J. Microbiol. Biotechnol. (2013), 23(11), 1636–1640
http://dx.doi.org/10.4014/jmb.1306.06047

jmb

Effect of Orally Administered *Lactobacillus brevis* HY7401 in a Food Allergy Mouse Model

Jeongmin Lee[1,2], Jieun Bang[1], and Hee-Jong Woo[1]*

[1]*Laboratory of Immunology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea*
[2]*Division of Zoonoses, Center for Immunology and Pathology, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongwon-gun, Chungcheongbuk-do, 363-951, Republic of Korea*

Received: June 19, 2013
Revised: August 23, 2013
Accepted: August 27, 2013

First published online
August 28, 2013

**Corresponding author*
Phone: +82-2-880-1262;
Fax: +82-2-877-8284;
E-mail: hjwoo@snu.ac.kr

pISSN 1017-7825, eISSN 1738-8872

We had found that orally administered *Lactobacillus* species were effective immune modulators in ovalbumin (OVA)-sensitized mice. To validate these findings, we investigated the effects of orally administered *Lactobacillus brevis* HY7401 in OVA-T cell receptor transgenic mice. This strain showed a tendency to induce Th1 cytokines and inhibit Th2 cytokines. All assayed isotypes of OVA-specific antibody were effectively reduced. Systemic anaphylaxis was also relatively reduced with the probiotic administration. These results reveal that *L. brevis* HY7401 might be useful to promote anti-allergic processes through oral administration.

Keywords: Food allergy, probiotics, immunomodulation, Th1 Th2 cytokines, OVA-TCR transgenic mice

Probiotics are live microorganisms that can provide health benefits by improving intestinal microfloral balance. A number of well-characterized strains of lactobacilli and bifidobacteria are used as probiotic supplements to reduce the risk of gastrointestinal disorders and as adjunctive treatments for cancer or infectious diseases and allergies [1, 5]. The regulatory roles of probiotics in allergic responses have been demonstrated by their suppressive or immunomodulatory effects on immune responses, including lymphocyte proliferation, cytokine release, and antigen-specific immunoglobulin production. Modulation of immune activity and enhancement of intestinal barrier function have received the most attention [4].

Previously, we screened *Lactobacillus* species for immunomodulatory effects; in the results, prospective data were obtained from *L. brevis* HY7401 (LB) administration [9]. In this study, to confirm our previous findings, we investigated the effects of oral administration of this strain as an allergic immune modulator in ovalbumin (OVA)-T cell receptor (TCR) transgenic mice. OVA-TCR transgenic mice, which carry an MHC class II restricted rearranged TCR transgene, $Tg(DO_{11.10})_{10}Dlo$, react to OVA peptide antigen (323 to 339 amino acid residues) [11]. These mice raise immune reactions to orally administered OVA without adjuvant and increase total and OVA-specific IgE

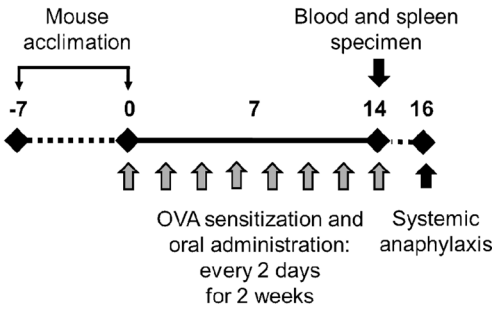

Fig. 1. Time line of OVA sensitization in mice.
All animal experiments were performed under the control of Seoul National University Institutional Animal Care and Use Committee in accordance with the laboratory's animal ethics guidelines. Mice were acclimatized for a week before the first sensitization on day 0. Sensitizations were performed by oral administration with 10 mg of OVA in PBS every 2 days for 2 weeks. The lyophilized probiotics were suspended and orally administered (2 mg per mouse) simultaneously with OVA sensitization. After oral administration, the mice were euthanized for collection of blood and splenocytes. Systemic anaphylaxis was induced by intravenous injection of OVA at 2 days after the end of oral administration.

production. Other well-characterized probiotics including *L. casei* YIT9029 strain Shirota (LC) and *Bifidobacterium longum* HY8001 (BL) (Korea Yakult Co. Ltd.) were used as comparators.

