Role of multidrug resistance-associated protein 1 in the pathogenesis of allergic airway inflammation

Masakata Yoshioka,1,2* Hironori Sagara,4* Fumiyuki Takahashi,1,2* Norihiro Harada,1,2,3 Kazuto Nishio,5 Akio Mori,6 Hiroko Ushio,3 Kazue Shimizu,1,2 Takenori Okada,4 Mayumi Ota,4 Yoichi M. Ito,7 Osamu Nagashima,1,2,3 Ryo Atsuta,1,2,3 Yoshihiro Suzuki,8 Takeshi Fukuda,4 Yoshinosuke Fukuchi,1,2 and Kazuhsa Takahashi1,2

1Department of Respiratory Medicine, 2Research Institute for Diseases of Old Ages, and 3Atoxy (Allergy) Research Center, Juntendo University School of Medicine, Bunkyo-Ku, Tokyo; 4Department of Pulmonary Medicine and Clinical Immunology, Dokkyo University School of Medicine, Tochigi; 5Department of Genome Biology, Kinki University School of Medicine, Osaka; 6Clinical Research Center for Allergy and Rheumatology, National Samagahara Hospital, Kanagawa; 7Department of Biostatistics, School of Public Health, The University of Tokyo, Tokyo; and 8Department of Analytical Biochemistry, Meiji Pharmaceutical University, Kiyose, Tokyo, Japan

Submitted 14 January 2008; accepted in final form 14 October 2008

Yoshioka M, Sagara H, Takahashi F, Harada N, Nishio K, Mori A, Ushio H, Okada KS, Ota M, Ito YM, Nagashima O, Atsuta R, Suzuki T, Fukuda T, Fukuchi Y, Takahashi K. Role of multidrug resistance-associated protein 1 in the pathogenesis of allergic airway inflammation. Am J Physiol Lung Cell Mol Physiol 296:L30–L36, 2009. First published October 17, 2008; doi:10.1152/ajplung.00026.2008.—Multidrug resistance-associated protein 1 (MRP1) is a cysteinyl leukotriene (CysLT) export pump expressed on mast cells. CysLTs are crucial mediators in allergic airway inflammation. However, biological significance of MRP1 in allergic airway inflammation has not yet been elucidated. In this study, we sensitized wild-type control mice (mrp1+/+) and MRP1-deficient mice (mrp1−/−) to ovalbumin (OVA) and challenged them with OVA by aerosol. Airway inflammation and goblet cell hyperplasia after OVA exposure were reduced in mrp1−/− mice compared with mrp1+/+ mice. Furthermore, CysLT levels in bronchoalveolar lavage fluid (BALF) from OVA-exposed mrp1−/− mice were significantly lower than those from OVA-exposed mrp1+/+ mice. Levels of OVA-specific IgE, IL-4, and IL-13 in BALF were also decreased in OVA-exposed mrp1−/− mice. IgE-mediated release of CysLTs from murine bone marrow-derived mast cells was markedly impaired by MRPl deficiency. Our results indicate that MRP1 plays an important role in the development of allergic airway inflammation through regulation of IgE-mediated CysLT export from mast cells.

cysteinyl leukotrienes; mast cell

MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 (MRP1) is a 190-kDa transmembrane protein belonging to the ATP-binding cassette transporter superfamily (15). The MRP1 gene was isolated from the doxorubicin-resistant human small cell lung cancer cell line H69AR (6), and subsequent in vitro studies established that MRP1 mediates the cellular excretion of many drugs and confers multidrug resistance of cancer cells (3). MRP1 has been shown to be expressed in various human tissues and cells, including mast cells (10). MRP1 transports glutathione S-conjugates of endogenous and xenobiotic lipophilic compounds across the cellular membrane into the extracellular space (15). Among these transport substrates, leukotriene C4 (LTC4) is a high-affinity endogenous glutathione S-conjugate substrate for the MRP1 (19) and is excreted from mast cells, which play an important role in the pathogenesis of allergy and asthma (2, 20).

