

Percutaneous Application of Peptidoglycan from *Staphylococcus aureus* Induces Mast Cell Development in Mouse Spleen

K. Matsui A. Nishikawa

Department of Immunobiology, Meiji Pharmaceutical University, Tokyo, Japan

Key Words

Atopic dermatitis · *Staphylococcus aureus* ·
Peptidoglycan · Mast cell

Abstract

Background: Previously, we found that percutaneous application of peptidoglycan (PEG) from *Staphylococcus aureus* increased the number of mast cells in the dermis, as seen in patients with atopic dermatitis. However, the origin of the mast cells infiltrating the dermis was not known. Our aim was to determine the origin of the skin mast cells induced by the percutaneous PEG application in mice. **Methods:** PEG was applied to barrier-disrupted abdominal skin of mice every 5 days. Twenty days after the first application, mast cells were detected in the spleen cells by alcian blue-safranin staining. Expression of mast cell proteases and cytokines in the skin or spleen was investigated by reverse transcription-polymerase chain reaction. **Results:** Percutaneous application of PEG increased the numbers of alcian blue-positive and safranin-negative mast cells in the spleen. Reverse transcription-polymerase chain reaction analysis of the spleen showed that these mast cells expressed mouse mast cell protease (MMCP)-1, MMCP-4, MMCP-5, and mast cell carboxypeptidase, suggesting that they were immature cells positioned developmentally between mucosal mast cells and connective tissue mast cells. In vitro long-term culture of spleen cells in the presence of PEG induced mast cell development. The features of these mast cells

were coincident with those seen in the spleens of PEG-applied mice. Furthermore, when mice were intradermally injected, their skin showed increased levels of expression of mRNAs of MMCP-4, MMCP-5, and mast cell carboxypeptidase, but not MMCP-1. Similar mRNA expression was observed in the skin of PEG-applied mice.

Conclusions: Percutaneous application of PEG may lead to the development of mast cells in the spleens of mice and contribute to an increase in mast cell numbers in the dermis, as seen in the skin lesions of atopic dermatitis patients.

Copyright © 2006 S. Karger AG, Basel

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease with immunopathologic features that vary depending on the duration of the lesions. Chronic skin lesions of AD patients show an increased number of inflammatory cells such as mast cells, eosinophils, and mononuclear cells in the dermis and superficial *Staphylococcus aureus* colonization [1]. *S. aureus* can be isolated from 75–96% of skin lesions of AD patients, whereas the skin of only 5–10% of healthy individuals is colonized by this organism [2, 3]. Previously, we found that the rate of detection of *S. aureus* on the lesional skin of AD patients was higher than on the non-lesional skin of these patients [3]. Furthermore, the bacterial cell count of *S. aureus* on the lesional skin of AD patients was significantly higher

than that on the skin of healthy individuals or on the non-lesional skin of AD patients. However, there was no significant difference in the rates of detection of *S. aureus* producing superantigenic exotoxin, which is believed to be the most important pathogenic factor, on the lesional and non-lesional skin of AD patients.

Gram-positive bacterial cell walls are composed of highly cross-linked peptidoglycan (PEG) decorated to a variable extent with teichoic acid polymers. The latter are linked to plasma membrane phospholipids, of which lipoteichoic acid (LTA) and PEG are the major surface components [4, 5]. Gram-positive bacteria contain no lipopolysaccharide, and LTA and/or PEG are thought to be major inflammatory products in their cell walls. Therefore, we hypothesized that LTA and/or PEG from *S. aureus* may be pathogenic factors that are more important than superantigenic exotoxins in AD patients [6, 7]. In a previous study, we found that percutaneous application of PEG induced higher numbers of mast cells in murine skin, as is seen in AD patients [8]. However, the origin of the mast cells infiltrating the dermis is not known. In the present work, we describe how percutaneous invasion of PEG leads to the development of immature mast cells in the mouse spleen, and we hypothesize that these mast cells may be the origin of the skin mast cells in PEG-applied mice.

Materials and Methods

PEG derived from *S. aureus* was obtained from Fluka (Buchs SG, Switzerland). The PEG was reconstituted in phosphate-buffered saline (PBS), pH 7.4, at a concentration of 1 mg/ml and sonicated for 1 h before use.

Mice

Female specific pathogen-free BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan) and used at 6–8 weeks of age. They were housed in plastic cages in a clean, air-conditioned room at 24°C and allowed free access to a standard laboratory diet and water. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of Meiji Pharmaceutical University, Tokyo.

Percutaneous Application of PEG

Percutaneous application of PEG to mice was performed as described previously [8]. Briefly, the skin of each mouse was barrier-disrupted by repeatedly (eight times) applying adhesive cellophane tape to the shaved abdominal skin, then pulling it off. Thereafter, 100 µl PEG solution (1 mg/ml in PBS) was applied topically to a 10-cm² area of barrier-disrupted abdominal skin at 0, 5, 10, and 15 days. PEG non-challenged control mice were treated by applying 100 µl PBS to the barrier-disrupted skin in the same manner.