In animal experiments, 30 8-week-old female OVA-TCR transgenic mice (The Jackson Laboratory, Bar Harbor, ME, USA) were randomly divided into five groups: negative control with PBS treatment, positive control with OVA sensitization and without bacteria administration, group 1 with LC administration, group 2 with BL administration, and group 3 with LB administration. The procedures and schedules for OVA sensitization, bacteria administration, and sample collection are presented in Fig. 1 [13].

First, the levels of total and OVA-specific immunoglobulin were measured by sandwich ELISA and indirect ELISA, respectively. The procedures were as described previously [9] and all assays were repeated three times independently. The results are shown in Fig. 2; the values are expressed as

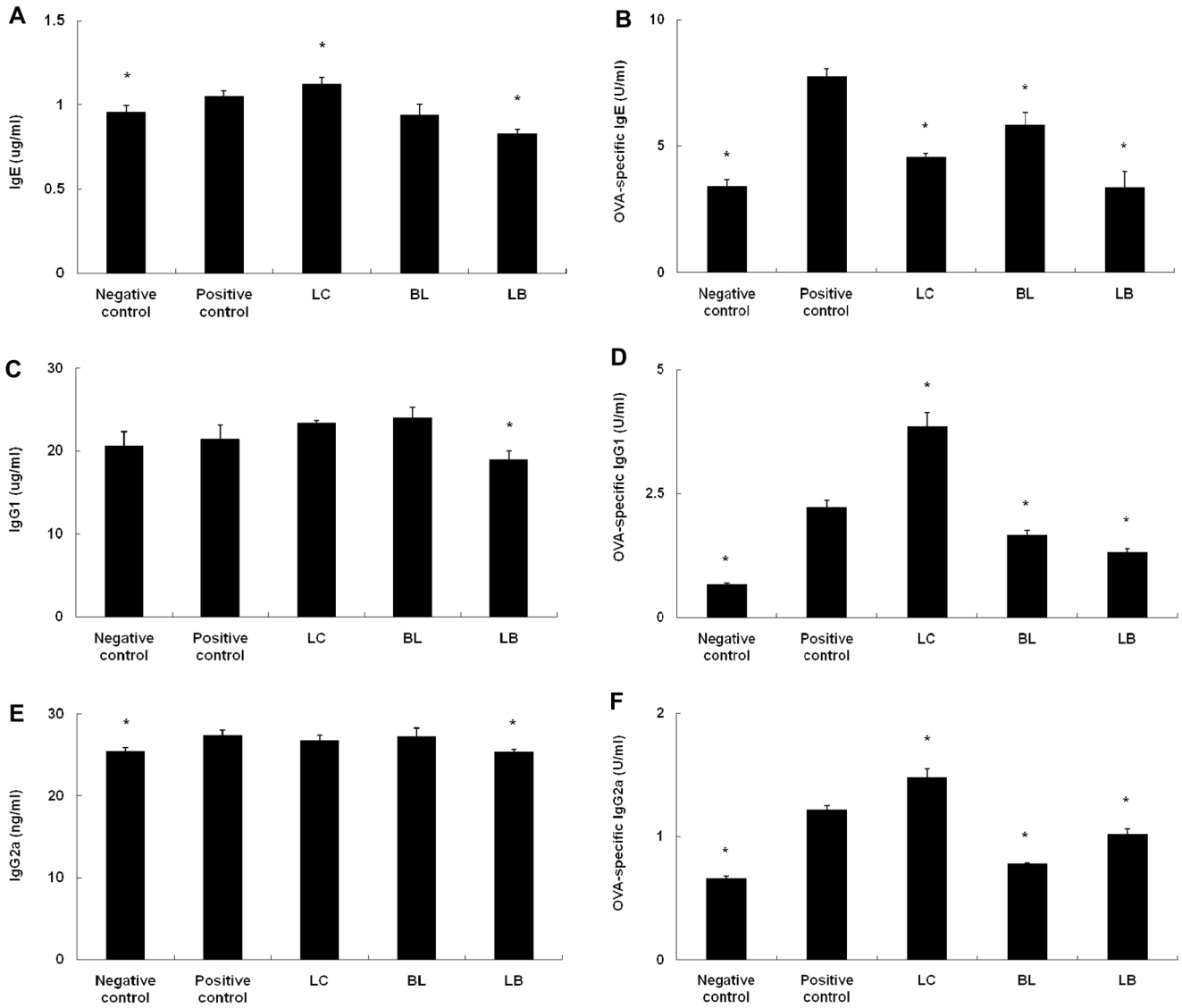

Fig. 2. Oral administration of probiotic bacteria and production of total or OVA-specific IgE (**A** and **B**), IgG1 (**C** and **D**), and IgG2a (**E** and **F**) in serum from OVA-sensitized mice.

Total and OVA-specific imunoglobulins were determined by sandwich ELISA and indirect ELISA, respectively. Serum titers of OVA-specific immunoglobulin were expressed as relative ELISA units, referring to a laboratory standard sera pool; negative control (PBS-treated mice); positive control (OVA-sensitized mice); oral administration with *L. brevis* HY7401 (LB), *L. casei* YIT9029 (LC), and *B. longum* HY8001 (BL). Statistical significance between positive control and each group is indicated with an asterisk ($P < 0.05$).

the mean ± standard deviation. IgE is the most critical molecule in allergic responses. Although OVA administration increased total and OVA-specific IgE production, LB administration reduced total and OVA-specific IgE to levels similar to those in the negative controls (Figs. 2A and 2B). T-helper type 2 (Th2) cytokines, including IL-4 and IL-5, induce antibody switching to IgG1 as well as IgE production; thus, the IgG1 level is involved in immune responses in allergy incubation [19]. In our study, total IgG1 levels did not differ in both negative and positive controls, and a slight decrease of total IgG1 was observed from LB administration (Fig. 2C). OVA-specific IgG1 showed more notable changes. OVA administration significantly increased OVA-specific IgG1 production; this effect was reduced when OVA was administered with LB or BL, but the effect was greater with LC (Fig. 2D). IgG2a is mainly induced by T-helper type 1 (Th1) cytokines; total and OVA-specific IgG2a production was triggered by OVA stimulation and was reduced significantly when LB was administered (Figs. 2E and 2F).

