-16 (Rand et al., 1991), regulated on activation, normal T cell expressed and secreted RANTES (Kameyoshi et al., 1992), eotaxin (Rothenberg et al., 1995), macrophage inflammatory protein-1 alpha (MIP-1 alpha) (Lukacs et al., 1995), and monocyte chemotactic protein-3 (MCP-3) (Dahinden et al., 1994). Studies of human eosinophils have demonstrated that signaling through CCR3 receptors is a prominent pathway leading to chemotaxis, although several other receptor-ligand interactions also appear to mediate eosinophil recruitment (Borchers et al., 2002). Eosinophils may play significant roles in gastrointestinal pathology.

In food allergic colitis, marked eosinophilia is observed in the gut lamina propria (Jenkins et al., 1984). The eosinophil cationic protein (ECP) concentration in feces also significantly increased after challenge with cow's milk in atopic dermatitis patients



who were positive for the challenge test (Majamaa et al., 1996). Furthermore, a number studies have shown that eosinophils are increased in the mucosae of the digestive tract following diseases of the digestive tract (Denis et al., 2001; Lampinen et al., 2001). Recently, it has been shown in active ulcerative colitis that there is increased influx of activated eosinophils to the intestinal mucosa and an increased release of eosinophil-derived proteins, such as ECP (Lampinen et al., 2001), suggesting that eosinophilic infiltration may contribute to tissue injury and intestinal inflammation. Eosinophils have been shown to express the gene encoding RANTES, and both IL-4 and RANTES have previously been shown to be expressed by mature eosinophils (Velazquez et al., 2000a,b).

RANTES and related molecules constitute the C-C class of the beta chemokine supergene family with inflammatory properties. Compared with the other members, RANTES is a potent chemoattractant and activator of eosinophils and plays significant roles in allergic inflammation (Fujisawa et al., 2000; Saito and Chihara, 2001). Previously, it was shown that RANTES levels are increased in the airways of patients with asthma, and RANTES was found to play an important role in lung allergic inflammation, lung leukocyte infiltration, bronchial hyperresponsiveness, and the recruitment of eosinophils (Baggiolini and Dahinden, 1994; Berkman et al., 1996; Conti and Digioacchino, 2001; Hasegawa et al., 2001; Velazquez et al., 2000a,b). In the skin, levels of RANTES were significantly elevated at antigen-challenge sites (Zweiman et al., 1997), and cutaneous injection of RANTES caused eosinophil recruitment (Beck et al., 1997; Conti and Digioacchino, 2001). Furthermore, a recent study showed that keratinocytes of patients with atopic dermatitis invariably responded with significantly earlier and higher RANTES expression compared to that in psoriasis patients after tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma administration (Giustizieri et al., 2001). In addition, Rajakulasingam et al. (1997) reported that nasal allergen challenge provoked significant increases in RANTES mRNA and protein levels. In the gut, it was demonstrated that RANTES mRNA was expressed in the colon of patients with atopic dermatitis (Yamada et al., 1996). All of these findings suggest a crucial role of RANTES in both acute and chronic inflammatory conditions and that eosinophil infiltration is a key player in the pathological mechanism(s).

In our previous study, we reported that IL-5 played an important role in eosinophil recruitment to the lesion site in the gut of OVA-sensitized mice after challenge (Bae et al., 1999). Despite the increased knowledge about the role of chemokines in allergy, the mechanisms underlying chemotactic factors in intestinal food allergy are not fully understood. We hypothesized that RANTES is activated following food allergy, resulting in gastrointestinal eosinophil infiltration.

MATERIALS AND METHODS

Animals and Immunizations

Seven-week-old female BALB/c mice (CLEA Co., Tokyo, Japan) were used in this study. The mice were housed in the animal facilities of Korea University Medical School and provided with a commercial rodent diet OVA free. They were maintained on a 12 h:12 h light-dark cycle and water was provided ad libitum. All experiments were performed in accordance with the animal use and ethics guidelines of Korea University.