Induction of Production of Mast Cells from Mouse Spleen Cells and Transplantation of Mast Cells into the Skin

Mast cells were induced by the long-term culture of mouse spleen cells, as described by Hu et al. [9]. Briefly, the spleen cells were adjusted to a concentration of 5×10^6 cells/ml in RPMI 1640 medium with *L*-glutamine (Sigma, St. Louis, Mo., USA) containing 10% fetal bovine serum (FBS; Sigma; Lot 118H84041), 25 mM Hepes (Sigma), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco RBL, Grand Island, N.Y., USA). The cultures (1 ml/well) were incubated in 24-well culture plates (Nunc, Roskilde, Denmark) in the presence of PEG (0–1.0 µg/ml) at 37°C in a humidified atmosphere with 5% CO₂. In some experiments, the cultures were incubated with 5 µg/ml anti-interleukin (IL)-3, anti-IL-6, or anti-tumor necrosis factor (TNF)-α polyclonal antibody (Pepro-Tech, London, UK) in the presence of 1 µg/ml PEG. Half of the total volume of the culture medium was changed every 4 days. To maintain their concentrations in the culture, the PEG and antibody were also replenished at the time the medium was changed. Twenty days after the beginning of culture, mast cells were counted by a staining method using alcian blue-safranin, as described elsewhere [10, 11]. To obtain purified mast cells, the mast cells in the culture were separated by Histopaque-1077 (Sigma) from dead cells and small lymphocytes, adjusted to 5×10^4 cells/ml in the culture medium, and a 20-µl volume was injected into the mouse dermis.

Reverse Transcription-Polymerase Chain Reaction Assay

To determine the levels of expression of mRNAs for mouse mast cell protease (MMCP)-1, MMCP-2, MMCP-4, MMCP-5, mast cell carboxypeptidase (MC-CPA), IL-3, IL-6, and TNF-α in the spleen, mast cells, or skin, total RNA was extracted from each sample by the single-step method with TRI-Reagent (Molecular Research Center, Cincinnati, Ohio, USA). Then, cDNA was synthesized from 8 µg of total RNA using a First-Strand cDNA Synthesis Kit (Amersham Biosciences, Piscataway, N.J., USA). Polymerase chain reaction (PCR) was performed using the following primers: β-actin (540 bp; purchased from Clontech, Palo Alto, Calif., USA) 5' primer, 5'-GTGGGCCGCTCTAGGCACCA-3', and 3' primer, 5'-CTCTTTGATGTCACGCACGATTTC-3'; IL-3 (489 bp; purchased from Stratagene, La Jolla, Calif., USA) 5' primer, 5'-GTTCTTGCCAGCTCTACCACCAGC-3', and 3' primer, 5'-CACGGTTCACGGTTAGGAGAGAC-3'; IL-6 (299 bp) [12] 5' primer, 5'-CTCTGCAAGAGACTTCCATC-3', and 3' primer, 5'-ATCCAGTTTGGTAGCATCC-3'; TNF-α (276 bp; purchased from Stratagene) 5' primer, 5'-ATGAGCAGACAAAGCATG-ATC-3', and 3' primer, 5'-TACAGGCTTGCTACTCGAATT-3'; MMCP-1 (460 bp) [13] 5' primer, 5'-GGAAACTGGAGAGAA-AGAACCCTAC-3', and 3' primer, 5'-GACAGCTGGGGACAGA-ATGGGG-3'; MMCP-2 (525 bp) [13] 5' primer, 5'-ATTTTCAT-TGCCTAGTTCCTCTGAC-3', and 3' primer, 5'-CAGGATG-AGAACAGGCTGGGAT-3'; MMCP-4 (454 bp) [13] 5' primer, 5'-GTAATTCCTCTGCCTCGTCCTTC-3', and 3' primer, 5'-GG-ACAGGATGGACACATGCTTT-3'; MMCP-5 (418 bp) [13] 5' primer, 5'-GGCAGAACAAACGTGAATGAGCC-3', and 3' primer, 5'-AAGAACCTTCTGGAAGTCAGGG-3'; MC-CPA (689 bp) [14] 5' primer, 5'-ACACAGGATCGAATGTGGAG-3', and 3' primer, 5'-TAATGCAGGACTTCATGAGC-3'. Each PCR was performed using a GeneAmp PCR System 9700 (Perkin-Elmer Co., Norwalk, Conn., USA) with a 25-µl reaction mixture comprising 1.5 µl cDNA (corresponding to 0.8 µg of total RNA starting material), 200 µM deoxynucleotide triphosphate mixture, 400 nM

of each PCR primer, and 25 U/ml Ex Taq DNA polymerase (Takara, Shiga, Japan). The reaction conditions were as follows: one 4-min cycle at 94°C, 20–35 cycles comprising 45 s at 94°C, 45 s at 61°C, and 2 min at 72°C, followed by one 7-min cycle at 72°C. PCR products were separated on a 2% agarose gel containing ethidium bromide.

Statistics

Data were expressed as means $\pm$ SD, and differences between means were analyzed using Student's *t* test with a two-tailed test of significance. Differences at $p < 0.05$ were considered significant.

Results

Mast Cell Development in the Spleen in Response to Percutaneous PEG Application

The first experiments were carried out to examine the development of mast cells in the spleen after percutaneous application of PEG to mice. Mice received 100 μ g PEG applied to a 10-cm² area of barrier-disrupted abdominal skin every 5 days, and after 20 days, the spleen cells were processed by alcian blue-safranin staining to detect mast cells. Figure 1 shows the numbers of mast cells among 1×10^5 spleen cells from each mouse. Significantly more alcian blue-positive and safranin-negative mast cells (fig. 1a) were detected in the spleens of PEG-applied mice; only a few mast cells were detected in the spleens of untreated and PBS-applied mice, suggesting that PEG application to the skin induced the appearance of mucosal mast cells (MMCs) as alcian blue-positive cells in the spleen. To examine the properties of these mast cells in more detail, we determined their protease patterns. In untreated and PBS-applied mice, MMCP-4, MMCP-5, and MC-CPA mRNAs were weakly and spontaneously expressed in the spleen. However, in the spleens of PEG-applied mice, the levels of expression of these mRNAs were increased, and the expression of MMCP-1 mRNA was newly induced (fig. 2).

