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Research Paper

Cytotoxicity of murine eosinophil major basic proteins: the functional analysis of the toxicity by expressing recombinant proteins in both bacterial and mammalian cells

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Abstract

Lifestyle-related diseases such as diabetes and allergies are increasing in Asia due to the westernization of food and increase of urbanization along with decreasing physical activity. In particular, it is suggested that less opportunity to encounter microbes increases the chances of being allergic to pollen or house dust. Eosinophils play a major role in the pathogenesis and reconstruction of the tissues in allergic reactions and helminthic infection. Eosinophil major basic protein (MBP), that is stored in the granules of eosinophils and released in response to allergic stimuli or parasite infection, causes damages to the parasites or tissues where the immune response occurred. However, the role of MBP is not still clear in many cases. In this paper, we studied the cytotoxic activity and the localization of MBP, when it is expressed in bacterial cells and mammalian cells. MBP is synthesized as a slightly acidic "pre-proform (pre-pro-mature)" protein that is cleaved to a basic "mature MBP" to be stored in the granules of eosinophils. To determine the cytotoxic activity of each portion of the pre-pro-mature MBP, we expressed recombinant mouse pre-pro MBP, mature MBP, and pre-pro-mature (full) MBP in *E. coli* cells. The growth of these bacterial transfectants was suppressed to the various levels by expressed proteins, suggesting the toxicity of each molecule. The degree of toxicity of each recombinant protein was confirmed by electron microscopy. Acidic pre-pro MBP was most toxic followed by full MBP and mature MBP. We also expressed recombinant mouse pre-pro-mature MBP, pre-mature MBP, and full MBP in Chinese hamster ovary cells. Cellular localization of each protein was examined using laser scanning microscopy. Although most cells expressing pre-pro MBP died, the cells expressing pre-mature MBP and full MBP were alive. Full MBP was expressed uniformly in the cytoplasm and mature MBP was localized in many small vesicles in the cytoplasm. The role of MBP as a regulator of other proteins by changing the combination of disulfide bonds is also discussed.

Key words: eosinophils, proform MBP, mature MBP, cytotoxicity of MBP, expression of MBP

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INTRODUCTION

In general, it is reported that Northern European countries have higher mortality rates from asthma than Southern European counties over the age range of 5 to 44 (Holgate et al. 1993). It is speculated that Southern European countries have more microbes in the atmosphere. Usually, these bacterial antigens induce the Th1 immune response to decrease the allergic diseases. For example, lipopolysaccharides of *Escherichia coli* (*E. coli*) stimulate macrophages to induce Th1 (type 1 T helper) cells to secrete interferon-γ that induces B cells to produce IgG and suppress the production of interleukin (IL)-4 which induces B cells to produce IgE. Recently, Herbst et al. (2011) reported that mice grown in germ-free conditions develop severe eosinophilia in the airway inflammation and this phenotype could be reversed by recolonizing germ-free mice with commensal flora of specific pathogen-free mice.

Lifestyle-related diseases such as diabetes and allergies are increasing in Asia due to the consumption of an energy dense diet, increased urbanization along with decreasing physical activity, and less opportunities to encounter microbes because of the increase in indoor life, especially among children. Eosinophils play a major role in the pathogenesis of allergic diseases. Indeed, Bisgaard et al. (2011) reported that bacterial diversity in the early intestinal flora 1 and 12 months after birth was inversely associated with the risk of allergic sensitization, peripheral blood eosinophils, and allergic rhinitis.

Eosinophils have four basic proteins. Three of them exist in the matrix of the granule: eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). Eosinophil major basic protein (MBP) makes up 50% of the granule proteins. Eosinophil MBP was first recognized to be a potent helminthotoxin and cytotoxic against bacteria, protozoa, and mammalian cells *in vitro* (Gleich et al. 1984). Eosinophil MBP causes release of histamine from mast cells and basophils, activates neutrophils and alveolar macrophages, and is implicated in the damage of epithelial cells of the lung. It also interacts in a synergistic fashion with IL-1α or TGF-β_1, to enhance the IL-6-type cytokine production by lung fibroblasts (Rochester et al. 1996).