The mice were initially divided into two groups. Group 1 (GRP1) had a total of seventy animals and group 2 (GRP2) had a total 35. GRP1 mice were immunized systemically with OVA 1 μ g in 5 mg of alum as previously described by Tomoe et al. (1992). Group 2 (GRP2) served as negative controls and were injected with alum 5 mg without OVA. The specific IgE antibody in the serum of the sensitized mice was evaluated by passive cutaneous anaphylaxis (PCA), using 7-week-old male Sprague-Dawley rats (CLEA Co.). Positive sensitization was achieved by injecting 1:2 dilutions (beginning at 1:40) of serum from the immunized mice (50 μ l) into the shaved dorsal skin of the rats. After 48 h, the rats received an intravenous injection of OVA 1 mg/1% Evans blue dye 0.5 ml in phosphate-buffered saline (PBS, 137 mM NaCl, 2.4 mM KCl, 10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , pH 7.4). The rats were killed 10 min after the injection, and blue discoloration 5 mm or more in diameter at the injection site was taken as confirmation of a positive reaction.

Antigen Challenge

For antigen challenge, GRP1 animals were further divided into two groups of seventy mice each and designated as group (n = 35) 1 test (GRP1-TEST) and group (n = 35) 1 control (GRP1-CON). The group 2 controls were redesignated as group (n = 35) 2 control (GRP2-CON). Challenges were performed 2 weeks after the final sensitization by delivering OVA through an intragastric cannula. All mice were fasted 18 to 24 h prior to antigen provocation. As a control, oral challenge was also performed using PBS in GRP1-CON and GRP2-CON. The mice were killed 3, 6, 12, and 24 h after antigen challenge, and tissue samples were quickly removed, frozen, and stored at -80°C until used for immunohistochemistry, reverse-transcriptase polymerase chain reaction (RT-PCR) and Southern blotting.

Eosinophil Immunohistochemistry

The jejunum (10 cm proximal to the cecum) was removed from all mice 3, 6, 12, and 24 h after antigen challenge. Cryostat sections (5 μ m) were made from the snap-frozen tissue and stained with chromotrope 2R antibody at 1:000 dilution according to the manufacturer's protocol. Briefly, frozen tissue sections were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature and incubated with 10% goat serum plus 0.3% Triton X-100 in PBS for 60 min to block nonspecific binding. The sections were incubated with the primary antibody at a dilution of 1:200 in PBS, 5% goat serum, and 0.1% Triton X-100 at 4°C overnight, followed by goat anti-rabbit IgG [R&D, MN, USA] at a dilution of 1:300 at room temperature for 2 hours. Hematoxylin was used to stain for eosinophils. Eosinophilic density was estimated as the mean number of cells in 10 microscopic fields at $\times 400$ magnification.

RNA Extraction, RT-PCR, and Southern Blotting

Tissues were washed three times in ice-cold PBS. Total RNA was extracted using the Fast PrepTM FP120 and Fast RNATM Kit Green (BIO 101, Vista, CA, USA). Samples were treated for 10 min at 37°C with 1 μ l (0.75 Units) of RNase-free DNase in 1 $\times$ DNase buffer to digest genomic DNA. cDNA synthesis was performed using



Superscript II (Gibco BRL, Rockville, MD, USA) by reverse transcription in a reaction mixture containing first-strand buffer, 2 μ l of 0.1 M dithiothreitol, 1 μ l of 10 mM dNTP, 100 units of M-MLV reverse transcriptase, and 1 μ l of 100 ng/ μ l Oligo-(dT) primers (Borchers et al., 2002; Dahinden et al., 1994; Jenkins et al., 1984; Kameyoshi et al., 1992; Lukacs et al., 1995; Majamaa et al., 1996; Rothenberg et al., 1995). Three microliters of reverse-transcriptase product was used as a template for primers designed to amplify full-length coding sequences of RANTES (GeneBank accession no.: M77747). The primers were: sense 5'-GCTCCAATCTTGCAGTCGTGTT; and antisense 5'-ATTTCTTGGGTTTCGTGGTTCG. PCR amplifications were carried out in a final volume of 100 μ l containing 1 $\times$ reaction buffer, 2 mM MgCl₂, 0.4 mM each of four dNTPs, 0.5 μ M each of a pair of the primers, 0.5 units of *Taq* DNA polymerase, and 3 μ l of cDNA. The amplification reactions were performed in a thermocycler for 35 cycles, which consisted of 94°C for 1 min, 56°C for 1 min, and 72°C for 1.5 min, followed by a final extension at 72°C for 10 min (Takara Ex Taq™, Takara Shuzo, Shiga, Japan). PCR products were electrophoresed through 1% agarose gel impregnated with ethidium bromide 0.5 μ g/ml. The resulting bands were visualized by transillumination at 312 nm and photographed with a Kodak Digital Science camera. The amplified products were blotted onto Hybond™ N+ membrane (Amersham, Buckinghamshire, UK) using standard methods. An oligonucleotide probe of mouse RANTES was labeled with peroxidase complexes, hybridized, and detected using ECL™ direct nucleic acid labeling and detection systems (Amersham) according to the manufacturer's protocol.