Bronsclhial asthma is a common disorder in adults and children and remains poorly understood and difficult to manage (4). Airway inflammation is a hallmark of this disease (4). Previous studies have indicated that cysteinyl leukotrienes (CysLTs) such as LTC4, LTD4, and LTE4, originally termed slow-reacting substance of anaphylaxis, are crucial mediators in the pathogenesis of allergic asthma (8). LTC4 is synthesized by and excreted from mast cells and is rapidly converted to LTD4 and then to LTE4 (5). CysLTs induce airway smooth muscle contraction, increase vascular permeability and mucus secretion, and may recruit more inflammatory cells to the airway in allergic asthma (13). However, the importance of MRP1, which is the LTC4 export pump on mast cells, for allergic airway inflammation remains poorly defined.

To elucidate the role of MRP1 in the pathogenesis of allergic airway inflammation in vivo, we used an ovalbumin (OVA) sensitization and airway challenge protocol and compared MRP1-deficient mice (mrp1−/−) with wild-type control mice (mrp1+/+) in a well-established model. We also cultured bone marrow-derived mast cells (BMMCs) from mrp1−/− and mrp1+/+ mice and stimulated them with IgE and anti-IgE antibody. The biological significance of MRP1 involvement in allergic airway inflammation and IgE-dependent export of CysLTs from mast cells is discussed.

MATERIALS AND METHODS

Animals. MRP1-deficient mrp1−/− mice were generated by gene targeting in embryonic stem cells as described previously (31). Mrp1−/− mice originally on the genetic background (129/Ola)/FVB (50:50) were backcrossed 12 times with FVB mice to obtain >99% FVB genetic background. Normal FVB mice were used as wild-type controls (mrp1+/+). Mrp1−/− and mrp1+/+ mice (male, 6–8 wk of age) were purchased from Taconic Laboratories (Germantown, NY). Mice were maintained in a limited access barrier and housed in a humidity (55 ± 10%)- and temperature (24 ± 2°C)-controlled room under a
ROLE OF MRP1 IN ALLERGIC AIRWAY INFLAMMATION

12:12-h light-dark cycle. The study protocol was reviewed and approved by the Juntendo University and Dokkyo University School of Medicine Committee on Animal Care and complies with National Institutes of Health guidelines for animal care.

Sensitization and airway challenge. Mice were sensitized on days 0 and 14 by an intraperitoneal injection of 50 μg of OVA (Sigma, St. Louis, MO) and 2 mg of aluminum hydroxide (Wako Pure Chemical Industries, Osaka, Japan) in 200 μL of PBS. Nonsensitized mice received only aluminum hydroxide in PBS. On days 22, 24, 26, and 28, the sensitized mice were challenged with aerosolized 1% OVA 30 ml for 30 min. The nonsensitized mice received PBS only. Bronchoalveolar lavage and histological analysis of the lungs were performed 48 h after the last aerosol challenge.

Histological analysis of lung. The murine lungs were infused and fixed with 10% formalin and then embedded in paraffin. Sections of lung histology in both groups (Fig. 1, A–F). However, this inflammation following OVA exposure was reduced in mrp1−/− mice compared with mrp1+/+ mice (Fig. 1, C and D). To evaluate the extent of inflammation, we employed a semiquantitative scoring system as described previously (9). As shown in Fig. 1G, blinded semiquantitative grading of the lung sections revealed a statistically significant difference in the degree of airway inflammation between the mrp1−/− and mrp1+/+ mice (P = 0.0143). In addition, blinded semiquantification of goblet cell staining with PAS also revealed attenuated mucus scores in OVA-exposed mrp1−/− mice compared with OVA-exposed mrp1+/+ mice (Fig. 1H) (P = 0.0431). These data indicate that airway inflammation and goblet cell hyperplasia are reduced in OVA-exposed mrp1−/− mice compered with OVA-exposed mrp1+/+ mice.