Induction of Mast Cell Development from PEG-Stimulated Spleen Cells

Subsequently, to determine whether PEG had the ability to induce mast cell development in vitro, spleen cells were cultured with PEG for 20 days and the number of mast cells was counted. As shown in figure 3b, when spleen cells were cultured long-term with PEG, the number of mast cells per culture increased and was correlated with the PEG concentration. Incubation of spleen cells with medium only did not induce the development of any mast cells in the culture. The mast cells induced by long-term

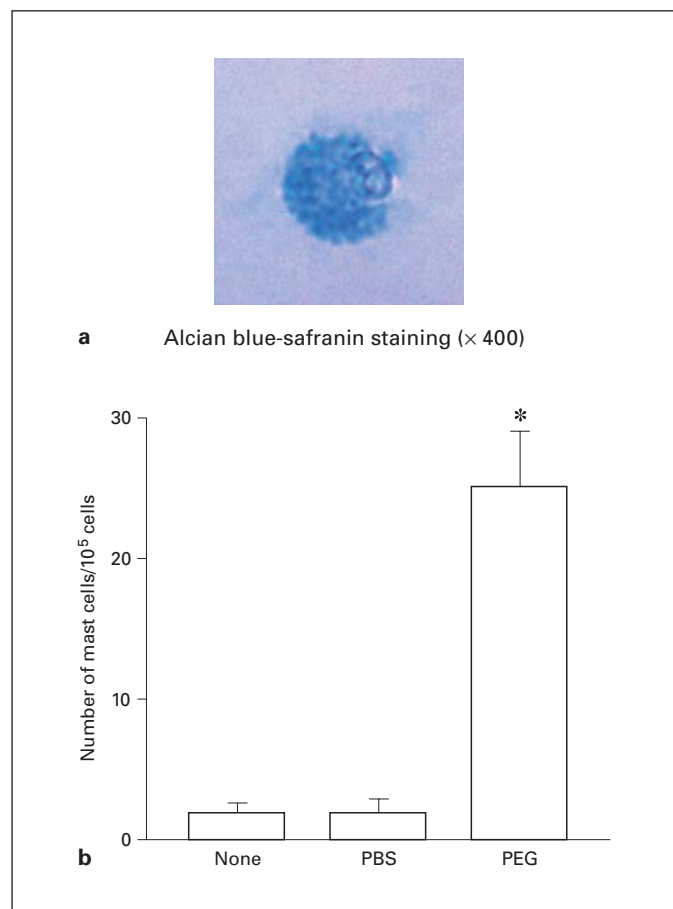


Fig. 1. Morphology and quantification of mast cells in the spleens of PEG-applied mice. Mice received 100 μ l PEG solution (1 mg/ml) applied to a 10-cm² area of barrier-disrupted abdominal skin every 5 days. PBS-treated mice received 100 μ l PBS percutaneously. Spleen cells were prepared 20 days after the first application, stained with alcian blue-safranin (a), and the number of mast cells was counted (b). The results are expressed as means of the number of mast cells $\pm$ SD ($n = 6$) in 10^5 spleen cells. * $p < 0.005$, compared with PBS application.

culture with PEG were confirmed to be alcian-blue positive and safranin negative (fig. 3a). Furthermore, these mast cells expressed mRNAs for MMCP-1, MMCP-4, MMCP-5, and MC-CPA (fig. 4). The properties of staining with alcian blue-safranin and the patterns of mRNA expression of the cultured mast cells were very similar to those of mast cells in the spleens from PEG-applied mice.

Involvement of Inflammatory Cytokines in Mast Cell Development

Next, to determine whether IL-3, IL-6, and TNF- α , which are inflammatory cytokines supporting mast cell in-

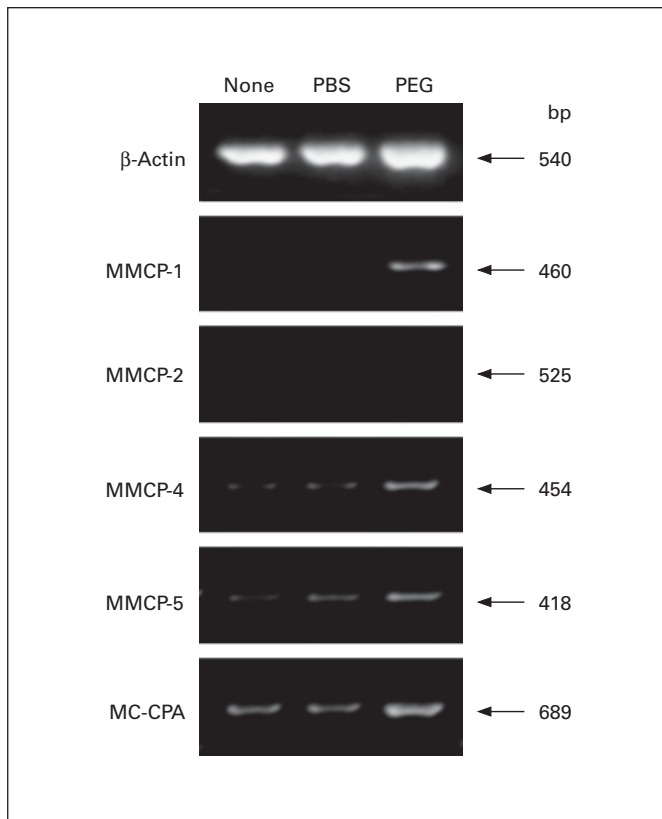


Fig. 2. MMCP and MC-CPA mRNA expression in spleens of PEG-applied mice. Mice received 100 μ l PEG solution (1 mg/ml) applied to a 10-cm² area of barrier-disrupted abdominal skin every 5 days. PBS-treated mice received 100 μ l PBS percutaneously. After 20 days, cytoplasmic RNA was extracted from the spleen of each mouse, reverse-transcribed, and amplified by PCR using β -actin, MMCP-1, MMCP-2, MMCP-4, MMCP-5, and MC-CPA primer sets. Data shown are representative results of four independent experiments.