Statistical Analysis

The data are expressed as means $\pm$ standard deviation (SD). Statistical significance was assessed using the paired Student's *t*-test for comparison of mean values between the time course and using one-way ANOVA with Tukey's post hoc test for comparisons of mean values among the three groups. A *P* value of at 0.05 was considered to represent statistical significance.

RESULTS

Morphological Changes and Eosinophil Infiltration

Immunization with OVA elevated the total IgE level and induced the production of OVA-specific IgE antibodies in the sera of treated mice. Compared with baseline OVA-specific IgE levels before challenge (640 ± 12.43 optical density [OD] units), OVA-challenged mice showed significantly elevated IgE level 640 over OD units after intragastric challenge, which were not observed in control animals in GRP1-CON and GRP2-CON.

Histopathological examinations of the intestines confirmed an anaphylactic response in the gut after the oral challenge with OVA, as revealed by marked edema of the intestinal villi (Figure 1a). Examination of the tissue sections revealed that the intestinal mucosa had been infiltrated by eosinophils in the sensitized specimens. This finding suggests that the intestinal mucosa was in an inflammatory state, probably



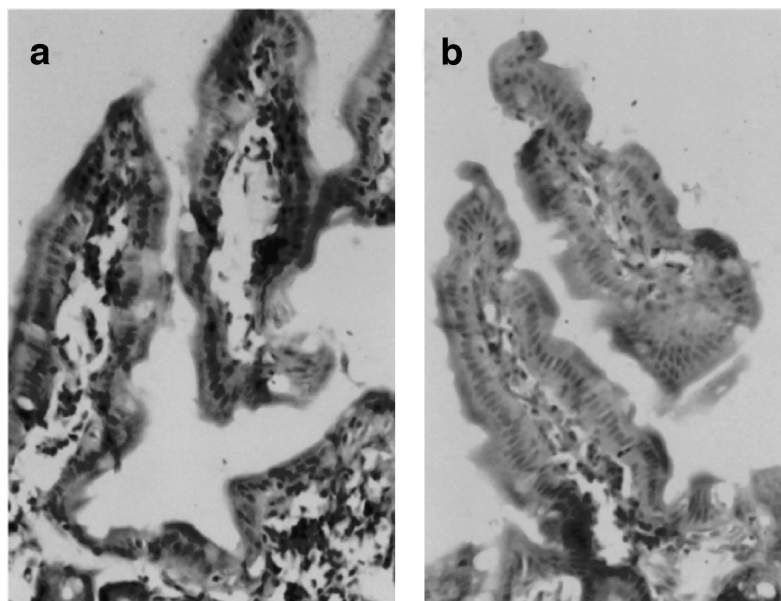


Figure 1. Morphological section of the jejunum showing marked edema of villi and eosinophil infiltration in GRP1-TEST (OVA-sensitized) mice (a) and absence of edema with little or no eosinophil infiltration in GRP1-CON and GRP2-CON (b).

induced by cytokines released by eosinophils. PCA analysis was positive at 300-fold dilution only in OVA-sensitized mice, while it was negative in GRP1-CON and GRP2-CON (data not shown). Positive cells were counted in 10 villi under light microscopy. Activated cells in small bowel epithelium 6 h after challenge in GRP1-TEST (Figure 2) showed an increase of 35.07 ± 6.47 as compared with GRP1-CON (14.53 ± 4.26 positive cells/microscopic field, $**P < 0.01$) and GRP2-CON 16.87 ± 3.93 positive cells, $**P < 0.01$). No edema of the intestinal villi was seen in GRP1-CON and GRP2-CON, and only a few eosinophils infiltrated the gut compared with GRP1-TEST (Figure 1B and Figure 2). Analysis revealed that the chemotactic activity was higher in sensitized mice (GRP1-TEST) than in controls (Figure 2, $**P < 0.01$). The chemotactic activity correlated with the levels of RANTES mRNA expression. Eosinophil infiltration in GRP1-TEST peaked at 6 h and declined thereafter. This time course of eosinophil infiltration is similar to the results of our previous study (Figure 2) (Bae et al., 1999).

