Journal of Allergy & Therapy
ISSN: 2155-6121

Matsui et al., J Allergy Ther 2017, 8:2
DOI: 10.4172/2155-6121.1000256

Research Article OMICS International

Peptidoglycan Synergistically Augments Production of Allergic Mediators from Murine Mast Cells in Combination with Muramyldipeptide

Katsuhiko Matsui*, Keisuke Shigehara and Reiko Ikeda

Department of Microbial Science and Host Defense, Meiji Pharmaceutical University, Tokyo, Japan

***Corresponding author:** Katsuhiko Matsui, Department of Microbial Science and Host Defense, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan, Fax: (+81)-42-495-8677; Tel: (+81)-42-495-8677; E-mail: kmatsui@my-pharm.ac.jp

Received date: April 25, 2017; **Accepted date:** May 08, 2017; **Published date:** May 18, 2017

Abstract

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by superficial *Staphylococcus aureus* colonization and an increased number of mast cells in the lesional skin, the immunopathologic features varying according to lesion duration.

Objective: The present study was conducted to clarify the effects of *S. aureus* cell wall components on production of allergic mediators from murine mast cells.

Methods: Peptidoglycan (PEG) and/or muramyldipeptide (MDP) were/was used to stimulate murine mast cells, and the resulting culture supernatants were assayed for Th1 and Th2 chemokines and histamine release. Chemokine production was assessed using reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). Histamine release was measured using a competitive ELISA.

Results: PEG stimulation induced production of the Th1 chemokine, CXCL10, and the Th2 chemokine, CCL17 by mast cells. Although MDP did not induce production of these chemokines, it synergistically enhanced the PEG-stimulated production of CCL17, but not CXCL10, from the mast cells. Histamine release was also enhanced in the presence of MDP.

Conclusion: The present results suggest that, in AD patients, *S. aureus* colonization may exacerbate acute allergic inflammation through up-regulation of CCL17 production and histamine release from PEG- and MDP-stimulated mast cells.

Keywords: Atopic dermatitis; Immunopathologic; Mast cells; Histamine; Inflammation

Introduction

Patients with atopic dermatitis (AD) show an increased number of inflammatory cells such as mast cells, eosinophils and mononuclear cells in the dermis, as well as superficial *Staphylococcus aureus* colonization, the immunopathologic features of the disease varying according to lesion duration [1]. *S. aureus* can be isolated from 96-100% of AD skin lesions, whereas only 0-10% of healthy individuals shows skin colonization by this organism [2,3]. Previously, we have shown that the detection rate of *S. aureus* on AD lesional skin is higher than that on non-lesional skin, the former showing a significantly higher *S. aureus* cell count than the latter [3]. However, the production of super antigenic exotoxin by *S. aureus*, considered the most important pathogenic factor in AD, does not differ between lesional and non-lesional skin.

The cell walls of Gram-positive bacteria contain highly cross-linked peptidoglycan (PEG) decorated to various degrees with teichoic acid polymers, some of which are linked to plasma membrane phospholipids to form lipoteichoic acid (LTA), another major cell wall component [4,5]. Gram-positive bacteria lack lipopolysaccharide (LPS), and LTA and/or PEG are considered to be their major inflammatory cell wall components. Accordingly, we have hypothesized that the pathogenetic role of *S. aureus* LTA and/or PEG may be more important than super antigenic exotoxins [6-8]. We have previously demonstrated that PEG induces an increase in the number of dermal mast cells in mice, similar to that in AD patients [8]. However, it has not been clarified whether PEG and its low-molecular-weight fragment, muramyldipeptide (MDP; N-acetylmuramyl-L-alanyl-D-isoglutamine), the minimum bioactive structure of PEG, influence the activation of mast cells. In the present study, therefore, we investigated the effects of PEG and/or MDP on allergy-related chemokine production and histamine release from murine mast cells.

Materials and Methods

PEG and MDP

S. aureus PEG was obtained from Fluka (Buchs SG, Switzerland). It was reconstituted at 1 mg/mL in phosphate-buffered saline (PBS) at pH 7.4, and sonicated for 1 h before use. MDP was purchased from EMD Biosciences (La Jolla, CA, USA) and reconstituted at 1 mg/mL in PBS at pH 7.4.