Structurally, eosinophil MBP is initially translated as a slightly acidic pre-proform (pre-pro-mature: full) protein and post-translationally modified to make a mature MBP. Murine acidic pro-MBP has 90 amino acids with an isoelectric point (IEP) of ~3.8 in which many glutamic acids and aspartic acids are included. Mature MBP (117 amino acids) contains many basic amino acids, including arginine with IEP of ~11 (Barker et al. 1988, Swaminathan et al. 2001, Acharya et al. 2014). The combination of the acidic and the basic portion in the pro-mature MBP form has a slightly acidic IEP of ~6.1. Previous studies have shown that conversion of full MBP to mature MBP is the key process in protecting eosinophils from the toxic mature MBP during the processing of MBP to be stored in the granule of eosinophils, suggesting that the acidic portion of full MBP masks the toxic effects of mature MBP (Acharya et al. 2014).

It has primarily been the potent toxicity of mature MBP against bacteria, parasites, and mammal cells that has been studied (Gleich et al. 1979, Gleich et al. 1984, Lehrer et al. 1989). Here, we have compared the relative effectiveness of mature MBP and acidic pro-MBP in terms of toxicity and localization when they are expressed in *E. coli* and Chinese hamster ovary (CHO) cells. Following that we have discussed the role of MBP not only in toxic activity against cells but also in its ability to regulate the function of other molecules such as metalloproteinase or angiotensinogen.

MATERIALS AND METHODS

Animals

IL-5 transgenic mice (C3H/HeN-TgN(IL-5)-Imeg) were developed by ourselves (Tominaga et al. 1991) and maintained in our animal facility in a specific pathogen free condition. Male IL-5 transgenic mice, 8-15 weeks old, were used to prepare eosinophils. All experiments were approved by the Animal Care and Use Committee of Kochi Medical School, Kochi University.

Preparation of MBP cDNA

Eosinophils were prepared from the spleen of IL-5

transgenic mice (C3H/HeN-TgN (IL-5) Imeg: IL-5TG, Tominaga et al. 1991). Messenger RNA was prepared from ten million eosinophils using QuickPrep Micro mRNA Purification Kit (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, England). The synthesis of cDNA was performed using Super Script Preamplification System for first Strand cDNA Synthesis Kit (GIBCO-BRL, Bethesda, MD). Pre is the signal peptide of MBP (16 amino acids). The pro-domain is the highly acidic portion (90 amino acids). Mature MBP is the highly basic MBP-1 protein (117 amino acids). PCR was performed to get pre-pro MBP cDNA, mature MBP cDNA, and pre-pro mature (full) MBP cDNA using the following primers:

Pre-pro MBP cDNA:
(sense primer: GGGAATTCCCTCTACTTCTGGCTCTT;
antisense primer: GGAGATCTTGTGTCCTCTTCCTTGG),
Mature MBP cDNA:
(sense primer: GGCATATGACCTGTCGCTACCTCCTA;
antisense primer: GGCTCGAGCATGAATTTGAACTCC),
Pre-pro-mature (full) MBP cDNA:
(sense primer: GGCATATGGACAAAGCCAAGATGAAAT;
antisense primer: GGCTCGAGGTGAGATAGACGCCAGTG).

Constructs for expressing MBP in *E. coli*

Expression plasmid pFLAG-1 (IBI-A Kodak Co., New Haven, CT) was used to express murine MBPs suing *E. coli* strain BL21. MBP PCR products were modified with linkers and cloned into pFLAG-1 expression vector that had OmpA, signal sequence for the secretion of FLAG fusion protein to peri plasmic space (Otto et al. 1995). This pFLAG-1 expression vector was driven by a strong *tac* promoter, which allowed the expression of FLAG-fused proteins using inducer, isopropyl-β-D-thiogalactopyranoside (IPTG).

Growth curve of MBP expressing *E. coli*

E. coli BL21 transfected with pFLAG-1-pre-pro MBP, pFLAG-1-mature MBP, pFLAG-1-pre-pro-mature (full) MBP, and pFLAG-1 vector were cultured in LB medium to reach about 1.0 of the optical density at 600 nm (OD_{600}), then IPTG (final 1 mM) was added. The OD_{600} of each culture was monitored every hour till four hours after the inoculation of cells.