RANTES mRNA Expression

The results of RT-PCR and Southern blotting demonstrated that RANTES mRNA expression was increased in the gut of GRP1-TEST, but not in GRP1-CON and GRP2-CON, suggesting OVA-specific induction of RANTES mRNA in the gut (Figure 3). Our results show that sustained transcript expression was detected for up to 24 h postsensitization. The expression of translated RANTES transcripts was confirmed to

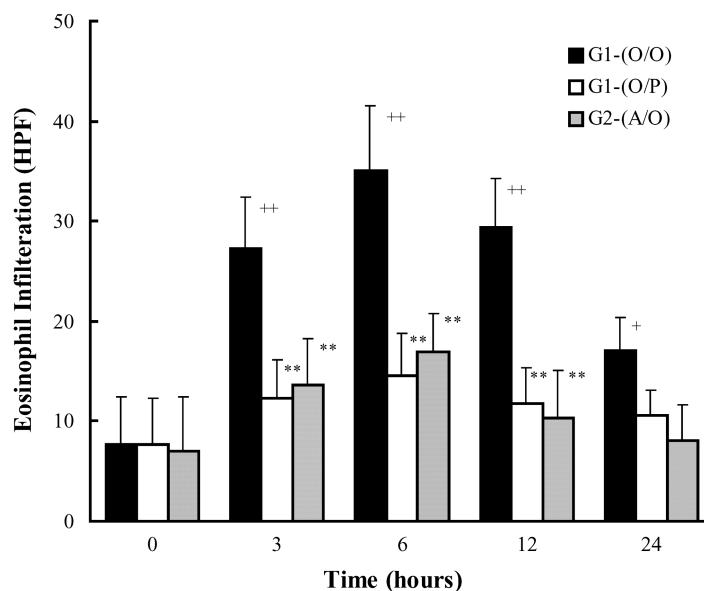


Figure 2. Extent of eosinophil infiltration in the gut of GRP1-TEST, GRP1-CON, and GRP2-CON (mean $\pm$ SD). O/O: OVA sensitization-OVA challenge (GRP1-TEST), O/P: OVA sensitization-PBS challenge (GRP1-CON), A/O: alum sensitization-OVA challenge (GRP2-CON). HPF: high-power field ($\times 400$). + $P < 0.05$ and ++ $P < 0.01$, significantly different in the O/O (GRP1-TEST) at 3, 6, 12 and 24 hours. ** $P < 0.001$, significantly different in the O/O (GRP1-TEST) and O/P (GRP1-CON) or A/O (GRP2-CON).