Mice

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

Preparation of mast cells and stimulation

Mast cells were induced by long-term (16-20 days) culture of mouse spleen cells in RPMI 1640 medium containing L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 10% fetal bovine serum (Sigma-Aldrich), 25 mM Hepes (Sigma-Aldrich), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco RBL, Grand Island, NY, USA) (RPMI 10) supplemented with 10 ng/mL TNF-α (R&D systems, Minneapolis, MN, USA), as described by Hu et al. [9]. Purified mast cells were separated from dead cells and small lymphocytes in the culture using Histopaque-1077 (Sigma-Aldrich) and then further expanded in RPMI 10 supplemented with 20 ng/mL IL-3 (Peprotech, Rocky Hill, NJ, USA) and 20 ng/mL IL-4 (Peprotech) for 2 weeks. These mast cells were adjusted to 1×10^6 cells/mL in RPMI 10, and then incubated in the presence of PEG (10 μg/mL) and/or MDP (10 μg/mL) at 37°C in a humidified 5% CO_2 atmosphere.

Detection of mRNA expression and quantification of chemokines

For determination of mRNA expression for Th1/Th2-type chemokine and toll-like receptor 2 (TLR2)/nucleotide-binding oligomerization domain-2 (NOD2), mRNA was extracted from mast cells after stimulation with PEG and/or MDP for 24 h using a Quick Prep Micro mRNA purification kit (GE Healthcare, Buckinghamshire, UK). The cDNA was then synthesized from 160 ng of the mRNA using a first-strand cDNA synthesis kit (GE Healthcare). Polymerase chain reaction (PCR) was performed using a GeneAmp PCR System 9700 (Perkin-Elmer, Norwalk, CT, USA) in a 25-μL reaction volume containing 1.5 μL of cDNA (corresponding to 16 ng of the original mRNA) as described previously, and the PCR products were separated on a 2% agarose gel containing ethidium bromide [7]. The primers used for amplification of β-actin, CXCL9/Mig, CXCL10/IP-10, CCL17/TARC, CCL22/MDC, TLR2 and NOD2 have been described elsewhere [7,10-15]. The culture supernatants of mast cells were also collected after incubation with PEG and/or MDP for 48 h, and their chemokine concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits for quantification of murine CXCL10 and CCL17 (R & D Systems).

Histamine release assay

For histamine release assay, 1×10^6 mast cells were suspended in 0.2 mL RPMI 10, then stimulated at 37°C for 1 h with 10 μg/mL PEG and/or 10 μg/mL MDP. The cell stimulation was stopped by cooling in iced water, and then the supernatant was removed for histamine assay. Histamine release was measured using a competitive ELISA kit for histamine (Immunotech Beckman Coulter, Marseille, France).

Statistical analysis

The data were expressed as means (± SD), and differences between means were analyzed using two-tailed Student's *t* test. Differences at $p<0.05$ were considered to be statistically significant.

Results

Influence of PEG and MDP on Th1/Th2-type chemokine production from mast cells

CXCL9 and CXCL10 are structurally related Th1-type chemokines that share a common receptor, CXCR3 [16], whereas CCL17 and CCL22 are Th2-type chemokines that share the receptor CCR4. To investigate whether PEG and/or MDP influences the mRNA expression of these chemokines in murine mast cells, we performed reverse transcription (RT)-PCR analysis. After 24 h of culture in the presence of PEG, but not MDP, mast cells began to express mRNA for CXCL10 and CCL17, but not that for CXCL9 and CCL22 (Figure 1A). Furthermore, the expression of mRNA for CCL17, but not that for CXCL10, was enhanced in the presence of MDP. To determine whether cultured mast cells produced CXCL10 and CCL17 at the protein level and secreted them into the culture medium, we performed ELISA using culture medium after 48 h of incubation. As shown in Figure 1B and C, PEG-stimulated, but not MDP-stimulated, mast cells produced significant levels of CXCL10 and CCL17, and MDP enhanced the PEG-induced production of CCL17, but not that of CXCL10. These results indicate that the synergistic effects of MDP are more specific to Th2-type chemokine production.