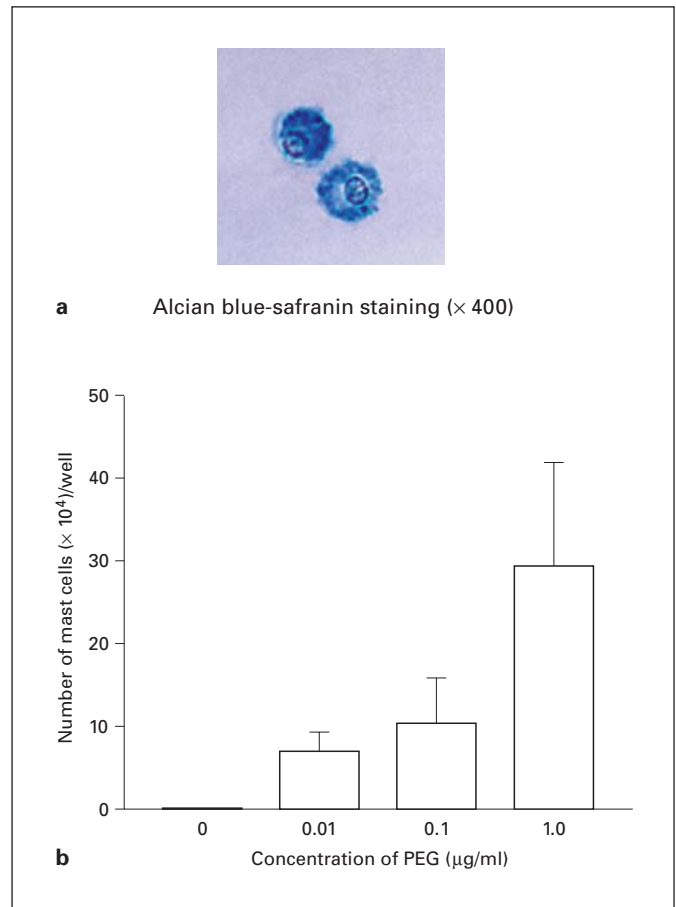
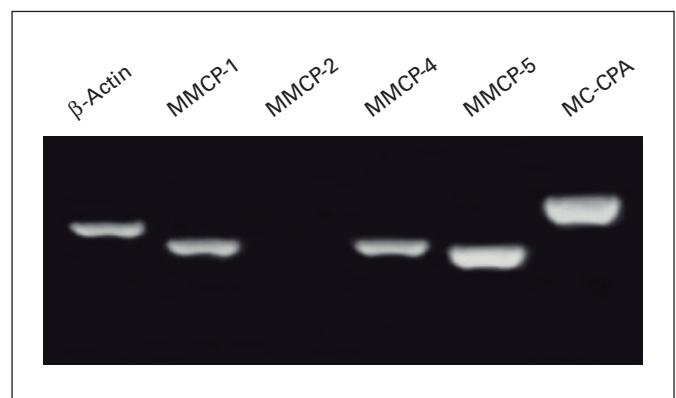


Fig. 3. Morphology and quantification of mast cells induced by long-term culture of mouse spleen cells with PEG. Mouse spleen cells (5×10^6 cells/ml) were incubated with different doses of PEG in 24-well culture plates. To maintain its concentration in the culture, PEG was replenished at the time of changing half the volume of the medium. Mast cells were stained by alcian blue-safranin on day 20 (**a**) and the number of mast cells was counted (**b**). Results are expressed as means $\pm$ SD ($n = 6$) of the number of mast cells per well.

Fig. 4. MMCP and MC-CPA mRNA expression in mast cells induced by long-term culture of mouse spleen cells with PEG. Mouse spleen cells (5×10^6 cells/ml) were incubated with 1 μ g/ml PEG in 6-well culture plates. To maintain its concentration in the culture, PEG was replenished at the time of changing half of the volume of the medium. Mast cells were recovered by Histopaque-1077 on day 20, and MMCP and MC-CPA mRNA expression by mast cells was confirmed as described in figure 2. Data are shown as representative results of four independent experiments.