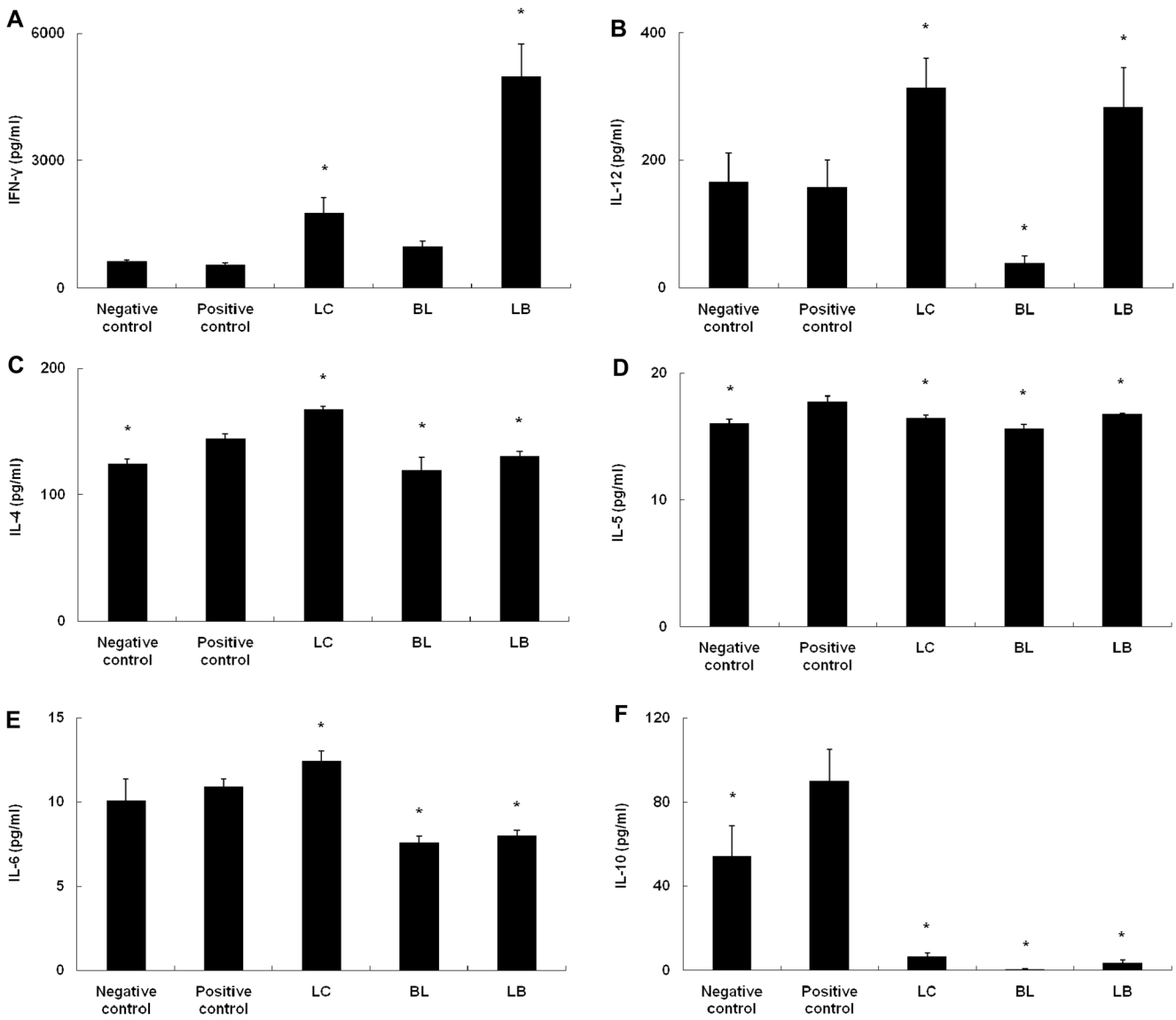

Fig. 3. Oral administration of probiotics and cytokine release from splenocytes isolated from OVA-sensitized mice.
The isolated splenocytes were cultured with OVA for restimulation and the culture supernatants were used to measure cytokines including IFN-γ (**A**), IL-12 (**B**), IL-4 (**C**), IL-5 (**D**), IL-6 (**E**), and IL-10 (**F**) by sandwich ELISA. All samples were tested in triplicate. Statistical significance between positive control and each group is indicated with an asterisk ($P < 0.05$).

To examine cytokine release with orally administered probiotics, splenocytes were tested. The spleen was collected from mice in each experimental group and single-cell suspensions were prepared. Restimulation was performed using 10 μg of OVA. The concentrations of cytokines, including IFN-γ, IL-4, IL-5, IL-6, IL-10, and IL-12, were determined by sandwich ELISA in culture supernatants after 48 h incubation [19].