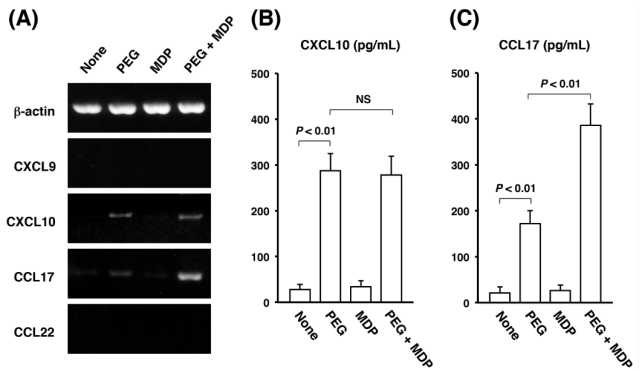

Figure 1: Effect of PEG and MDP on Th1-type and Th2-type chemokine production by murine mast cells. Murine mast cells (1×10^6/mL) were incubated with 10 μg/mL PEG in the presence or absence of 10 μg/mL MDP. (A) After incubation for 24 h, cytoplasmic mRNA was extracted from 1×10^6 cells, reverse-transcribed and PCR-amplified using primer sets for β-actin, CXCL9, CXCL10, CCL17 and CCL22. The data shown are representative results of four independent experiments. (B) Assay of 48 h culture supernatants for CXCL10 production using ELISA. The results are expressed as means ± SD (n=6). (C) Assay of 48 h culture supernatants for CCL17 production using ELISA. The results are expressed as means ± SD (n=6).

Expression of TLR2 and NOD2 in mast cells

It is well known that PEG and MDP signal through TLR2 and NOD2, respectively [17-19]. Therefore, expression of TLR2 and NOD2 by mast cells was also examined by RT-PCR to confirm their capacity to respond to PEG and MDP. As shown in Figure 2, mast cells spontaneously expressed mRNA for both TLR2 and NOD2, and the expression level of the former, but not that of the latter, was slightly increased by PEG stimulation. However, MDP-stimulated mast cells showed no increase in TLR2 or NOD2 mRNA expression, and simultaneous stimulation with PEG and MDP did not synergistically increase the expression of either.

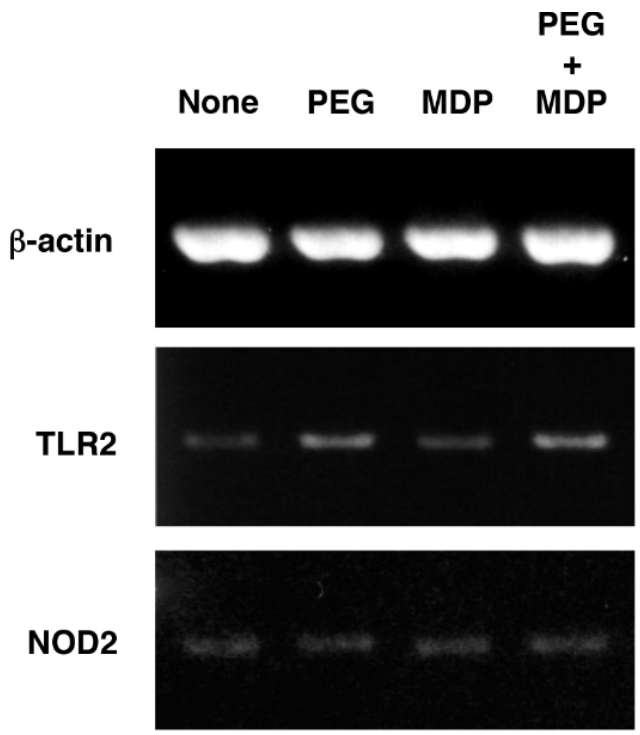

Figure 2: Effect of PEG and MDP on TLR2 and NOD2 mRNA expression in murine mast cells. Murine mast cells (1×10^6/mL) were incubated with 10 μg/mL PEG in the presence or absence of 10 μg/mL MDP. After 24 h of incubation, cytoplasmic mRNA was extracted from the mast cells (1×10^6 cells), reverse-transcribed and PCR-amplified using primer sets for β-actin, TLR2 and NOD2. The data shown are representative results of four independent experiments.

Influence of PEG and MDP on histamine release from mast cells

We examined whether PEG and MDP were able to trigger mast cell degranulation by determining the release of histamine from mast cells after 1 h of incubation with PEG and/or MDP. As shown in Figure 3, significant histamine release from mast cells was elicited by stimulation with PEG, but not MDP. However, PEG in combination with MDP caused a marked increase of histamine release in comparison with PEG stimulation alone. This suggests that MDP has the ability to synergistically enhance the PEG-induced hypersensitivity reaction through degranulation of mast cells.