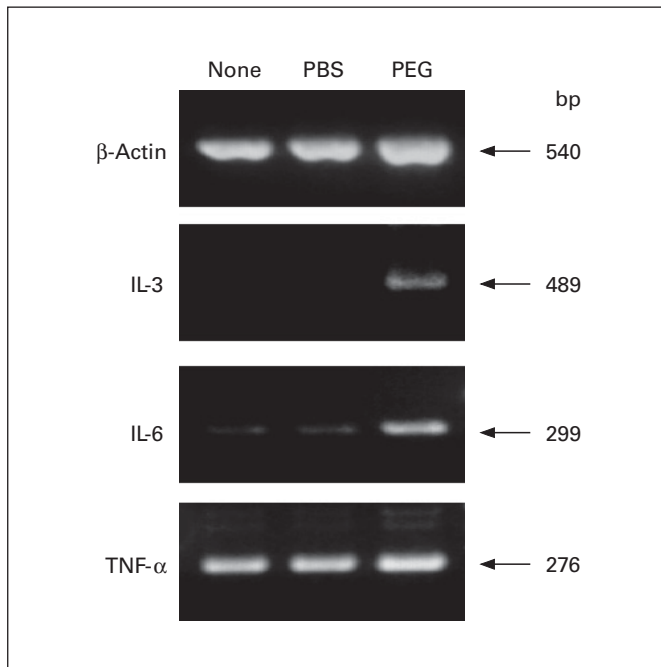


Fig. 5. IL-3, IL-6, and TNF- α mRNA expression in spleens from PEG-applied mice. Mice received 100 μ l PEG solution (1 mg/ml) applied to a 10-cm² area of barrier-disrupted abdominal skin every 5 days. PBS-treated mice received 100 μ l PBS percutaneously. After 20 days, cytoplasmic RNA was extracted from the spleen of each mouse, reverse-transcribed, and amplified by PCR using β -actin, IL-3, IL-6, and TNF- α primer sets. Data shown are representative results of four independent experiments.

duction, are produced in the spleens of PEG-applied mice, cytokine mRNA expression in the spleen 20 days after PEG application every 5 days, starting on day 0, was confirmed by RT-PCR analysis. Figure 5 shows that in the spleens of PEG-applied mice, levels of IL-3 and IL-6 mRNAs increased, but not that of TNF- α mRNA. Furthermore, mast cell development in vitro in response to PEG stimulation was inhibited in the presence of anti-IL-3 antibody and anti-IL-6 antibody, but not anti-TNF- α antibody (fig. 6). The combination of anti-IL-3 and anti-IL-6 antibodies acted to synergistically inhibit mast cell development.

Expression of MMCP and MC-CPA in the Skin of PEG-Applied Mice and Mast Cell-Injected Mice

We previously reported that percutaneous application of PEG increases the number of mast cells in the dermis [8]. Therefore, we compared the patterns of expression of mRNAs of MMCP and MC-CPA in the skin of PBS- and PEG-applied mice. As shown in figure 7a, the skin of

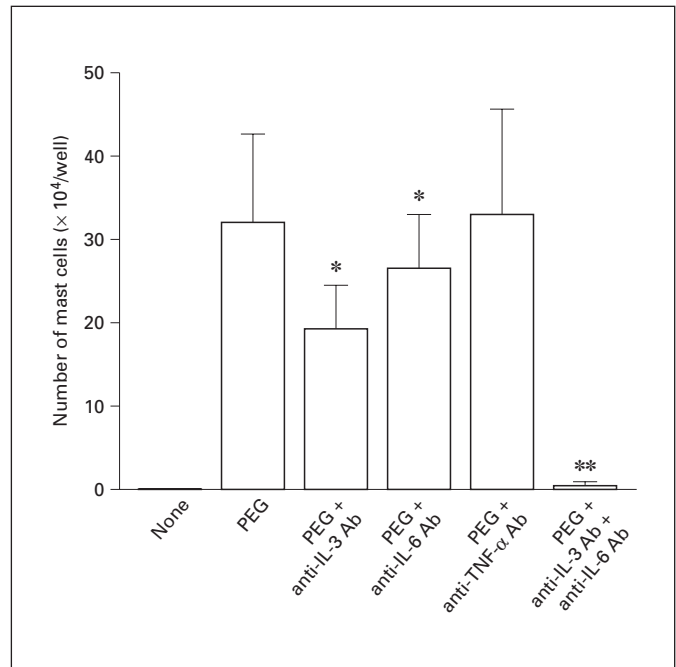
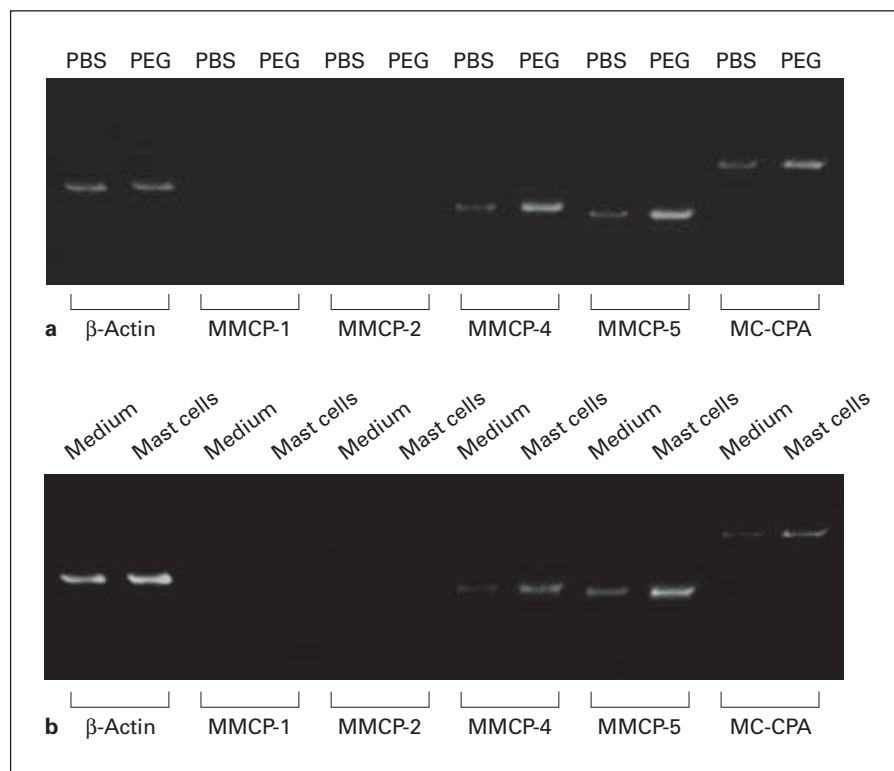


Fig. 6. Neutralization of PEG-induced mast cell development. Mouse spleen cells (5×10^6 cells/ml) were incubated with 1 μ g/ml PEG in the presence of 5 μ g/ml anti-IL-3, anti-IL-6, or anti-TNF- α antibody (Ab) in 24-well culture plates. To maintain their concentrations in the culture, PEG and each neutralizing antibody were replenished at the time of changing half the volume of the medium. Mast cells were stained by alcian blue-safranin on day 20 and the numbers of mast cells were counted. Results are expressed as means $\pm$ SD ($n = 6$) of the number of mast cells per well. * $p < 0.05$, ** $p < 0.001$, compared with PEG treatment.

