

ORIGINAL ARTICLE

***Listeria* infection inhibits IgE production in regional lymph nodes by suppressing chemotaxis of basophils to lymph nodes**

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ABSTRACT

Infection with *Listeria* induces a dominant shift to the Th1 immune response and inhibits the Th2 response. Papain is frequently utilized in animal models of allergies. Papain administration induces chemotaxis of basophils to regional lymph nodes (LNs) and production of interleukin (IL)-4 by basophils, resulting in a Th2-dominant status and increased IgE production in LNs. In this model, production of immunoglobulin (Ig) E by LN cells is primarily controlled by IL-4 produced by basophils. Based on this model, it was postulated that *Listeria monocytogenes* (Lm) infection suppresses IgE production by LN cells. Therefore, the effects of Lm infection on a papain-induced mouse model of allergies were investigated. Following s.c. injection of papain, basophils transiently migrated to draining LNs because of the effects of chemokine (C–C) motif ligand (CCL) 24 and secreted IL-4, inducing a Th2 response. Lm infection blocked recruitment of basophils into the popliteal LNs by inhibiting CCL24 production. Papain-induced class switch recombination (CSR) to IgE is inhibited by Lm infection, whereas CSR to IgG1 is not affected by the same treatment. Therefore, the CSR of IgG1 to IgE is basophil-dependent, whereas the CSR of IgM to IgG1 is basophil-independent. Hence, Lm infection suppresses CSR to IgE without affecting CSR to IgG1.

Key words basophil, chemokine (C–C) motif ligand 24, *Listeria*, Th2 response.

According to the set of lymphokines they produce, CD4⁺ T cells are divided into two types; namely, IFN- γ -producing Th1 T cells and IL-4-producing Th2 T cells (1–3). Differentiation of T cells into Th1 or Th2 cells is controlled by cytokines produced during the early phase of the innate immune response (4–9). Th2 is thought to be involved in allergic responses. Viral and bacterial infections lead to activation of innate immunity followed by induction of a Th1 T cell subset that is involved in an antigen-specific fashion under the influence of IL-12 and

IFN- γ . Lm infects DCs/macrophages and modulates the host immune response in an antigen-independent manner. We have previously demonstrated that Lm infection affects functional CD4⁺ T cell subset differentiation to inhibit the Th2 response (10).

In light of this background, we postulated that Lm infection regulates the IgE response mediated by Th2. To evaluate this possibility, we used a papain-induced IgE production system (11). Papain, a serine protease, is a potent inducer of Th2 responses. Papain administration

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Received 17 July 2013; revised 10 September 2013; accepted 17 September 2013.

List of Abbreviations: B6, C57BL/6; CCL, chemokine (C–C) motif ligand; CD, cluster of differentiation; cfu, colony forming unit; CSR, class switch recombination; DC, dendritic cell; IFN, interferon; Ig, immunoglobulin; IL, interleukin; *L innocua*, *Listeria innocua*; Lm, *Listeria monocytogenes*; LN, lymph nodes; LPS, lipopolysaccharide; mAb, monoclonal antibody; PLN, popliteal lymph node; SEM, standard error of the mean.

induces basophil chemotaxis to regional LNs and production of IL-4 by basophils, resulting in a Th2-dominant status and increased IgE production in regional LNs. In this model, production of IgE by LN cells is primarily controlled by IL-4 produced by basophils. Based on this model, we postulated that *Listeria* infection suppresses IgE production by LN cells.

In this study, we evaluated whether *Lm* infection modulates the IgE response induced by papain injection via a basophil-mediated pathway. We observed that *Lm* infection inhibits papain-induced Th2 responses and suppresses IgE production. *Lm* infection inhibits recruitment of basophils into the PLNs following s.c. injection of papain into the rear footpads. Therefore, *Lm* infection inhibits papain-induced IgE, but not IgG1, production of PLN cells by inhibiting basophil recruitment to the PLNs.

MATERIALS AND METHODS

Mice

Eight to 10-week-old B6 and BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). Three to five mice were used per group. The mice were reared under specific pathogen-free conditions in the animal facility of Ehime University Graduate School of Medicine. All mice were used in accordance with the institutional guidelines for animal experimentation.