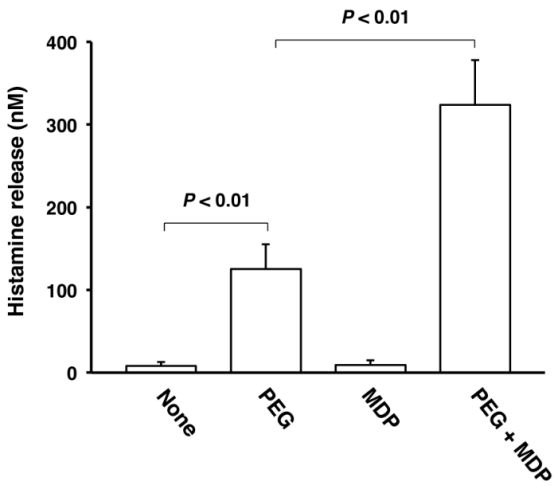

Figure 3: Effect of PEG and MDP on histamine release from murine mast cells. Murine mast cells (1×10^6/0.2 mL) were incubated with 10 μg/mL PEG in the presence or absence of 10 μg/mL MDP. After 1 h of stimulation, the supernatants were analyzed for histamine release from mast cells by a competitive ELISA. The results are expressed as means ± SD (n=6).

Discussion

Chronic skin colonization with *S. aureus* is a well-documented feature of AD. Most studies focusing on the role of *S. aureus* in the skin of AD patients have centered on staphylococcal exotoxins [20]. However, as about half of the *S. aureus* strains isolated from AD patients are not capable of super antigens production, the roles of these strains in AD skin lesions have remained unclear [3,21,22]. Percutaneous application of a cell wall component of *S. aureus*, PEG, has been shown to induce a Th2 immune response similar to that in AD patients [23]. As the number of Th2 cells is markedly increased in both peripheral blood and acute skin lesions of AD patients, the development of Th2 cells appears to be important for triggering AD, and *S. aureus* strains not producing toxins such as super antigen appear to be capable of triggering Th2-type inflammation in AD lesions.

In the present study we examined the effects of PEG from *S. aureus* and its low-molecular-weight fragment, MDP, on murine mast cells. Chemokines are involved in the recruitment of inflammatory cells to immune reaction sites. Here we found that, in combination with MDP, PEG enhanced the production of the Th2-type chemokine CCL17 from mast cells in comparison with PEG stimulation alone, but not that of the Th1-type chemokine CXCL10. CCL17 has been thought to elicit migration of Th2 cells to sites of inflammation [12]. Infiltration of Th2 cells into the skin lesions of AD patients occurs in the acute phase of inflammation with concomitant induction of an early allergic reaction [1]. Histamine also works as a primary mediator of the early allergic reaction, and histamine released by degranulation of sensitized mast cells/basophils causes acute allergic symptoms. Here we found that the release of histamine from PEG-stimulated mast cells was also enhanced in the presence of MDP, suggesting that it is important to eliminate the source of PEG and MDP for relief of the acute inflammation phase in AD patients. Since it is well known that PEG is

a TLR2 agonist and MDP a NOD2 agonist, both TLR2 stimuli and NOD2 stimuli would result in synergistic release of allergic mediators from mast cells [17-19]. RT-PCR using TLR2 and NOD2 primer sets predicted the presence of TLR2 and NOD2 in mast cells and suggested that increased production of CXCL10, CCL17 and histamine release elicited by stimulation with PEG might be partially associated with an increase of TLR2 expression on mast cells. However, the synergistic effects of simultaneous stimulation with PEG and MDP on CCL17 production and histamine release from mast cells were not explained by the levels of TLR2 and NOD2 expression, and the mechanisms responsible remain to be clarified in a further study.

As the density of *S. aureus* in skin lesions of AD patients exceeds 1×10^7 organisms/cm^2, the concentrations of 10 μg/mL PEG and 10 μg/mL MDP used for *in vitro* stimulation would likely be possible *in vivo* [2]. Therefore, sustained *S. aureus* colonization in AD patients would stimulate skin mast cells to induce a Th2-type immune response and hypersensitivity reaction through PEG and MDP, which are cell components common to both super antigenic exotoxin-producing and -non-producing *S. aureus* strains. Since the skin of most AD patients shows superficial *S. aureus* colonization and barrier disruption due to reduced levels of filaggrin [24], PEG and MDP from *S. aureus* would be expected to penetrate the skin and possibly play a critical role in the perpetuation of skin inflammation through production of CCL17 and histamine release from mast cells.