PEG-applied mice expressed mRNAs for MMCP-4, MMCP-5, and MC-CPA at increased levels compared with those seen in the skin of normal (data not shown) or PBS-applied mice. However, mRNAs of MMCP-1 and MMCP-2 were not detected in any of the groups, including the PEG-applied mice.

As described above, the mast cells induced by long-term culture of spleen cells with PEG expressed mRNAs for MMCP-1, MMCP-4, MMCP-5, and MC-CPA. These mast cells in culture were purified by Histopaque-1077 and injected into the dermis of normal mice. Twenty days later, the skin was cut out and processed for detection of mRNAs of MMCP and MC-CPA. Figure 7b shows that the skin of mice injected with mast cells had increased levels of expression of mRNAs of MMCP-4, MMCP-5, and MC-CPA compared with the skin from culture medium-injected mice. However, expression of MMCP-1 mRNA, which was detected in the cultured mast cells, was not observed.

Fig. 7. MMCP and MC-CPA mRNA expression in the skin from a PEG-applied mouse and an MMC-injected mouse. Mice received 100 μ l PEG solution (1 mg/ml) applied to a 10-cm² area of barrier-disrupted abdominal skin every 5 days. PBS-treated mice received 100 μ l PBS percutaneously. After 20 days, cytoplasmic RNA was extracted from the skin of each mouse, reverse-transcribed, and amplified by PCR using β -actin, MMCP-1, MMCP-2, MMCP-4, MMCP-5, and MC-CPA primer sets (**a**). Another group of mice was injected intradermally with 20 μ l culture medium or MMC suspension (5×10^4 cells/ml), as induced by long-term culture of mouse spleen cells with PEG (fig. 4), and skin samples were removed 20 days later and processed for RT-PCR using the same primer sets (**b**). Data shown are representative results of four independent experiments.



Discussion

Mast cell numbers are increased in the lesional skin of AD patients [15–17] and release inflammatory chemical mediators, such as histamine, leukotrienes, and prostaglandins, upon immunoglobulin-E-mediated stimulation with specific allergens. Furthermore, mast cells produce a wide variety of cytokines, suggesting their active participation in the late-phase reaction and the chronic stage of inflammation [18, 19]. We previously reported that the skin of mice to which PEG had been applied showed an increased number of mast cells compared with the skin of mice that had only received PBS [8]. In addition, our previous observations suggested that the induction of mast cell migration into the dermis in response to PEG application depended on PEG-induced transforming growth factor- β_1 production in the epidermis. However, the origin of the mast cells whose appearance was induced by PEG application to the skin was not clarified.

Our present study showed that percutaneous PEG application to mice induced the appearance of alcian blue-positive mast cells in the spleen. Furthermore, long-term culture of spleen cells in vitro in the presence of PEG induced alcian blue-positive and safranin-negative mast

cells. However, the production of these immature mast cells in the lymph nodes was not induced by PEG application (data not shown). Culture of lymph node cells in vitro with PEG did not induce mast cell production either. Since an alternative route, intradermal injection of PEG, was able to induce alcian blue-positive and safranin-negative mast cells in the spleen (data not shown), it was thought that PEG applied percutaneously translocated to the regional lymph nodes and then to the spleen through the bloodstream. To our knowledge, no reports have been found about mast cell development in the human spleen. However, in the murine system, it has been confirmed that mast cells are derived from progenitors that originate in bone marrow and can then reside in the spleen [20]. The progenitors in the spleen are present in very small numbers and cannot be identified as mast cells by morphology and histochemistry. Based on the data presented, spleen cells include progenitors for mast cells, and percutaneous application of PEG to mice resulted in the development of MMCs as alcian blue-positive mast cells in the spleen.

Mouse mast cells are classified into two subtypes, MMCs and connective tissue mast cells (CTMCs), both of which are derived from common precursor cells [21].

Mature mast cells retain more intracellular granules containing mast cell-specific proteases than do undifferentiated mast cells [22–24]. Whereas the proteases in MMCs are primarily MMCP-1 [25] and MMCP-2 [26], in CTMCs, the main granular proteases are MMCP-4 [27], MMCP-5 [28], and MC-CPA [29]. The expression of each mast cell protease changes during differentiation. Therefore, examination of the expression of granular proteases is one way to assess the subtypes of mast cells. Interestingly, the spleens from the PEG-applied mice expressed MMCP-1 mRNA in addition to the CTMC markers, MMCP-4, MMCP-5, and MC-CPA. The pattern of MMCP and MC-CPA mRNA expression in the mast cells induced by long-term culture of spleen cells with PEG was coincident with that seen in the spleens of PEG-applied mice. These results suggest that mast cells that develop in the spleens of PEG-applied mice are immature cells developmentally positioned between MMCs and CTMCs, and that they may be induced by PEG-dependent differentiation of mast cell progenitors in the spleen.