Experimental infection of pathogens

Listeria monocytogenes (EGD strain) and an avirulent strain of *Listeria innocua* were provided by Dr. M. Mitsuyama, Kyoto University, Kyoto, Japan. The bacteria were grown to exponential phase at 37 °C in brain heart infusion broth, washed three times and resuspended in PBS. *Lm* at 1×10^3 cfu were injected s.c. into the mice's rear footpads. Avirulent *L. innocua* at 1×10^6 cfu were similarly injected as controls.

Reagent and antibody

Papain was purchased from Sigma-Aldrich (St. Louis, MO, USA) and diluted to 1 mg/mL with 0.05 M citrate buffer pH 4.5. Diluted papain was injected immediately. Anti-CD28 mAb (PV-1) was a generous gift from Dr. R. Abe, Tokyo Science University, Noda, Japan. Anti-CD3 mAb (2C11) was purified from 2C11 ascites cells. Recombinant mouse cytokines were purchased from Genzyme (Cambridge, MA, USA).

In vitro stimulation of T and B cells

Spleen cells were cultured in the presence or absence of anti-CD3 mAb and anti-CD28 mAb at 37 °C in a 5% CO₂

humidified air atmosphere for 1 day. The amount of IFN- γ and IL-4 produced by the culture was determined by ELISA.

In the papain administration experiments, 1×10^5 PLN cells were stimulated either with a mixture of anti-CD3 mAb and anti-CD28 mAb for 1 day to assess IL-4 and IFN- γ or with 1 μ g/mL of LPS for 5 days to assess IgG1, IgG2a and IgE.

Enzyme-linked immunosorbent assay

Sandwich ELISA established with mAbs purchased from PharMingen (San Diego, CA, USA) was used to detect IFN- γ and IL-4 in the culture supernatant. The amount of IgG1 and IgG2a was detected by sandwich ELISA established with mAbs purchased from PharMingen. Mouse myeloma protein MOPC21 (IgG1) was purchased from Sigma Chemical (St. Louis, MO, USA) and used as a standard. Mouse IgE was detected by ELISA MAX standard SET (BioLegend, San Diego, CA, USA).

RNA isolation and mRNA detection by reverse transcription-enzyme-linked immunosorbent assay

Total cellular RNA was isolated with TRIzol according to the manufactures' protocol. Equal amounts of RNA (1 μ g) were reverse transcribed using reverse transcriptase (MMLV, Promega, Madison, WI, USA). Amplification of β -actin and Ig β was used for sample normalization. PCR primers used for analysis were as follows: productive IgG1, 5'-CTCTGGCCCTGCTTATTGTTG-3' and 5'-ATAGACGATGGGGGTGTTTCG-3'; productive IgE, 5'-CTCTGGCCCTGCTTATTGTTG-3' and 5'-CAGTGCCTTTTACAGGGCTTC-3'; productive IgG2a, 5'-CTCTGGCCCTGCTTATTGTTG-3' and 5'-GCTGGGCCAGGTGCTCGAGGTT-3'; Ig β , 5'-ATGGTGAAGTTTCACTGCTACAC-3' and 5'-AACGTGCTGATCCTAAGACTAGA-3'; β -actin, 5'-ATGG GTCAGAA-GGACTCC-3' and 5'-CCCAAGAAGGAAGGCTGG-3'; IL-4, 5'-TCCTGCTCTTCTTCTCGAATGTACC-3' and 5'-ACGAGTAATCCATTTGCATGATGCTC-3'; IFN- γ , 5'-TGCAGACACACTGCATCTTGG-3' and 5'-CGGGTACCATGAGCTCATTGAATG-3'; mcpt-8, 5'-GGTACAGAGTCCAAACCCCACT-3' and 5'-GTGTCCCAGTACACAAGCGACAAC-3'.

The PCR products were visualized on a 2% agarose gel containing ethidium bromide under ultraviolet illumination. The relative expression of the gene was measured by NIH image software.

Statistical analysis

Student's unpaired *t*-test was used for the statistical analysis.