Inflammatory cell recruitment in BALF. The recovery of cells from the BALF of PBS-exposed mrp1−/− and mrp1+/+ mice revealed a predominance of alveolar macrophages in both groups, without any significant differences (data not shown). Aerosol challenge of mice with OVA induced a marked increase in the total cell numbers compared with control groups with PBS (Fig. 2). However, the total cell numbers in BALF were significantly decreased in OVA-exposed mrp1−/− mice compared with OVA-exposed mrp1+/+ mice. However, OVA-exposed mrp1−/− mice had significantly lower numbers of eosinophils and lymphocytes than the mrp1+/+ mice (P = 0.0243 and 0.0187, respectively). The numbers of macrophages and neutrophils were not significantly different between groups. These results imply that mrp1−/− mice show reduced recruitment of inflammatory cells, especially eosinophils and lymphocytes, into the airway lumen after OVA challenge compared with mrp1+/+ mice.

CysLT levels in BALF. To investigate the role of MRP1 as a CysLT export pump in vivo, we measured total CysLT levels in BALF from mrp1−/− and mrp1+/+ mice exposed to PBS or OVA aerosol. As shown in Fig. 3, levels of CysLTs in BALF from OVA-exposed mrp1−/− mice were significantly lower than those in mrp1+/+ mice (Cayman Chemicals). The cells were resuspended in lysis buffer, homogenized, centrifuged, and then collected for determination of intracellular CysLT. Each experiment was performed in triplicate.

Statistics. Data are means ± SD and were analyzed using the unpaired t-test. Differences between means were considered statistically significant at P < 0.05.

RESULTS

Histopathology of the lungs of mrp1−/− and mrp1+/+ mice. To investigate the biological significance of MRPI in allergic airway inflammation, we sensitized mrp1−/− and mrp1+/+ mice to OVA and challenged them with OVA by aerosol. Control mice received PBS. The lungs from mrp1−/− and mrp1+/+ mice exposed to PBS aerosol showed normal lung histology in both groups (Fig. 1, A and B). Sensitization and subsequent exposure to OVA resulted in peribronchial and perivascular inflammation both in the mrp1−/− and mrp1+/+ mice, and excessive production of airway mucus glycoproteins by goblet cells in airway epithelium was observed (Fig. 1, C–F). However, this inflammation following OVA exposure was reduced in mrp1−/− mice compared with mrp1+/+ mice (Fig. 1, C and D). To evaluate the extent of inflammation, we employed a semiquantitative scoring system as described previously (9).

Generation of murine BMMCs. BMMCs were generated from the femoral bone marrow cells of mrp1−/− and mrp1+/+ mice and maintained in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated FCS, 100 μM 2-mercaptoethanol, 10 μM MEN-nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% pokeweed mitogen-stimulated spleen-conditioned medium as a source of macrophage colony-stimulating factor. BMMCs of mrp1−/− mice (Fig. 1, A and B). Sensitization and subsequent exposure to OVA resulted in peribronchial and perivascular inflammation both in the mrp1−/− and mrp1+/+ mice, and excessive production of airway mucus glycoproteins by goblet cells in airway epithelium was observed (Fig. 1, C–F). However, this inflammation following OVA exposure was reduced in mrp1−/− mice compared with mrp1+/+ mice (Fig. 1, C and D). To evaluate the extent of inflammation, we employed a semiquantitative scoring system as described previously (9).

Statistics. Data are means ± SD and were analyzed using the unpaired t-test. Differences between means were considered statistically significant at P < 0.05.

RESULTS

Histopathology of the lungs of mrp1−/− and mrp1+/+ mice. To investigate the biological significance of MRPI in allergic airway inflammation, we sensitized mrp1−/− and mrp1+/+ mice to OVA and challenged them with OVA by aerosol. Control mice received PBS. The lungs from mrp1−/− and mrp1+/+ mice exposed to PBS aerosol showed normal lung histology in both groups (Fig. 1, A and B). Sensitization and subsequent exposure to OVA resulted in peribronchial and perivascular inflammation both in the mrp1−/− and mrp1+/+ mice, and excessive production of airway mucus glycoproteins by goblet cells in airway epithelium was observed (Fig. 1, C–F). However, this inflammation following OVA exposure was reduced in mrp1−/− mice compared with mrp1+/+ mice (Fig. 1, C and D). To evaluate the extent of inflammation, we employed a semiquantitative scoring system as described previously (9).