Expression of recombinant MBPs fused with EGFP in CHO cells

Enhanced green fluorescent protein (EGFP) gene was prepared from pEGFP-C2 Vector (Clontech Lab., Mountain View, CA) and inserted between *Nhe*I site and *Kpn*I site of pCXN2 (Niwa et al. 1991, a generous gift of Dr. Jun-ichi Miyazaki, Division of Stem Cell Regulation Research, Osaka University Medical School), derivative of pCAGGS that had chicken β-actin promoter and cytomegalovirus enhancer, CMV-IE. Pre-pro-mature (full) MBP cDNA, pre-pro MBP cDNA, and pre-mature MBP cDNA were inserted into the *Nhe*I site of pCXN2-EGFP. EGFP was fused with the carboxyl-terminal of each MBP protein to express MBP-EGFP proteins. These MBP expression constructs were expressed in CHO cells using TransIt-2 (TAKARA BIO INC., Kusatsu, Shiga, Japan) according to the manufacturer's protocol. CHO cells transfected with pCXN2-pre-pro MBP-EGFP, pCXN2-pre-mature MBP-EGFP, pCXN2-pre-pro-mature (full) MBP-EGFP, and pCXN2-EGFP were cultured in the presence of 400 μg/ml of G418 to eliminate non-transfectants. The Expression of EGFP was confirmed by FACScan (Becton-Dickinson and Co., Mountain View, CA).

SDS-PAGE and Western Blots

E. coli cells were solubilized in lysis buffer (8 M urea in 10 mM Na-phosphate Buffer, pH 7.2). CHO cells were solubilized in lysis buffer (0.5% NP40, 50 mM Tris-HCl, pH 8.0, 250 mM NaCl, 5 mM EDTA, 1mM DTT, 1 mM PMSF, 10 mg/ml leupeptin, 10 mg/ml aprotinin, 20 mM NaF, 20 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM benzamidine). Proteins separated on 8-16% gradient SDS-PAGE gels were transferred onto a PVDF membrane (Bio Rad Lab., Hercules, CA). In case of *E. coli* cell extracts, membranes were probed with mouse anti-FLAG M2 antibodies followed by alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (Southern Biotechnology Associates Inc., Birmingham, AL). Proteins bound with anti-FLAG antibodies and AP-conjugated goat anti-mouse IgG were visualized using CDP-Star (GE Healthcare, UK Ltd, Little Chalfont, Buckinghamshire, England) and Hyper-Film ECL (Amersham Biosciences, GE Healthcare, UK Ltd,

England) according to the manufacturer's protocol.

In the case of CHO cell extracts, membranes were probed with rabbit anti-EGFP antibodies (Bioss Inc., Woburn, MA) followed by AP-conjugated goat anti-rabbit IgG. All blots were probed with rabbit anti-human beta-actin antibodies as a loading control. AP-conjugated goat anti-rabbit antibodies are purchased from Sigma-Aldrich (St Louis, MO) and the intensity of each band of dried gels was obtained by exposing to Hyper-Film ECL (Amersham Biosciences, GE Healthcare, UK Ltd, England).

Electron microscopy of *E. coli* expressing MBPs

E. coli cells were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 for 1h at 4°C. They were suspended 1% agarose in 0.9% NaCl at 37°C. After being centrifuged, they were cooled in ice water and cut into small pieces. Then, they were treated with 1% OsO_4 in 0.1 M phosphate buffer, pH 7.3 for 1h at 4°C. After being dehydrated, they were embedded in Epon 812 (TAAB Laboratories Equipment, Berkshire, England). Thin sections were stained with uranyl acetate and lead citrate, and examined using an electron microscope (H7100, Hitachi, Japan).