IFN-γ, which is produced in Th1 cells and known to inhibit allergic responses [17], showed no difference when administered with OVA. However, it dramatically increased in the LB-administered group (Fig. 3A). IFN-γ induces antibody switching to IgG2a; however, in this study, highly elevated IFN-γ by LB administration (Fig. 3A) did not seem to induce IgG2a class switching (Fig. 2F), since both Th1 and Th2 responses can be involved in IgG2a production. In addition, IL-12, which is secreted from antigen-presenting cells and known to activate Th1 cytokine secretion [21], was significantly increased in the LB- and LC-administered groups and decreased in the BL-administered group (Fig. 3B).

Among Th2 cytokines, IL-4, which induces B-cell class switching to produce IgE [20], was decreased in the LB- and BL-administered groups (Fig. 3C). IL-5, which is produced by Th2 cells and enhances IL-4-dependent IgE production [14], was slightly reduced in all experimental groups, but the differences were not exceptional (Fig. 3D). IL-6, secreted from T cells and monocytes, has a role in IL-4–dependent IgE synthesis [3]. IL-6 was significantly reduced in the LB- and BL-administered groups in comparison with the positive control (Fig. 3E). IL-10, which inhibits Th1 responses [2], was dramatically reduced when all experimental bacteria were administered with probiotics (Fig. 3F).

After 2 weeks of administration of OVA and probiotics, OVA was intravenously injected *via* the tail vein to induce systemic anaphylaxis. The results are summarized in Table 1. Severe anaphylaxis (+++) was not observed in this study. LC and BL showed weak anaphylactic reaction with less activity or standing still with an increased respiratory rate. However, no sign of shock was observed in only one animal in the LB group.

We briefly investigated the effects of oral administration of *L. brevis* HY7401 as a putative immune modulator in OVA-TCR transgenic mice. The interpretation of cytokine responses still remains ambiguous and is inconsistent with the results of immunoglobulin production, such as highly induced Th1 cytokines and the negative result of IgG2a production. Although significant strain dependence and different mechanisms may be involved [6–8, 12, 15], our data preliminarily demonstrate that *L. brevis* HY7401 induces Th1 cytokines and inhibits Th2 cytokines. Moreover, all tested isotypes of OVA-specific antibody were effectively reduced. Systemic anaphylaxis was relatively reduced with probiotic administration although it was not significant because of the small number of mice tested. This investigation suggests specific probiotic bacteria such as *L. brevis* HY7401 can be used to promote anti-allergic processes through immune modulation. Further studies are needed to validate the mechanisms of controlling or improving allergic hypersensitivity with this strain.

Table 1. Induction of anaphylaxis[a].

Group	No. of mice showing anaphylactic reaction[b]			
	-	+	++	+++
Negative control	3	0	0	0
Positive control	0	0	3	0
LC	0	3	0	0
BL	0	3	0	0
LB	1	2	0	0

[a]Systemic anaphylaxis was induced by intravenous injection of OVA (1 mg in PBS per mouse) *via* the tail vein after 2 weeks of administration of OVA and probiotics [18].

[b]The extent of anaphylactic shock was measured as follows: no sign of shock (-), less activity or standing still with an increased respiratory rate (+), slight or no response to stimuli (++), and lie-down and suffering by anaphylactic shock (+++) [10, 16].

Acknowledgments

This work was supported by a research grant from Korea Yakult Co., Ltd to H.-J. W. None of the authors have any conflicts of interest to declare.

References

1. Borchers AT, Selmi C, Meyers FJ, Keen CL, Gershwin ME. 2009. Probiotics and immunity. *J. Gastroenterol.* **44:** 26-46.
2. Frossard CP, Eigenmann PA. 2008. The role of IL-10 in preventing food-induced anaphylaxis. *Expert Opin. Biol. Ther.* **8:** 1309-1317.
3. Gajewski TF, Goldwasser E, Fitch FW. 1988. Anti-proliferative effect of IFN-gamma in immune regulation. II. IFN-gamma inhibits the proliferation of murine bone marrow cells stimulated with IL-3, IL-4, or granulocyte-macrophage colony-stimulating factor. *J. Immunol.* **141:** 2635-2642.
4. Gill H, Prasad J. 2008. Probiotics, immunomodulation, and health benefits. *Adv. Exp. Med. Biol.* **606:** 423-454.
5. Huang J, Zhong Y, Cai W, Zhang H, Tang W, Chen B. 2010. The effects of probiotics supplementation timing on an