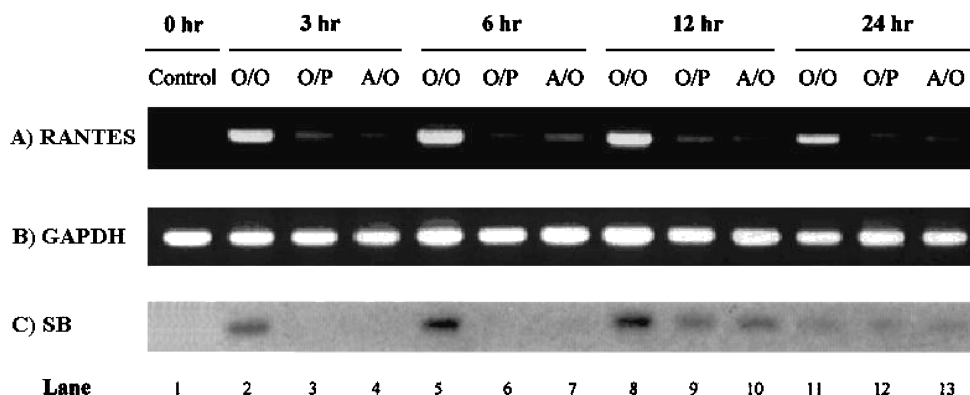


Figure 3. Time course of RANTES mRNA expression in the gut after oral challenge based on RT-PCR = 280 bp RANTES (A upper panel), GAPDH (B middle panel), and Southern blotting (SB; C lower panel) data. Lane 1: untested control, lanes 2, 5, 8, and 11, OVA sensitization-OVA challenge (GRP1-CON), lanes 3, 6, 9, and 12: OVA sensitization-PBS challenge (GRP1-CON), lanes 4, 7, 10, and 13: alum sensitization-OVA challenge (GRP2-CON).

be elevated at 3 and 6 h postchallenge, but declined over the next 18 h to low levels at 24 h (Figure 3).

DISCUSSION

RANTES is considered a crucial factor for eosinophil infiltration in several organs (Baggiolini and Dahinden, 1994; Beck et al., 1997; Berkman et al., 1996; Hasegawa et al., 2001; Velazquez et al., 2000a,b; Zweiman et al., 1997), although the role RANTES plays in eosinophil recruitment in the allergic gut tissue is not fully understood. The purpose of this study was to investigate whether RANTES plays a role in OVA-mediated food allergy in a mouse model by recruiting eosinophils to gastrointestinal lesion sites. Following antigenic challenge, in the OVA-sensitized mice, but not in controls, we observed: 1) marked edema of intestinal villi and eosinophil infiltration of the gut; 2) a peak in eosinophil infiltration 6 h after OVA challenge, supporting our previous results (Bae et al., 1999); 3) increased RANTES mRNA expression that peaked at 6 h and declined over the next 18 h; and 4) a peak increase in RANTES mRNA expression that coincided with increased tissue eosinophil infiltration.

In this study, we observed that an increase in local gut expression of RANTES resulted in increased eosinophil influx to the lamina propria after OVA challenge in sensitized mice. Eosinophils are recognized as central effector cells in the inflamed asthmatic airway (Iwamoto et al., 1992). Eosinophils release toxic basic proteins and lipid mediators such as cysteinyl-leukotrienes that cause bronchial epithelial injury and airflow obstruction (Sampson, 2000). The inflammatory role of RANTES in attracting eosinophils to tissue lesions has previously been reported in the skin and the lungs (Iwamoto et al., 1992; Nakajima et al., 1992). Several other factors are reported to induce RANTES expression. For example, RANTES is strongly induced by respiratory syncytial virus infection in cultures of upper airway epithelial cells (Chung and Kim, 2002). Katayama et al. (2002) also showed that RANTES was induced by TNF- α and was markedly increased by the addition of IFN- γ , resulting in increased eosinophil chemoattractant activity.

As in the skin and lung, our present results showed that local antigen-induced RANTES mRNA expression in the gut was observed as early as 3 h after oral challenge, peaked at 6 h, and declined after 24 h. Peak RANTES expression coincided with peak eosinophil recruitment. This time course of RANTES expression differed from those reported in other studies. In allergic subjects, when RANTES was injected intradermally recruitment of eosinophils was induced with peak responses at 24 h (Beck et al., 1997). In addition, Lukacs et al. demonstrated that accumulation of eosinophils within the lung peaked 24 h after allergen challenge (Lukacs et al., 1996). The time course of local antigen-induced RANTES mRNA expression synchronized with that of eosinophil infiltration in this study. Ronthenberg et al. (1995) demonstrated constitutive expression of eotaxin mRNA in the gut of mice. Yamada et al. (1996) reported RANTES mRNA expression in the colon of patients with atopic dermatitis.

Considering the inflammatory roles of cytokines and chemokines, eosinophil-selective cytokines and chemokines including IL-5, eotaxin, and RANTES may represent targets for novel therapies (Sampson, 2000). In vitro (Lukacs et al., 1996) and in vivo (Venge et al., 1996), pretreatment with specific antibodies for RANTES



induced a significant attenuation in eosinophil infiltration (Katayama et al., 2002). Furthermore, RANTES-mediated chemokine production was inhibited by pertussis toxin, indicating that G-protein-coupled chemokine receptors participate in RANTES signaling (Luo et al., 2002). In this study, we did not attempt to raise RANTES-specific antibodies to determine whether they would inhibit eosinophil influx into the intestine.