Hu et al. [9] reported a method for induction of MMCs by long-term culture of mouse spleen cells without the use of exogenous IL-3, which supports mast cell proliferation. They also demonstrated that FBS was important, that by this system, sera could be divided into mast cell-inducing and non-mast cell-inducing sera, and that lipopolysaccharide contamination of the FBS was responsible for mast cell induction [30]. Subsequently, they found that endogenous IL-3 production in the culture was essential, although IL-3 alone was not enough, and that inflammatory cytokines such as TNF- α or IL-6 were also important for mast cell development [31]. Therefore, in our in vitro experiments, non-mast cell-inducing FBS was used and the influence of PEG on mast cell development was confirmed. The in vitro mast cell development induced by PEG was significantly inhibited by the combination of anti-IL-3 and anti-IL-6 antibodies. Furthermore, in in vivo experiments, we confirmed the induction of IL-3 and IL-6 mRNA expression in the spleens of PEG-applied mice. These results suggest that the mast cell development induced by percutaneous PEG application is associated with PEG-induced IL-3 and IL-6 production in the spleen. Since PEG is well known as a toll-like receptor 2 (TLR2) agonist [32], signaling through TLR2 on splenic macrophages and/or dendritic cells would be associated with IL-3 and IL-6 production in the spleen. Removal of macrophages actually reduced the mast cell development in the spleen [31]. However, the possibility that TLR2 may exist on mast cell progenitors and support their maturation has remained to be definitively elucidated.

Levi-Schaffer et al. [33] showed that suspension-cultured mast cells started to synthesize heparin proteoglycan when the mast cells were transferred onto a monolayer of skin-derived fibroblasts. Their report suggests that the phenotypes of mast cells are determined by the anatomical microenvironment in which their final differentiation occurs, and that fibroblasts would be a component of the microenvironment that induces the differentiation of cultured mast cells into CTMCs. When mast cells induced in vitro by PEG stimulation were transplanted into the dermis of mice, the expression of MMCP-1 mRNA, a marker of MMCs, in the skin had disappeared 20 days later, although the expression of mRNAs of MMCP-4, MMCP-5, and MC-CPA, markers of CTMCs, was preserved in the skin. This pattern of expression of mRNAs of MMCP and MC-CPA in the skin was coincident with that in the skin of mice given percutaneous PEG. These results suggest that the percutaneous application of PEG induces IL-3 and IL-6 production in the spleen and that immature mast cells subsequently develop, move to the dermis, and differentiate into CTMCs which are defective in MMCP-1. Probably, migration of immature mast cells into the dermis is supported by transforming growth factor- β_1 production by PEG-stimulated skin keratinocytes, as described previously [8].

Previous reports have not fully explained the role of *S. aureus* in mast cell induction in the skin lesions of AD patients. As the skin of most AD patients shows superficial *S. aureus* colonization and barrier disruption due to reduced ceramide levels [34], a bacterial cell wall component such as PEG would be expected to penetrate their skin. As the density of *S. aureus* in AD patients exceeds at least 1×10^7 organisms/cm² of their skin lesions [35], sustained *S. aureus* colonization could lead to elevated blood levels of PEG to affect the spleen. Our present results provide the evidence that the increase in mast cell numbers in the spleen is related to PEG-induced inflammatory mediators and suggest that *S. aureus* colonization plays a critical role in perpetuating skin tissue inflammation through the excessive induction of development of mast cells by PEG. Therefore, a combination of antimicrobial treatment and anti-inflammatory therapy in at least a subgroup of AD patients, irrespective of whether they show clinical signs of superinfection, may be considered as a new therapeutic strategy for AD.