Confocal laser scanning microscopy of CHO cells expressing EGFP-MBPs

CHO cells transfected with pCXN2-pre-pro MBP-EGFP, pCXN2-pre-mature MBP-EGFP, pCXN2-pre-pro-mature (full) MBP-EGFP, and pCXN2-EGFP were observed using a laser scanning confocal microscope (LSM-410, Carl Zeiss Microscopy Co., Ltd, Oberkochen, Germany). Excitation of EGFP was performed at 488 nm using an Argon-ion laser. Comparison among transfectants was made with images of laser scanned differential interference contrast and an excitation wavelength of 488 nm.

RESULTS

Preparation of recombinant MBPs

Whole cell lysates of MBP transfectants with pFLAG-1-pre-pro-mature (full) MBP, pFLAG-1-pre-pro MBP (pre-pro MBP), pFLAG-1-mature MBP (mature MBP), and pFLAG-1 vector were prepared and analyzed by Western Blot using anti-FLAG M2 antibodies (Fig. 1). A major 33 kDa band was observed in the lysate of full MBP transfectant, though M. W. of OmpA-FLAG-full MBP was estimated to be 28754. It was assumed that 33 kDa band was the translated protein from this pFLAG-1 construct, as a single 33 kDa band was found after purifying FLAG-full MBP proteins using anti-FLAG M2 antibodies. A major 16 kDa band was detected in mature MBP transfectant as expected. In pFLAG-1-pre-pro MBP transfectant, 22 kDa and 11 kDa band were newly detected compared with pFLAG-1vector transfectant. As our pre-pro MBP cDNA encoded 10 amino acids shorter protein compared with full length of pre-pro MBP (based on the position of antisense primer), the 11 kDa band corresponded to the translated material of the FLAG-pre-pro MBP gene. The 22 kDa band could be interpreted as a dimer.

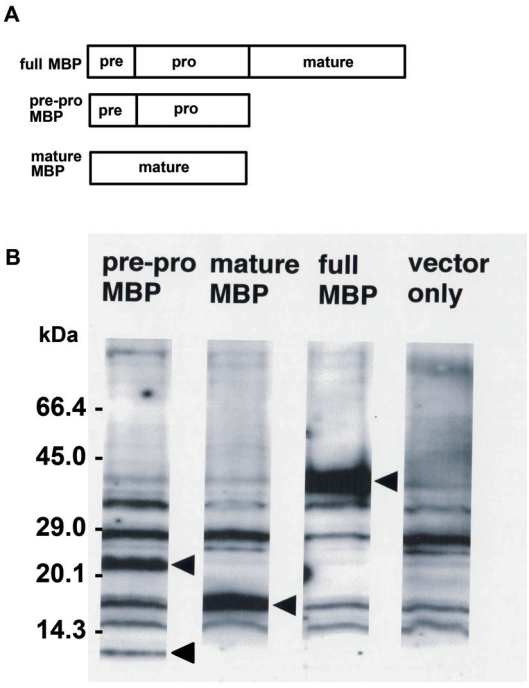

Fig. 1. Western blot analysis of the expressed MBPs in *E. coli*. A: Schematic figures of full MBP, pre-pro MBP, and mature MBP expressing each portion of MBP. B: Whole cell lysates were prepared from transfectants expressing pFLAG-1-pre-pro MBP, pFLAG-1-mature MBP, pFLAG-1-full MBP, and pFLAG-1 vector. MBP proteins were expressed under the control of *tac* promoter. Fusion proteins tagged with FLAG peptides were detected with anti-FLAG antibodies as described in Materials and methods.

Amino-terminal sequence including the fusion site between FLAG and MBP was confirmed by determining the sequence of each recombinant protein. Amino-terminal sequence of each recombinant protein was in agreement with that predicted from the cDNA sequence of each MBP clone (data not shown).

Suppression of bacterial growth of recombinant strains of *E. coli*

Cytotoxic activity of each recombinant MBP against *E. coli* strain was examined by measuring the growth of each transfectant. Bacterial growth was examined by measuring the optical density at 600 nm (OD_{600}) after inducing the expression of each recombinant MBP protein by adding IPTG to the culture (Fig. 2). Bacterial growth of expressing pre-pro MBP was suppressed completely. The growth of full MBP-expressing strain was suppressed significantly. The suppression of the growth of mature MBP-expressing strain was marginal. Expression of FLAG alone did not suppress the growth of this strain.