In conclusion, our results demonstrated an important role of RANTES in eosinophil infiltration of the gut, as previously reported for the skin and lung. In a study similar to ours, Bae et al. (1999) reported marked eosinophil infiltration of the lamina propria was induced by oral challenge in Balb/c mice intraperitoneally sensitized with OVA, which peaked 6 h after the oral challenge. However, Bae and co-workers did not investigate any involvement of RANTES. Our present findings revealed that OVA induces rapid and transient RANTES mRNA expression and showed that RANTES may play a central role in the pathogenesis of food-mediated gastrointestinal allergy.

ACKNOWLEDGMENTS

This study was partially supported by Grant-in-Aid for Scientific Research College of Medicine, Soonchunhyang University and Grant-in-Aid for JSPS Follows (No. P02525 for T. M.) from Japan Society for promotion of Science (JSPS).

REFERENCES

- Bae, S. J., Tanaka, Y., Hakugawa, J., Katayama, I. (1999). Interleukin-5 involvement in ovalbumin-induced eosinophil infiltration in mouse food-allergy model. *J. Dermatol. Sci.* 21(1):1–7.
- Baggiolini, M., Dahinden, C. A. (1994). CC chemokines in allergic inflammation. *Immunol. Today* 15(3):127–133.
- Beck, L. A., Dalke, S., Leiferman, K. M., Bickel, C. A., Hamilton, R., Rosen, H., Bochner, B. S., Schleimer, R. P. (1997). Cutaneous injection of RANTES causes eosinophil recruitment: comparison of nonallergic and allergic human subjects. *J. Immunol.* 159(6):2962–2972.
- Berkman, N., Krishan, V. L., Gilbey, T., Newton, R., O'Connor, B., Barnes, P. J., Chung, K. F. (1996). Expression of RANTES mRNA and protein in airways of patients with mild asthma. *Am. J. Respir. Crit. Care Med.* 154(6 Pt 1):1804–1811.
- Borchers, M. T., Ansay, T., DeSalle, R., Daugherty, B. L., Shen, H., Metzger, M., Lee, N. A., Lee, J. J. (2002). In vitro assessment of chemokine receptor-ligand interactions mediating mouse eosinophil migration. *J. Leukoc. Biol.* 71(6):1033–1041.
- Chung, H. L., Kim, S. G. (2002). RANTES May be predictive of later recurrent wheezing after respiratory syncytial virus bronchiolitis in infants. *Ann. Allergy, Asthma, & Immun.* 88(5):463–467.
- Conti, P., DiGioacchino, M. (2001). MCP-1 and RANTES are mediators of acute and chronic inflammation. *Allergy Asthma Proc.* 22(3):133–137.
- Dahinden, C. A., Geiser, T., Brunner, T., von Tscharnner, V., Caput, D., Ferrara, P., Minty,