Statistics. Data are means ± SD and were analyzed using the unpaired t-test. Differences between means were considered statistically significant at P < 0.05.
we cultured BMMCs from role of MRP1 in IgE-mediated CysLT export from mast cells, mrp1 mechanism for the reduced airway inflammation in OVA-sol. As shown in Fig. 4, the levels of IL-4 and IL-13 were below the lower limit of infiltration (data not shown). As shown in Fig. 4, OVA-specific IgE levels in BALF from OVA-exposed mrp1+/− mice were significantly lower than those from OVA-exposed mrp1+/+ mice (P = 0.0003). These results indicate that MRP1 plays a crucial role in IgE-mediated export of CysLTs from activated mast cells.

**DISCUSSION**

Recent generation of mrp1−/− mice has enabled investigation of the biological function of MRP1 in vivo (30). Wijnholds et al. (31) demonstrated that ear swelling induced by topical application of arachidonic acid was dramatically reduced in the mrp1−/− mice. Importantly, they also revealed that BMMCs from mrp1−/− mice had a reduced capacity to excrete LTC4 after stimulation with calcium ionophore (31). Their report strongly suggests that MRP1 plays an important role in allergic inflammatory response in vivo. However, little is known of the implication of MRP1 in IgE-mediated transport of CysLTs from mast cells and the biological significance of MRP1 in allergic airway disease, including asthma.

In our study, we developed a murine allergic airway inflammation model by intraperitoneal OVA sensitization and airway challenge. We revealed that mrp1−/− mice showed decreased airway inflammation and goblet cell hyperplasia after OVA exposure. CysLT levels in BALF from OVA-exposed mrp1−/− mice were significantly lower than those from mrp1+/+ mice.
In addition, OVA-specific IgE, IL-4, and IL-13 levels in BALF were also decreased in OVA-exposed *mrp1*/*mrp1* mice. IgE-dependent release of CysLTs from murine BMMCs was markedly impaired due to MRP1 deficiency. These findings strongly imply that MRP1 plays a key role in the development of allergic airway disease through regulation of IgE-mediated CysLT export from mast cells. To our knowledge, our study is the first report to reveal that *mrp1*/*mrp1* mice are less sensitive to asthmatic response to allergen exposure by using a murine model.

IgE-mediated activation of mast cells in the airway leads to oxygenation of arachidonic acid by 5-lipoxygenase (5-LO) and generation of LTs (12). Among them, secreted CysLTs bind to CysLT receptors and induce bronchoconstriction, mucus hypersecretion, and eosinophil chemotaxis (12, 17). Therefore, inhibition of CysLT biosynthesis or receptor-mediated action is beneficial for patients with bronchial asthma (8). In our murine allergic airway inflammation model, CysLT-synthesizing cells including mast cells in *mrp1*/*mrp1* mice had a reduced capacity to secrete CysLTs, resulting in decreased CysLT levels in BALF. Suppression of CysLT production due to MRP1 deficiency reduced recruitment of eosinophils and mononuclear cells in the lungs. These findings suggest the possibility that MRP1 inhibitor may be useful as an anti-asthma drug to attenuate airway inflammation to allergen exposure by suppressing IgE-mediated CysLT production.