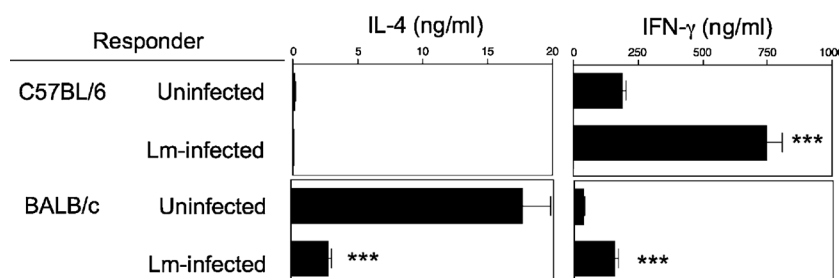


Fig. 1. Reciprocal cytokine production pattern in C57BL/6 and BALB/c mice. C57BL/6 and BALB/c mice were infected with Lm (1×10^3 cfu) in their rear footpads. PLN cells were prepared from Lm-infected mice 5 days after Lm infection. The PLN cells (1×10^5) were stimulated with anti-CD3 plus anti-CD28 for 24 hr and the amount of cytokines in the culture supernatant determined using ELISA. The data represent the findings of one of two experiments with similar results and are presented as mean $\pm$ SEM (***) $P < 0.001$.

RESULTS

Listeria infection prior to papain administration strongly suppresses immunoglobulin E, but not immunoglobulin G1 production

Listeria monocytogenes infection modulates the immune response to a Th1-dominant status and suppresses the Th2 response (Fig. 1). When cultures are stimulated with anti-CD3 antibodies plus anti-CD28 antibodies, T cells primarily produce the observed cytokines (10). In light of this data, we postulated that Lm infection regulates IgE responses mediated by Th2 responses. To evaluate this possibility, we used a papain-induced IgE production system (11). B6 mice are a Th1 dominant strain and the LN cells of untreated B6 mice produce small amounts of Th2 cytokines. Papain injection into the rear footpads of B6 mice strongly induced Th2 cytokine production by PLN cells (Fig. 2a), whereas Th1 cytokine production was profoundly inhibited. Lm injection into the rear footpads prior to papain administration suppressed production of IL-4 and IL-5 by PLN cells. In contrast, IFN- γ production was rather augmented by the same treatment. Augmented IFN- γ production was observed in the group injected with Lm but not papain, indicating that Lm injection interferes with the effects of papain (data not shown). No effects of Lm were observed in an avirulent mutant strain of Lm (*L. innocua*), suggesting that an active infective process is involved in the observed interference. This possibility was confirmed by evidence showing that many Lm reached the PLNs 2 days after injection of Lm into the rear footpads. In contrast, because these bacteria are unable to invade the cytosol, *L. innocua* barely reached the PLNs even after injection of 1000 times the amount of bacteria into the rear footpads of the mice (Fig. 2b).

The augmented Th2 cytokine production accompanied an increased degree of CSR to IgE and IgG1 (Fig. 3a). This resulted in an incremental increase in IgG1 and IgE production by PLN cells (Fig. 3b). Lm infection almost

completely inhibited CSR to IgE and resulted in reduced production of IgE by PLN cells (Fig. 3a,b). To our surprise, however, Lm infection did not interfere with CSR to IgG1 (Fig. 3a,b), thus suggesting that Th2 affects CSR to IgE and IgG1 differently.

Expression of chemokine (C-C) motif ligand 24 is accompanied by expression of Mcpt-8 in the popliteal lymph nodes following papain injection

Following injection of papain into the rear footpads, immune-reactive cells move into the PLNs under the influence of inflammatory chemokines. Expression of chemokines by PLN cells following papain injection was assessed (Fig. 4a and data not shown). mRNA expressions of several chemokines, including CCL2, CCL6, CCL12, CCL19, CCL24, CXCL1 and CXCL11, were upregulated by papain injection into the rear footpads. Of this type of chemokine, only CCL24 (eotaxin-2) was downregulated by injection of Lm prior to papain injection (Fig. 4a). It was also demonstrated that CCL11 (eotaxin) and CCL26 (eotaxin-3) are upregulated by papain injection and slightly downregulated by injection of Lm prior to papain injection (Fig. 4b). Papain administration upregulated expression of CCR3, myeloperoxidase and Mcpt-8, indicating that eosinophils, neutrophils and basophils, respectively, were recruited to the PLNs. Lm infection selectively inhibited incremental increase in expression of Mcpt-8 (Fig. 4c). Because CCL24 is a potent chemo-attractant for eosinophils, basophils and Th2 (12, 13), it has been speculated that basophils are responsible for the initiation of Th2 responses after papain injection (11, 14–16). Therefore, we compared the mRNA expressions of CCL24 and basophil-specific Mcpt-8 after papain injection (17). As shown in Figure 4d, expression of CCL24 and Mcpt-8 is upregulated 1 day after papain injection and sustained thereafter. Lm infection prior to papain injection suppressed mRNA expression of CCL24,