Conclusion

The acute allergic inflammation observed in AD patients may be enhanced by PEG and MDP from colonizing *S. aureus*. Therefore, irrespective of whether clinical signs of super infection are evident, antimicrobial treatment may be a promising new therapeutic strategy for at least a proportion of AD patients.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA (2004) New insights into atopic dermatitis. J Clin Invest 113: 651-657.
2. Guzik TJ, Bzowska M, Kasprowicz A, Czerniawska-Mysik G, Wójcik K, et al. (2005) Persistent skin colonization with *Staphylococcus aureus* in atopic dermatitis: relationship to clinical and immunological parameters. Clin Exp Allergy 35: 448-455.
3. Matsui K, Nishikawa A, Suto H, Tsuboi R, Ogawa H (2000) Comparative study of *Staphylococcus aureus* isolated from lesional and non-lesional skin of atopic dermatitis. Microbiol Immunol 44: 945-947.
4. Sutcliffe IC, Shaw N (1991) Atypical lipoteichoic acids of gram-positive bacteria. J Bacteriol 173: 7065-7069.
5. Fischer W (1994) Lipoteichoic acids and lipoglycans: Bacterial cell wall. (Ghuysen JM, Hackenbeck R, editors), Elsevier Science Publishing, Amsterdam, PP. 199-215.
6. Matsui K, Motohashi R, Nishikawa A (2000) Cell wall components of *Staphylococcus aureus* induce interleukin-5 production in patients with atopic dermatitis. J Interferon Cytokine Res 20: 321-324.
7. Matsui K, Nishikawa A (2002) Lipoteichoic acid from *Staphylococcus aureus* induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen. Clin Exp Allergy 32: 783-788.
8. Matsui K, Nishikawa A (2005) Percutaneous application of peptidoglycan from *Staphylococcus aureus* induces an increase in mast cell numbers in the dermis of mice. Clin Exp Allergy 35: 382-387.
9. Hu ZQ, Kobayashi K, Zenda N, Shimamura T (1997) Tumor necrosis factor-α-and interleukin-6-triggered mast cell development from mouse spleen cells. Blood 89: 526-533.
10. Park MK, Hoffmann KF, Cheever AW, Amichay D, Wynn TA, et al. (2001) Patterns of chemokine expression in models of *Schistosoma mansoni* inflammation and infection reveal relationships between type 1 and type 2 responses and chemokines *in vivo*. Infect Immun 69: 6755-6768.
11. Lacroix-Lamande S, Mancassola R, Naciri M, Laurent F (2002) Role of gamma interferon in chemokine expression in the ileum of mice and in a murine intestinal epithelial cell line after *Crystosporidium parvum* infection. Infect Immun 70: 2090-2099.
12. Xiao T, Fujita H, Saeki H, Mitsui H, Sugaya M, et al. (2003) Thymus and activation-regulated chemokine (TARC/CCL17) produced by mouse epidermal Langerhans cells is upregulated by TNF- α and IL-4 and downregulated by IFN- γ. Cytokine 23: 126-132.
13. Kuroda E, Sugiura T, Okada K, Zeki K, Yamashita U (2001) Prostaglandin E2 up-regulates macrophage-derived chemokine production but suppresses IFN-inducible protein-10 production by APC. J Immunol 166: 1650-1658.
14. Matsui K, Wirotesangthong M, Nishikawa A (2009) Analysis of signaling pathways involved in peptidoglycan-induced RANTES production from murine Langerhans cells. Int Arch Allergy Immunol 149: 167-172.
15. Mühlbauer M, Cheely AW, Yenugu S, Jobin C (2008) Regulation and functional impact of lipopolysaccharide induced Nod2 gene expression in the murine epididymal epithelial cell line PC1. Immunology 124: 256-264.
16. Olson TS, Ley K (2002) Chemokines and chemokine receptors in leukocyte trafficking. Am J Physiol Regul Integr Comp Physiol 283: R7-R28.
17. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2: 675-680.
18. Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21: 335-376.
19. Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, et al. (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2. J Biol Chem 278: 5509-5512.
20. Taskapan MO, Kumar P (2000) Role of staphylococcal superantigens in atopic dermatitis: from colonization to inflammation. Ann Allergy Asthma Immunol 84: 3-12.
21. Jappe U, Heuck D, Witte W, Gollnick H (1998) Superantigen production by *Staphylococcus aureus* in atopic dermatitis: No more than a coincidence? J Invest Dermatol 110: 844-846.
22. Zollner TM, Wichelhaus TA, Hartung A, Von Mallinckrodt C, Wagner TOF, et al. (2000) Colonization with superantigen-producing *Staphylococcus aureus* is associated with increased severity of atopic dermatitis. Clin Exp Allergy 30: 994-1000.
23. Matsui K, Nishikawa A (2011) Percutaneous application of peptidoglycan from *Staphylococcus aureus* induces infiltration of CCR4$^+$ cells into mouse skin. J Investig Allergol Clin Immunol 21: 354-362.
24. Brown SJ, McLean WH (2012) One remarkable molecule: filaggrin. J Invest Dermatol 132: 751-762.