- A., Baggiolini, M. (1994). Monocyte chemotactic protein 3 is a most effective basophil-and eosinophil-activating chemokine. *J. Exp. Med.* 179(2):751–756.
- Dearman, R. J., Caddick, H., Stone, S., Kenna, J. G., Basketter, D. A., Kimber, I. (2002). Immunogenic properties of rapidly digested food proteins following gavage exposure of mice: a comparison of ovalbumin with a potato acid phosphatase preparation. *Food Chem. Toxicol.* 40(5):625–633.
- Denis, M. A., Louis, R., Malaise, M., Belaiche, J., Louis, E. (2001). Increased response of blood eosinophils to various chemotactic agents in quiescent crohn disease. *Scand. J. Gastroenterol.* 36(2):190–195.
- Fujisawa, T., Kato, Y., Nagase, H., Atsuta, J., Terada, A., Iguchi, K., Kamiya, H., Morita, Y., Kitaura, M., Kawasaki, H., Yoshie, O., Hirai, K. (2000). Chemokines induce eosinophil degranulation through CCR-3. *J. Allergy Clin. Immunol.* 106(3):507–513.
- Giustizieri, M. L., Mascia, F., Frezzolini, A., De Pita, O., Chinni, L. M., Giannetti, A., Girolomoni, G., Pastore, S. (2001). Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J. Allergy Clin. Immunol.* 107(5):871–877.
- Hasegawa, S., Tashiro, N., Matsubara, T., Furukawa, S., Ra, C. (2001). A comparison of FcεsRI-mediated RANTES release from human platelets between allergic patients and healthy individuals. *Int. Arch. Allergy Immunol.* 125(Suppl 1):42–47.
- Iwamoto, I., Tomoe, S., Tomioka, H., Takatsu, K., Yoshida, S. (1992). Role of CD4+ T lymphocytes and interleukin-5 in antigen-induced eosinophil recruitment into the site of cutaneous late-phase reaction in mice. *J. Leukoc. Biol.* 52(5):572–578.
- Jenkins, H. R., Pincott, J. R., Soothill, J. F., Milla, P. J., Harries, J. T. (1984). Food allergy: the major cause of infantile colitis. *Arch. Dis. Child.* 59(4):326–329.
- Kameyoshi, Y., Dorschner, A., Mallet, A. I., Christophers, E., Schroder, J. M. (1992). Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J. Exp. Med.* 176(2):587–592.
- Katayama, H., Yokoyama, A., Kohno, N., Sakai, K., Hiwada, K., Yamada, H., Hirai, K. (2002). Production of eosinophilic chemokines by normal pleural mesothelial cells. *Am. J. Respir. Cell. Mol. Biol.* 26(4):398–403.
- Knippels, L. M., van der Kleij, H. P., Koppelman, S. J., Houben, G. F., Penninks, A. H. (2000). Comparison of antibody responses to Hen's egg and cow's milk proteins in orally sensitized rats and food-allergic patients. *Allergy* 55(3):251–258.
- Lampinen, M., Carlson, M., Sangfelt, P., Taha, Y., Thorn, M., Loof, L., Raab, Y., Venge, P. (2001). IL-5 and TNF-alpha participate in recruitment of eosinophils to intestinal mucosa in ulcerative colitis. *Dig. Dis. Sci.* 46(9):2004–2009.
- Lukacs, N. W., Streiter, R. M., Shaklee, C. L., Chensue, S. W., Kunkel, S. L. (1995). Macrophage inflammatory protein-1 alpha influences eosinophil recruitment in antigen-specific airway inflammation. *Eur. J. Immunol.* 25(1):245–251.
- Lukacs, N. W., Standiford, T. J., Chensue, S. W., Kunkel, R. G., Strieter, R. M., Kunkel, S. L. (1996). C-C Chemokine-induced eosinophil chemotaxis during allergic airway inflammation. *J. Leukoc. Biol.* 60(5):573–578.
- Luo, Y., Berman, M. A., Zhai, Q., Fischer, F. R., Abromson-Leeman, S. R., Zhang, Y., Kuziel, W. A., Gerard, C., Dorf, M. E. (2002). RANTES stimulates inflammatory cascades and receptor modulation in murine astrocytes. *Glia* 39(1):19–30.
- Majamaa, H., Miettinen, A., Laine, S., Isolauri, E. (1996). Intestinal inflammation in