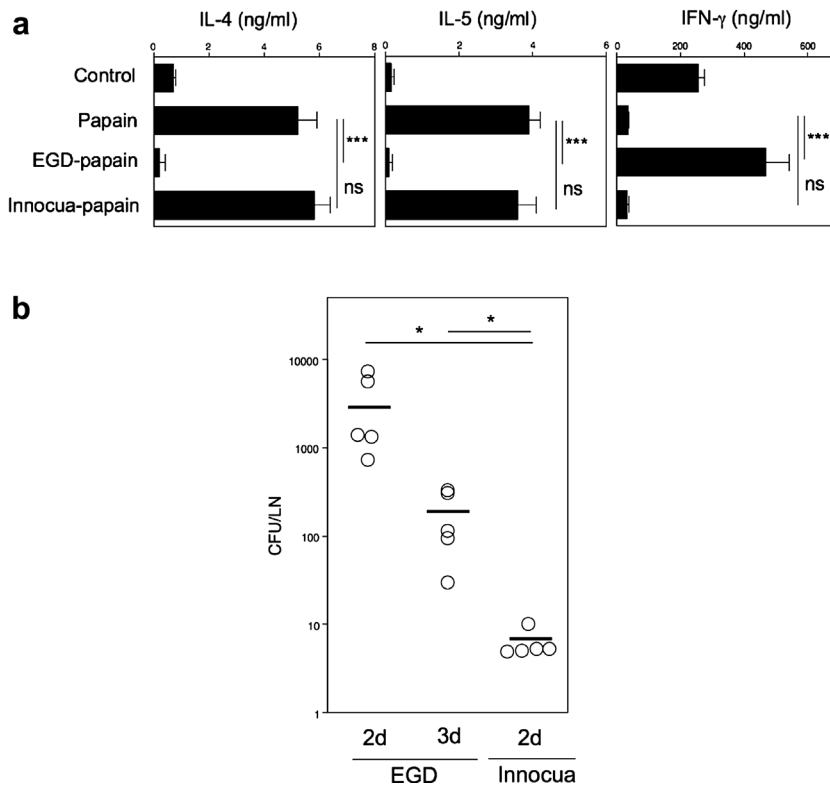


Fig. 2. Lm injection inhibits papain-induced Th2 cytokine production in PLN cells. (a) C57BL/6 mice were infected subcutaneously with *Listeria* (1×10^3 cfu of the EGD strain or 1×10^6 cfu of the *L. innocua* strain) in their rear footpads. The indicated mice were injected with 50 μ g of papain in the rear footpads on the same side as Lm injection the following day. PLN cells were prepared from the treated mice 5 days after papain injection. The PLN cells (1×10^5) were stimulated with anti-CD3 plus anti-CD28 for 24 hr and the amount of cytokines in the culture supernatant determined using ELISA. The data represent the findings of one of three experiments with similar results and are presented as mean $\pm$ SEM (** $P < 0.001$, ns, not significant). (b) The number of *Listeria* in the PLNs in (a) was calculated. The data represent the findings of one of three experiments with similar results (* $P < 0.02$).

this being accompanied by suppression of Mcpt-8 expression (Fig. 4e). Expression of IL-4 was slightly upregulated on day 1, strongly upregulated on day 3 and downregulated by preinfection with Lm prior to papain injection (Fig. 4f). In contrast, expression of IFN- γ was upregulated by Lm infection.