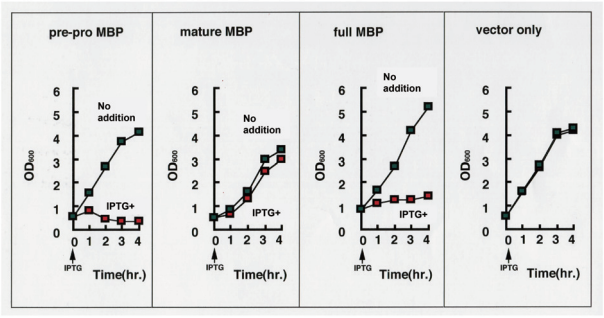

Fig. 2. Comparison of cell growth among transfectants expressing pFLAG-1-pre-pro MBP, pFLAG-1-mature MBP, pFLAG-1-full MBP, and pFLAG-1. Each transfectant was cultured in the presence or absence of IPTG that induced the production of MBPs.

After induction of each recombinant MBP by adding IPTG, the colony forming cells of each transfectant were counted every hour after the addition of IPTG (Fig. 3). Based on the colony numbers of each recombinant strain, the anti-bacterial activity of each recombinant MBP was estimated as follows from higher to lower level of toxicity: pre-pro MBP > full MBP > mature MBP.

Electron microscopy of bacteria expressing recombinant MBPs was examined (Fig. 4). *E. coli* cells

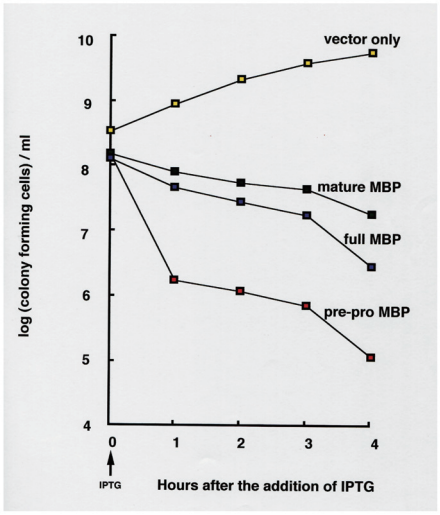

Fig. 3. Counts of *E. coli* cell colonies transfected with pFLAG-1-pre-pro MBP, pFLAG-1-mature MBP, pFLAG-1-full MBP, and pFLAG-1. After the induction of MBP expression by IPTG, bacterial colonies were counted as described in Materials and methods.

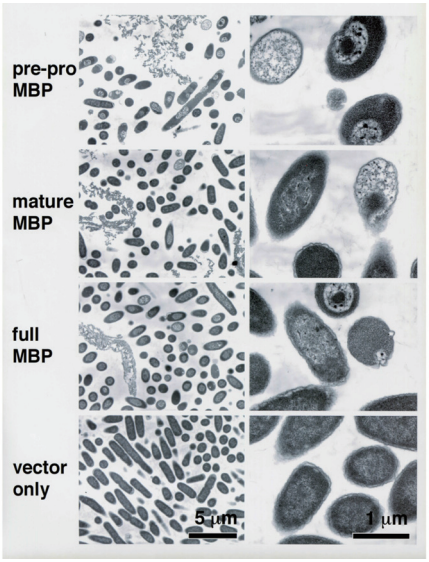

Fig. 4. Examination of *E. coli* transfectants by electron microscopy. *E. coli* cells transfected with pFLAG-1-pre-pro MBP, pFLAG-1-mature MBP, pFLAG-1-full MBP, and pFLAG-1 were examined by electron microscopy as described in Materials and methods.

transfected with acidic pre-pro MBP were damaged at the highest level. The internal structure of the cells was destroyed. Most of the cells were short and the inside of the cells were full of fiber-like structures and less dense than normal cells. *E. coli* cells transfected with full MBP and mature MBP had similar features and structures at lower level.