References

- ▶ 1 Leung DYM: Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. *J Allergy Clin Immunol* 1995;96:302–319.
- ▶ 2 Gloor M, Peters G, Stoika D: On the resident aerobic bacterial skin flora in unaffected skin of patients with atopic dermatitis and in normal controls. *Dermatologia* 1992;164:258–265.
- ▶ 3 Matsui K, Nishikawa A, Suto H, Tsuboi R, Ogawa H: Comparative study of *Staphylococcus aureus* isolated from lesional and non-lesional skin of atopic dermatitis. *Microbiol Immunol* 2000;44:945–947.
- ▶ 4 Lambert PA, Hancock IC, Baddiley J: Occurrence and function of membrane teichoic acids. *Biochim Biophys Acta* 1977;472:1–12.
- 5 Fischer W: Bacterial phosphoglycolipids and lipoteichoic acids; in Hannah DJ (ed): *Handbook of Lipid Research*. New York, Plenum, 1989, pp 123–234.
- ▶ 6 Matsui K, Motohashi R, Nishikawa A: Cell wall components of *Staphylococcus aureus* induce interleukin-5 production in patients with atopic dermatitis. *J Interferon Cytokine Res* 2000;20:321–324.
- ▶ 7 Matsui K, Nishikawa A: Lipoteichoic acid from *Staphylococcus aureus* induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen. *Clin Exp Allergy* 2002;32:783–788.
- ▶ 8 Matsui K, Nishikawa A: Percutaneous application of peptidoglycan from *Staphylococcus aureus* induces an increase in mast cell numbers in the dermis of mice. *Clin Exp Allergy* 2005;35:382–387.
- ▶ 9 Hu Z-Q, Yoshida T, Shimamura T: Induction and identification of mast cells from long-term culture of mouse spleen cells without conditioned medium. *J Immunol Methods* 1992;149:173–181.
- ▶ 10 Gilbert HS, Ornstein L: Basophil counting with a new staining method using alcian blue. *Blood* 1975;46:279–286.
- ▶ 11 Valchanov KP, Proctor GB: Enzyme histochemistry of tryptase in stomach mucosal mast cells of the mouse. *J Histochem Cytochem* 1999;47:617–622.
- ▶ 12 Kondo H, Ichikawa Y, Imokawa G: Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur J Immunol* 1998;28:769–779.
- ▶ 13 Miller HRP, Wright SH, Knight PA, Thornton EM: A novel function for transforming growth factor- β_1 : upregulation of the expression and the IgE-independent extracellular release of a mucosal mast cell granule-specific β -chymase, mouse mast protease-1. *Blood* 1999;93:3473–3486.
- ▶ 14 Tsai M, Miyamoto M, Tam SY, Wang ZS, Galli SJ: Detection of mouse mast cell-associated protease mRNA: heparinase treatment greatly improves RT-PCR of tissues containing mast cell heparin. *Am J Pathol* 1995;146:335–343.
- ▶ 15 Mikhail GR, Miller-Milinska A: Mast cell population in human skin. *J Invest Dermatol* 1964;43:249–254.
- ▶ 16 Mihm MCJ, Soter NA, Dvorak HF, Austen KF: The structure of normal skin and the morphology of atopic eczema. *J Invest Dermatol* 1976;67:305–312.
- ▶ 17 Damsgaard TE, Olesen AB, Sørensen FB, Thestrup-Pedersen K, Schiøtz PO: Mast cells and atopic dermatitis. Stereological quantification of mast cells in atopic dermatitis and normal human skin. *Arch Dermatol Res* 1997;289:256–260.
- ▶ 18 Harvima IT, Horsmanheimo L, Nankkariinen A, Horsmanheimo M: Mast cell proteinases and cytokines in skin inflammation. *Arch Dermatol Res* 1994;287:61–67.
- ▶ 19 Bradding P: Human mast cell cytokines. *Clin Exp Allergy* 1996;26:13–19.
- ▶ 20 Kitamura K, Yokoyama M, Matsuda H, Ohno T, Mori KJ: Spleen colony-forming cells as common precursor for tissue mast cells and granulocytes. *Nature* 1981;291:159–160.
- ▶ 21 Kobayashi T, Nakano T, Nakahata T, Asai H, Yagi Y, Tsuji K, Komiyama A, Akabane T, Kojima S, Kitamura Y: Formation of mast cell colonies in methylcellulose by mouse peritoneal cells and differentiation of these cloned cells in both the skin and the gastric mucosa of W/W^v mice: evidence that a common precursor can give rise to both 'connective tissue-type' and 'mucosal' mast cells. *J Immunol* 1986;136:1378–1384.
- ▶ 22 Kitamura Y: Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu Rev Immunol* 1989;7:59–76.
- ▶ 23 Denburg JA: Basophil and mast cell lineages in vitro and in vivo. *Blood* 1992;79:846–860.
- ▶ 24 Gurish MF, Ghildyal N, McNeil HP, Austen KF, Gillis S, Stevens RL: Differential expression of secretory granule proteases in mouse mast cells exposed to interleukin 3 and c-kit ligand. *J Exp Med* 1992;175:1003–1012.
- ▶ 25 Ghildyal N, McNeil HP, Stechschulte S, Austen KF, Silberstein D, Gurish MF, Somerville LL, Stevens RL: IL-10 induces transcription of the gene for mouse mast cell protease-1, a serine protease preferentially expressed in mucosal mast cells of *Trichinella spiralis*-infected mice. *J Immunol* 1992;149:2123–2129.
- ▶ 26 Ghildyal N, Friend DS, Nicodemus CF, Austen KF, Stevens RL: Reversible expression of mouse mast cell protease 2 mRNA and protein in cultured mast cells exposed to IL-10. *J Immunol* 1993;151:3206–3214.
- ▶ 27 Serafin WE, Sullivan TP, Conder GA, Ebrahimi A, Marcham P, Johnson SS, Austen KF, Reynolds DS: Cloning of the cDNA and gene for mouse mast cell protease 4. Demonstration of its late transcription in mast cell subclasses and analysis of its homology to subclass-specific neutral protease of the mouse and rat. *J Biol Chem* 1991;266:1934–1941.
- ▶ 28 McNeil HP, Frenkel DP, Austen KF, Friend DS, Stevens RL: Translation and granule localization of mouse mast cell protease-5. Immunodetection with specific antipeptide Ig. *J Immunol* 1992;149:2466–2472.
- ▶ 29 Serafin WE, Dayton ET, Gravalles PM, Austen KF, Stevens RL: Carboxypeptidase A in mouse mast cells. Identification, characterization, and use as a differentiation marker. *J Immunol* 1987;139:3771–3776.
- ▶ 30 Hu ZQ, Asano K, Yamazaki T, Shimamura T: Effects of lipopolysaccharide on mouse mast cell induction by a splenic cell culture system. *Infect Immun* 1994;62:3844–3849.
- ▶ 31 Hu ZQ, Kobayashi K, Zenda N, Shimamura T: Tumor necrosis factor- α - and interleukin-6-triggered mast cell development from mouse spleen cells. *Blood* 1997;89:526–533.
- ▶ 32 Re F, Strominger JL: Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* 2001;276:37692–37699.
- ▶ 33 Levi-Schaffer F, Austen KF, Gravalles PM, Stevens RL: Coculture of interleukin 3-dependent mouse mast cells with fibroblasts results in a phenotypic change of the mast cells. *Proc Natl Acad Sci USA* 1986;83:6485–6488.
- ▶ 34 Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A: Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? *J Invest Dermatol* 1991;96:523–526.
- ▶ 35 Leyden JJ, Marples RR, Klingman AM: *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 1974;90:525–529.