DISCUSSION

We studied the effects of *Listeria* infection on Th1 and Th2 immune responses and demonstrated that Lm infection influences differentiation of the functional CD4⁺ T cell subset to inhibit Th2 responses. *Listeria* infection in DCs/macrophages activates the *Il12p40* gene and induces a shift toward the Th1 subset (10). In addition, *Listeria*-infected DCs/macrophages produce a soluble factor that inhibits Th2 cell function (Saho Maruyama, unpublished observations). Therefore, the immune system shifts predominantly to Th1 during *Listeria* infection, resulting in protection of the host from pathogen infection. In light of this data, we postulated that Lm infection regulates IgE

responses mediated by Th2. To evaluate this possibility, we used a papain-induced IgE production system in which basophils play a central role in inducing IgE responses (15). Sokol *et al.* showed that basophils enter draining LNs by crossing a high endothelial venule (11). Tang *et al.* reported that basophils and DCs function cooperatively to stimulate Th2 cells in papain-induced Th2 responses requiring the DC-derived chemokine, CCL7 (14). In our study, however, CCL7 expression by PLN cells was not upregulated after papain injection. On the other hand, CCL24 was upregulated after papain injection, accompanied by upregulation of Mcpt-8 expression. Although we were unable to determine the primary producer of CCL24 in the present study, it has been reported that epithelial cells produce CCL24 in mice (18) and in humans (19, 20). These findings suggest that, following papain injection, basophils are recruited into the PLNs by CCL24 expressed by PLN cells.

Listeria monocytogenes infection strongly suppressed both IL-4 and IgE production induced by papain

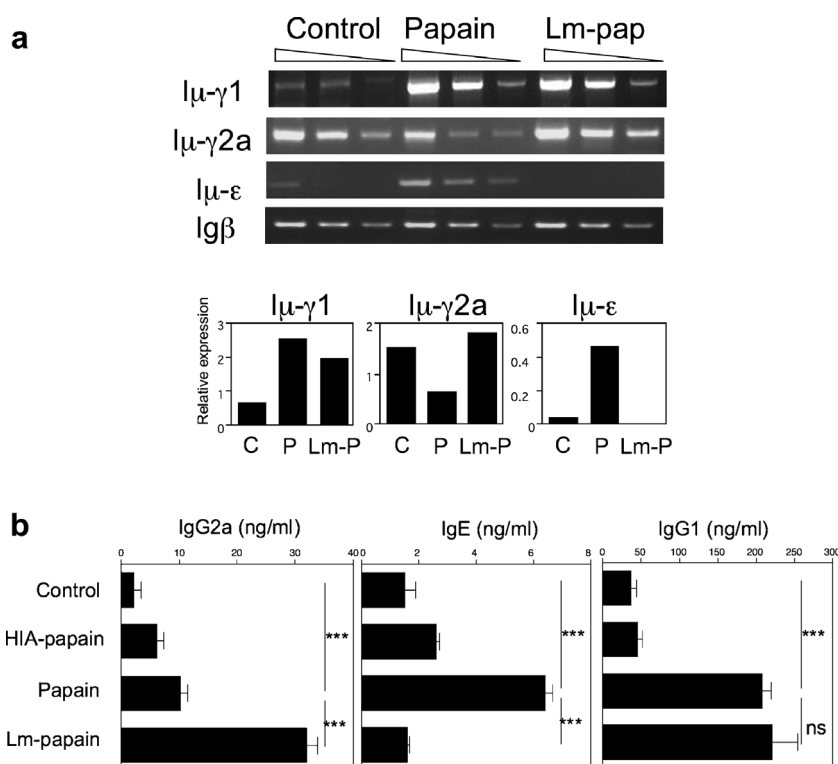


Fig. 3. Lm injection inhibits the papain-induced CSR to IgE but not IgG1 in PLN cells. (a) The treatment of the mice and preparation of the PLN cells were the same as those described in Figure 2. PLN cells (1×10^5) were stimulated with $1 \mu\text{g/mL}$ of LPS for 5 days. CSR to IgG1, IgG2a, and IgE was detected using PCR. The relative expressions of the CSR were calculated using NIH image and corresponding result was shown as bar graphs in parallel. The data represent the findings of one of four experiments with similar results. C, control; P, Papain; Lm-P, Lm-pap. (b) The amount of IgG1, IgG2a, and IgE in the culture supernatant of (a) was determined using ELISA. HIA: heat-inactivated papain. The data represent the findings of one of six experiments with similar results and are presented as the mean $\pm$ SEM (** $P < 0.001$, ns, not significant).