Expression of MBP-EGFP proteins in CHO cells

We prepared the following constructs for expressing MBPs in mammalian cells, pCXN2-pre-pro MBP-EGFP, pCXN2-pre-mature MBP-EGFP, and pCXN2-full MBP-EGFP. All constructs were prepared in pCXN2 and expressed under the control of chicken β-actin promoter/CMV-IE enhancer. EGFP was fused to the C-terminal end of MBP. Translated products detected by Western blot showed the following molecular weight bands: pre-pro MBP-EGFP; 41 kDa, pre-mature MBP-EGFP; 42 kDa, and full MBP-EGFP; 53 kDa (Fig. 5).

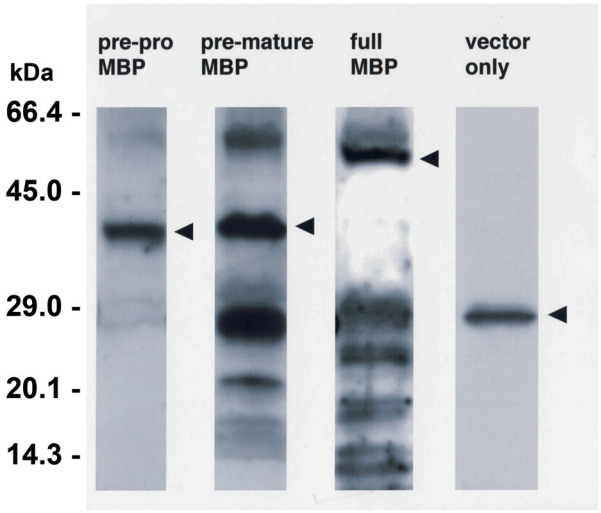

Fig. 5. Western blot analysis of expressed MBPs in CHO cells. CHO cells transfected with pCXN2-pre-pro MBP-EGFP, pCXN2-pre-mature MBP-EGFP, pCXN2-full MBP-EGFP, and pCXN2-EGFP vector were lysed and expressed MBP-EGFP fusion proteins were analyzed by Western blot as described in Materials and methods.

Localization of recombinant MBPs in Chinese hamster ovary cells

These recombinant proteins showed a characteristic localization in CHO cells under confocal laser scanning microscopy (Fig. 6). All cells expressing pre-mature MBP were adhered to dishes as vector transfectants and pre-mature MBP-EGFP (pre portion is expected to be