ovalbumin-sensitized rat model. *FEMS Immunol. Med. Microbiol.* **60:** 132-141.
6. Kim H, Kwack K, Kim DY, Ji GE. 2005. Oral probiotic bacterial administration suppressed allergic responses in an ovalbumin-induced allergy mouse model. *FEMS Immunol. Med. Microbiol.* **45:** 259-267.
7. Kim H, Lee SY, Ji GE. 2005. Timing of *Bifidobacterium* administration influences the development of allergy to ovalbumin in mice. *Biotechnol. Lett.* **27:** 1361-1367.
8. Kim JY, Choi YO, Ji GE. 2008. Effect of oral probiotics (*Bifidobacterium lactis* AD011 and *Lactobacillus acidophilus* AD031) administration on ovalbumin-induced food allergy mouse model. *J. Microbiol. Biotechnol.* **18:** 1393-1400.
9. Lee J, Bang J, Woo HJ. 2013. Immunomodulatory and anti-allergic effects of orally administered *Lactobacillus* species in ovalbumin-sensitized mice. *J. Microbiol. Biotechnol.* **23:** 724-730.
10. McCaskill AC, Hosking CS, Hill DJ. 1984. Anaphylaxis following intranasal challenge of mice sensitized with ovalbumin. *Immunology* **51:** 669-677.
11. Murphy KM, Heimberger AB, Loh DY. 1990. Induction by antigen of intrathymic apoptosis of CD4+CD8+TCRlo thymocytes *in vivo*. *Science* **250:** 1720-1723.
12. Ohno H, Tsunemine S, Isa Y, Shimakawa M, Yamamura H. 2005. Oral administration of *Bifidobacterium bifidum* G9-1 suppresses total and antigen specific immunoglobulin E production in mice. *Biol. Pharm. Bull.* **28:** 1462-1466.
13. Okamoto A, Kawamura T, Kanbe K, Kanamaru Y, Ogawa H, Okumura K, *et al*. 2005. Suppression of serum IgE response and systemic anaphylaxis in a food allergy model by orally administered high-dose TGF-beta. *Int. Immunol.* **17:** 705-712.
14. Pene J, Rousset F, Briere F, Chretien I, Wideman J, Bonnefoy JY, *et al*. 1988. Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen. *Eur. J. Immunol.* **18:** 929-935.
15. Peng S, Lin JY, Lin MY. 2007. Antiallergic effect of milk fermented with lactic acid bacteria in a murine animal model. *J. Agric. Food Chem.* **55:** 5092-5096.
16. Poulsen OM, Hau J, Kollerup J. 1987. Effect of homogenization and pasteurization on the allergenicity of bovine milk analysed by a murine anaphylactic shock model. *Clin. Allergy* **17:** 449-458.
17. Powrie F, Coffman RL. 1993. Cytokine regulation of T-cell function: potential for therapeutic intervention. *Immunol. Today* **14:** 270-274.
18. Shida K, Hachimura S, Ametani A, Ishimori M, Ling M, Hashiguchi M, *et al.* 2000. Serum IgE response to orally ingested antigen: a novel IgE response model with allergen-specific T-cell receptor transgenic mice. *J. Allergy Clin. Immunol.* **105:** 788-795.
19. Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, *et al.* 2002. *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clin. Exp. Allergy* **32:** 563-570.
20. Sokol CL, Barton GM, Farr AG, Medzhitov R. 2008. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9:** 310-318.
21. Temblay JN, Bertelli E, Arques JL, Regoli M, Nicoletti C. 2007. Production of IL-12 by Peyer patch-dendritic cells is critical for the resistance to food allergy. *J. Allergy Clin. Immunol.* **120:** 659-665.