administration. On the other hand, the avirulent strain *L. innocua* and a listeriolysin O-deficient mutant strain did not inhibit IL-4 or IgE production (Fig. 2a and data not shown). As shown in Figure 2b, the difference in the effects of these two Lm strains is attributable to the difference in the number of bacteria that reached the PLNs. These results suggest that *Listeria* escapes from phagosomes and reaches the regional LNs in order to modulate the immune response. The Lm-infected DCs/macrophages migrated into the PLNs to suppress CCL24 expression by PLN cells, resulting in inhibition of basophil recruitment into the PLNs. The inhibition of CCL24 expression in PLNs following Lm injection is interesting because, in contrast, expression of the other chemokines tested was upregulated. The suppression of CCL24 by Lm injection correlated with downregulation of Mcpt-8 expression. Therefore, CCL24 induced by papain injection is responsible for recruitment of Mcpt-8⁺ cells to the PLNs, whereas inhibition of CCL24 results in failure of Mcpt-8⁺ cell recruitment and Th2 responses in PLN cells. The mechanisms underlying the inhibition of production of CCL24 are yet to be clarified.

Immunoglobulin class-switch to IgE is preceded by two steps. It has recently been shown that immature B cells in the bone marrow and spleen class-switch to IgE in a direct μ to ϵ CSR, whereas mature B cells have a propensity to switch via an IgG1 intermediate (21). In the LNs, B cells are helped by follicular helper T cells in the germinal center and undergo CSR of IgM to IgG1, after which B cells move to the T cell zone and undergo CSR of IgG1 to IgE under the influence of Th2. It has been demonstrated in mice that high-affinity IgE antibodies are generated through an IgG1 intermediate (22). Recent studies have shown that DCs are able to directly control the CSR of B cells and suppress IgE production (23). Interestingly, Lm infection 1 day prior to papain administration strongly suppressed IgE, but not IgG1, production. We have yet to determine how Lm infection blocks the CSR of IgG1 to IgE.

According to the present findings, Lm-infected DCs/macrophages move to the regional LNs and inhibit secretion of CCL24 by LN cells, resulting in inhibition of recruitment of basophils to the LNs. Lm-infected DCs/macrophages may affect the CSR of IgG1 to IgE in