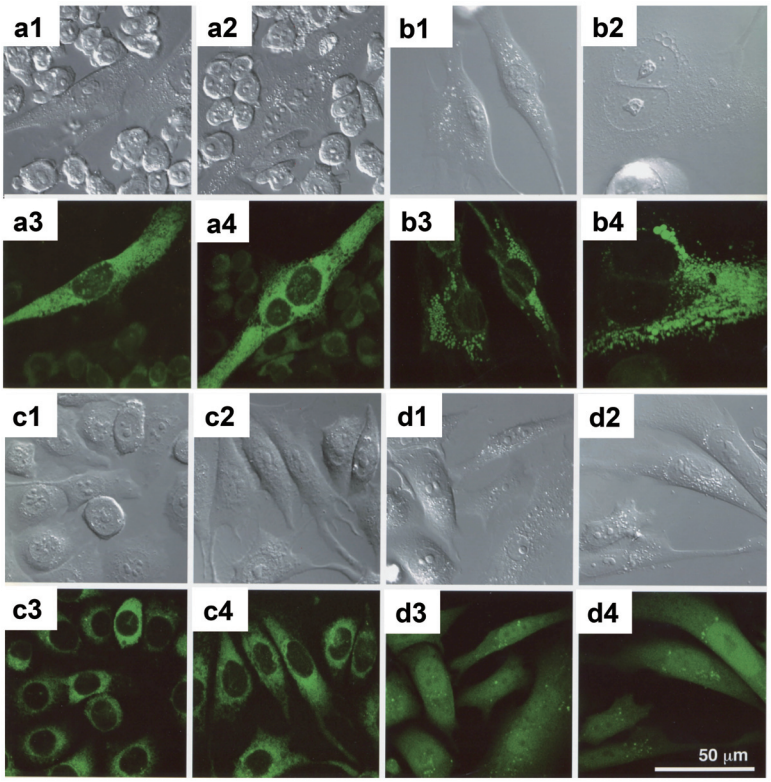

Fig. 6. Examination of CHO cell transfectants by laser scanning confocal microscope. CHO cells transfected with pCXN2-pre-pro MBP-EGFP (a1-a4), pCXN2-pre-mature MBP-EGFP (b1-b4), pCXN2-full MBP-EGFP (c1-c4), and pCXN2-EGFP (d1-d4) were observed using a laser scanning confocal microscope as described in Materials and methods. Images of laser scanned differential interference contrast: (a1, a2, b1, b2, c1, c2, d1, d2); Images with an excitation wavelength of 488 nm: (a3, a4, b3, b4, c3, c4, d3, d4).

removed) proteins were accumulated in the granule-like vesicles. In vector transfectants, EGFP is distributed in both nuclei and cytoplasm. In contrast to vector transfectants, MBPs conjugated with EGFP were distributed in cytoplasm. Compared to the adhered CHO cells transfected with pCXN2-EGFP, CHO cells expressed pre-pro MBP-EGFP were round-shaped and seemed to be in the process of cell death. In the adhered viable cells, acidic pre-pro MBP proteins were widely distributed in the cytoplasm. Compared with CHO cells expressed pre-pro MBP-EGFP, CHO cells expressing full MBP-EGFP were viable. However, the shape of the CHO cells expressing full MBP-EGFP was round compared with vector transfectants. These results suggest that mature MBP is not toxic in CHO cells and can be stored safely in the granule of these ovary cells.

DISCUSSION

In general, the cytotoxicity of MBP is evaluated by mixing MBP with cells *in vitro*. In contrast, we evaluated the toxicity by expressing pre-pro MBP, mature MBP, and full MBP (pre-pro-mature MBP) in both *E. coli* and CHO cells. It has been reported that both human MBP and eosinophil cationic protein (ECP) showed significant bactericidal activities against *E. coli* ML-35 and *S. aureus* 502A at concentrations from 1 to 10 μM (Lehrer et al. 1989). In another experiment, human MBP showed a half-maximal inhibitory concentration of ~18 nM against *E. coli* BL21 (Soragni et al. 2015). Spleen cells, mononuclear cells, intestinal cells, and skin cells were significantly killed at a concentration of 8.2×10^{-5} M (Gleich et al. 1979). It was reported that about 40% of Schistosomula of *Schistosoma mansoni* were dead after 20 hours incubation with human MBP at a concentration of 1.5×10^{-5} M. It was also reported that ECP was eight to ten times more potent than MBP in terms of their cytotoxicity assay. (Ackerman et al. 1985).

As pro portion of MBP is very acidic and mature MBP is basic, it has been speculated that toxicity of arginine-rich mature MBP may be neutralized with pro MBP. Indeed, acidic polyamino acids inhibit human eosinophil granule major basic protein toxicity. Poly-L-aspartic acid, ploy-D-glutamic acid, and poly-L-glutamic acid inhibited MBP toxicity (Barker et al. 1991).

Soragni et al. (2015) reported that crystallization in the eosinophil granules makes MBP non-toxic. They also reported that MBP toxicity is triggered by granule acidification, followed by extracellular aggregation, which mediates the damage to pathogens.

In our study, mature MBP expressed in CHO cells were stored in granules and cells were viable, although MBPs were not observed as crystallized proteins. We think that native molecules like heparin that inhibit the toxicity of MBP may bind mature MBP in the granules of CHO cells. Indeed, the killing of microfilariae of *Brugia malayi* by MBP was inhibited by heparin (Hamann et al. 1990). Furthermore, the binding of the most abundant repeating unit of heparin sulfate (1,4-dideoxy-O_2-sulfo-glucorinic acid-*N, O*6-disulfo-glucosamine) with eosinophil MBP was suggested (Swaminathan et al. 2001). Swaminathan et al. (2001) showed that eosinophil MBP belongs to the C-type lectin superfamily, though it lacks the Ca^{2+}-dependent carbohydrate binding site. This family contains molecules engaged primarily in natural immunity, such as dendritic cell-specific ICAM-grabbing non-integrin, langerin expressed on Langerhans cells, mannose-binding proteins, selectins, CD44, CD23, and NK-cell receptors like NKG2D (Zelensky et al. 2005).