- children with atopic eczema: faecal eosinophil cationic protein and tumor necrosis factor- α as non-invasive indicators of food allergy. *Clin. Exp. Allergy* 26(2):181–187.
- Nakajima, H., Iwamoto, I., Tomoe, S., Matsumura, R., Tomioka, H., Takatsu, K., Yoshida, S. (1992). CD4 + T-lymphocytes and Interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am. Rev. Respir. Dis.* 146(2):374–377.
- Ohtsuka, Y., Yamashiro, Y., Maeda, M., Oguchi, S., Shimizu, T., Nagata, S., Yagita, H., Yabuta, K., Okumura, K. (1996). Food antigen activates intraepithelial and lamina propria lymphocytes in food-sensitive enteropathy in mice. *Pediatr. Res.* 39(5):862–866.
- Poulsen, L. K., Hansen, T. K., Norgaard, A., Vestergaard, H., Stahl Skov, P., Bindslev-Jensen, C. (2001). Allergens from fish and egg. *Allergy* 56(Suppl 67):39–42.
- Rajakulasingam, K., Hamid, Q., O'Brien, F., Shotman, E., Jose, P. J., Williams, T. J., Jacobson, M., Barkans, J., Durham, S. R. (1997). RANTES in human allergen-induced rhinitis: cellular source a relation to tissue eosinophilia. *Am. J. Respir. Care Med.* 155(2):696–703.
- Rand, T. H., Cruikshank, W. W., Center, D. M., Weller, P. F. (1991). CD4-mediated stimulation of human eosinophils: lymphocyte chemoattractant factor and other CD4-binding ligands elicit eosinophil migration. *J. Exp. Med.* 173(6):1521–1528.
- Rothenberg, M. E., Luster, A. D., Leder, P. (1995). Murine eotaxin: an eosinophil chemoattractant inducible in endothelial cells and in interleukin 4-induced tumor suppression. *Proc. Natl. Acad. Sci. U. S. A.* 92(19):8960–8964.
- Saito, N., Chihara, J. (2001). Chemokines (RANTES, eotaxin, etc.). *Nippon Rinsho* 59(10):1900–1905.
- Sampson, A. P. (2000). The role of eosinophils and neutrophils in inflammation. *Clin. Exp. Allergy* 30(Suppl 1):22–27.
- Satter, M. A., Sakai, K., Ahmed, S., Yoshino, K., Yamamoto, S., Shimizu, Y., Ota, F. (2002). Low-protein diet induces oral tolerance to ovalbumin in mice. *J. Nutr. Sci. Vitaminol.* 48(1):51–58.
- Tomoe, S., Iwamoto, I., Yoshida, S., Tomioka, H. (1992). The in vivo depletion of CD4 + T cells prevents antigen-induced eosinophil infiltration into mouse skin. *Allergy* 41(6):699–703.
- van Halteren, A. G., van der Cammen, M. J., Biewenga, J., Savelkoul, H. F., Kraal, G. (1997). IgE and mast cell response on intestinal allergen exposure: a murine model to study the onset of food allergy. *J. Allergy Clin. Immunol.* 99(1 Pt 1):94–99.
- Velazquez, J. R., Lacy, P., Mahmudi-Azer, S., Bablitz, B., Milne, C. D., Denburg, J. A., Moqbel, R. (2000a). Interleukin-4 and RANTES expression in maturing eosinophils derived from human cord blood CD34 + progenitors. *Immunology* 101(3):419–425.
- Velazquez, J. R., Lacy, P., Moqbel, R. (2000b). Replenishment of RANTES mRNA expression in activated eosinophils from atopic asthmatics. *Immunology* 99(4):591–599.
- Venge, J., Lampinen, M., Hakansson, L., Rak, S., Venge, P. (1996). Identification of IL-5 and RANTES as the major eosinophil chemoattractants in the asthmatic lung. *J. Allergy Clin. Immunol.* 97(5):1110–1115.
- Warringa, R. A., Mengelers, H. J., Raaijmakers, J. A., Bruijnzeel, P. L., Koenderman, L.



- (1993). Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J. Allergy Clin. Immunol.* 91(6):198–1205.
- Yamada, H., Izutani, R., Chihara, J., Yudate, T., Matsukura, M., Tezuka, T. (1996). RANTES mRNA expression in skin and colon of patients with atopic dermatitis. *Int. Arch. Allergy Immunol.* 111(Suppl 1):19–21.
- Yamaguchi, Y., Hayashi, Y., Sugama, Y., Miura, Y., Kasahara, T., Kitamura, S., Torisu, M., Mita, S., Tominaga, A., Takatsu, K. (1988). Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J. Exp. Med.* 167(5):1737–1742.
- Yamashiro, Y., Ohtsuka, Y., Yabuta, K. (1994). The regulation of intestinal hypersensitivity reactions to ovalbumin by omega-3 fatty acid enriched diet: Studies of IEL and LPL in mucosal damage. *Acta Paediatr. Jpn.* 36(5):550–556.
- Zweiman, B., Kaplan, A. P., Tong, L., Moskovitz, A. R. (1997). Cytokine levels and inflammatory responses in developing late-phase allergic reactions in the skin. *J. Allergy Clin. Immunol.* 100(1):104–109.



Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Order Reprints" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

[Request Permission/Order Reprints](#)

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081IMM120027682>