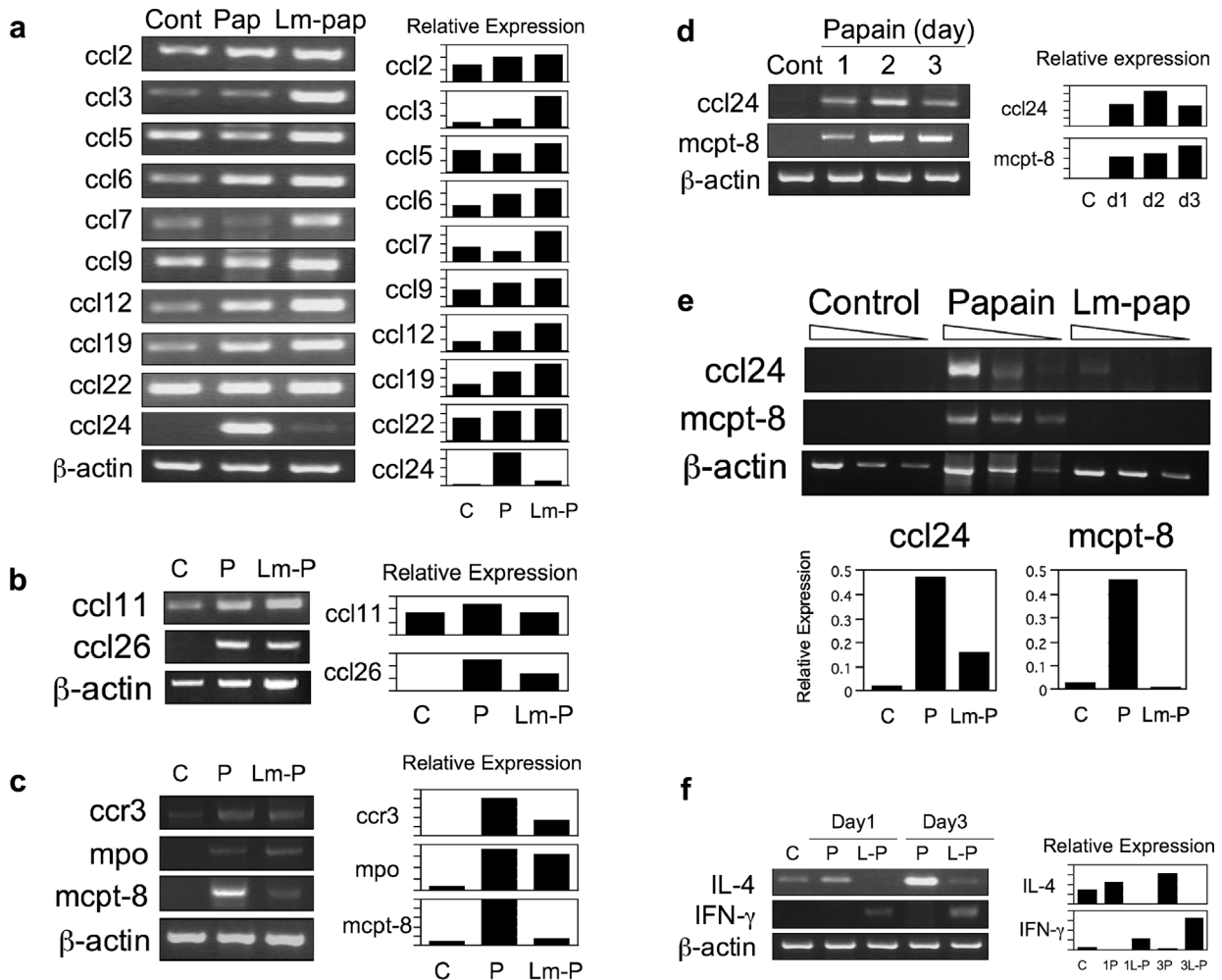


Fig. 4. Correlation between expression of CCL24 and of Mcpt-8. Mice were infected subcutaneously with Lm (1×10^3 cfu) in their rear footpads. The indicated mice were injected with 50 μ g of papain in their rear footpads on the same side as Lm injection the following day. PLN cells were prepared from the treated mice 3 days after papain injection or on the indicated day. Expression of the indicated genes was determined using RT-PCR. (a) CC chemokines. (b) Eotaxin-1 and eotaxin-3. (c) Cell type-specific markers. (d) Kinetics of CCL24 and Mcpt-8 expression. (e) Correlation between expression of CCL24 and of Mcpt-8. (f) IL-4 and IFN- γ . The relative expressions of the genes were calculated using NIH image software: the results are shown as bar graphs in parallel. The data represent the findings of one of two to five experiments with similar results. 1P, day 1 post papain; 1L-P, day 1 post Lm-pap; 3P, day 3 post papain; 3L-P, day 3 post Lm-pap; C, control; d1, day 1 post papain; d2, day 2 post papain; d3, day 3 post papain; Lm-P, Lm-pap; P, Papain.

the T cell zone but not in the germinal center. Although recruitment of basophils to regional LNs was completely inhibited by Lm pretreatment, papain-induced CSR of IgM to IgG1 was not suppressed by the same treatment (Fig. 3b). These results suggest that the CSR of IgM to IgE is basophil-dependent, whereas the CSR of IgM to IgG1 is basophil-independent. Therefore, Lm infection suppresses CSR to IgE without affecting CSR to IgG1.

ACKNOWLEDGEMENTS

We thank Dr. M. Mitsuyama, Kyoto University, Kyoto, Japan for providing us with wild type and mutant *Listeria*.

We thank Dr. R. Abe, Tokyo Science University, Noda, Japan for anti-CD28 monoclonal antibody and Dr. M. Yamashita (Toon city, Ehime, Japan) for helpful discussion. This work was supported in part by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

DISCLOSURE

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this manuscript.

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