Although *Brugia pahangi* was killed by MBP *in vitro*, even in the absence of MBP (C57BL/6-MBP-$1^{-/-}$), larvae of *Brugia pahangi* were eliminated from the peritoneal cavity of this strain of mouse (Ramalingam et al. 2005). They showed the evidence that eosinophils are important for clearance of this larva from hosts even in the absence of MBP. However, Specht et al. reported that 129/Svj-MBP-$1^{-/-}$ developed a significantly higher burden of *Litomosoides sigmodontis* worms. This result suggests that MBP is responsible for the protection against the infection of *Litomosoides sigmodontis* worms (Specht et al. 2006). This discrepancy may be explained by the difference of the background of mouse strains, or that of the susceptibility of parasite species to MBP-1.

Even in MBP-$1^{-/-}$ mice, airway hyper-responsiveness occurs, suggesting that MBP-1 is not involved in the pathogenesis of the disease (Denzler et al. 2000). Denzler et al. (2000) reported that the loss of mMBP-1 had no

effect on OVA-induced airway histopathologies or inflammatory cell recruitment.

Recently, it is speculated that a sudden increase of asthma is deeply related to children's adaptation to an indoor lifestyle, resulting in an increased sensitization to indoor antigens and diet, and decreased physical activity (Platts-Mills 2015). He even suggests that sensitization to peanut antigens can occur through the skin and may be caused by the higher level of permeability of the skin induced by the change of children's lifestyle.

In this paper, we have analyzed the molecular basis of the toxicity of the eosinophil major basic protein, MBP-1. Although there is no direct relation between the function of MBP and children's adaptation to the indoor lifestyle, we have to remember that proform MBP (full MBP) encoded by the gene *PRG2* is one of the most highly expressed proteins during human pregnancy (Weyer et al. 2011). It is reported that the pregnancy-associated major basic protein increases early in gestation and plateaus by week 20 at concentrations more than 10 times normal (Wasmoen et al. 1989). It is suggested that proform MBP can inactivate the enzymatic activity of metzincin metalloproteinase pregnancy-associated plasma protein A (PAPPA) by making disulfide bonds to control the folliculogenesis, placental development, atherosclelosis, wound healing, and bone remodeling (Weyer et al. 2011). Binding of proform MBP to PAPPA, that digests insulin-like growth factor (IGF) -binding protein to release IGF, results in the decrease of bioavailable IGF. In particular, IGF2 controls folliculogenesis, placental development, and fetal growth (Constância et al. 2002). It is also reported that the risk of delivering a low-birth-weight baby at full term may be determined by the placental activity of IGFs in very early pregnancy, because PAPPA, which is responsible for the release of IGF, is highly expressed in first-trimester trophoblasts (Smith et al. 2002). They showed a strong, positive correlation between first-trimester levels of PAPPA and birth weight at 38-41 weeks of gestation. Thus, proform MBP regulates the IGF signaling.

Proform MBP also makes disulfide bonds with prohormone angiotensinogen (AGT) identified as proform MBP/AGT and proform MBP/AGT/C3dg. It has been reported that the complexes of proMBP/AGT are present in the plasma of both normal and pregnant individuals (Oxvig et al. 1995). Generation of Ang-1, decapeptide angiotensin-1 is slowed down by construction of a complex of proform MBP/AGT, suggesting that proform MBP function as an inhibitor of Ang-1 generation (Weyer et al. 2011).

Glerup et al. (2005) analyzed the intra- and intermolecular disulfide rearrangements between preform MBP and PAPPA precisely. Their results suggest that proform MBP has a possibility to function as a regulator of many cysteine-containing proteins.

In summary, proform MBP consisting of pro portion of MBP and mature MBP regulates the growth and reproduction of tissues by controlling IGF signaling and the renin-angiotensin system. The function of MBP must be re-examined not only as a cytotoxic protein in the immune system but also as a regulator of cell growth and blood pressure. This work will shed light on the role of MBP in the regulation of growth and the function of cells involved in the repair system after the damage of tissues caused by allergic reactions.

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