Th2 inflammatory response is a central component of allergic airway inflammation. In our murine model, Th2 cytokine IL-4 and IL-13 production and lymphocyte recruitment in the lungs were significantly decreased in OVA-exposed *mrp1*/*mrp1* mice, resulting in decreased antigen-specific IgE production. Previous studies have demonstrated that OVA-induced airway eosinophil infiltration and goblet cell hyperplasia were markedly reduced in LTC4 synthase (LTC4S)-deficient mice compared with wild-type control mice (18). Importantly, antigen-specific IgE and Th2 cytokine expression in the lungs were also significantly reduced in OVA-exposed LTC4S-deficient mice, although delayed-type cutaneous hypersensitivity (Th1 cell-dependent response) was intact (18). Others have demonstrated that blockade of CysLT1 receptor reduced elevation of IL-4...
and IL-13 levels in BALF in OVA-exposed mice and attenuated airway inflammation (14). These previous findings provide direct evidence that CysLTs are involved in the regulation of Th2 immune response-dependent pulmonary inflammation. Our current findings in \textit{mrp1}^{-/-} mice are consistent with these prior reports, because MRP1 is involved in IgE-mediated LTC4 export from mast cells, and a lack of MRP1 resulted in the decrease of CysLT, antigen-specific IgE, IL-4, and IL-13 levels in the lungs of \textit{mrp1}^{-/-} mice. Impaired Th2 cytokine production due to MRP1 deficiency might be an important mechanism of reducing airway inflammation in our murine model.

Dendritic cells (DCs) are the most potent antigen presenting cells in the airways and initiate immune responses by presenting antigens to T cells (22). Previous studies have demonstrated that DCs express MRP1, and MRP1 regulates the migration of DCs by transporting LTC4, which promotes chemotaxis to the CCL19 (25). In a model of contact sensitization induced by topical application of FITC, DC migration was substantially attenuated in \textit{mrp1}^{-/-} mice compared with that observed in \textit{mrp1}^{+/+} mice (25). In addition, MRP1 transporter activity is also crucial for DC differentiation (27). These aforementioned observations on DCs may contribute to the decreased inflammatory response following OVA exposure that we examined in the lungs of \textit{mrp1}^{-/-} mice.

MRP1 was the first identified ATP-dependent export pump for LTC4. However, the members of the MRP subfamily, including MRP1–6 and MRP10–12, also mediate the ATP-dependent efflux of organic anions, including glutathione conjugates such as LTC4, across the plasma membrane into the extracellular space (7). We questioned whether the lack of MRP1 in mice would be compensated for by induction or altered expression of other ATP-binding cassette transporter subfamily members. However, van der Deen et al. (30) examined immunohistochemical expression of other transporters such as MRP2, -3, -4, -5, -6, and -9 and breast cancer resistance protein (Brcp) in murine lung tissues and observed no differences in expression of all these transporters in MRP1/MDR1a/1b-deficient mice compared with wild-type mice (30). MRP2, also named the canalicular multispecific organic anion transporter (cMOAT), and MRP1 share very similar substrates, including LTC4 (19). However, Wijnholds et al. (31) demonstrated that anti-cMOAT monoclonal antibody does not detect cMOAT protein on the mast cells in \textit{mrp1}^{-/-} and \textit{mrp1}^{+/+} mice, whereas cMOAT in the liver and kidney is readily visualized, and the same holds in \textit{mrp1}^{-/-} and \textit{mrp1}^{+/+} tissues. These previous findings strongly suggest that MRP1 (and/or MDR1) deficiency does not affect expression of other transporters in lung tissues in mice and supports our conclusion that inhibition of MRP1 might be a major cause of the impaired development of allergic airway inflammation in the lungs of \textit{mrp1}^{-/-} mice.

Recent studies have demonstrated that MRP4 can transport leukotrienes (LTB4 and LTC4) and contribute to the migration of DCs, like MRP1 (24) (28). Furthermore, MRP4 is expressed in the bronchial epithelial cells in the lungs (29). These previous findings indicate the possibility that other transporters, such as MRP4, also may be important in the lungs of murine allergic airway inflammation model in addition to MRP1, although expression of other ATP-binding cassette transporters was not altered in the lungs of MRP1-deficient mice. This may be the reason why the differences in the degree of airway inflammation between \textit{mrp1}^{-/-} and \textit{mrp1}^{+/+} mice were smaller than expected.

In this study, we also investigated the immunohistochemical expression of MRP1 in the lungs of patients with asthma and in a murine allergic airway inflammation model. MRP1 staining was observed in the cytoplasm and on the plasma membrane of the mast cells, and its expression was also found on macrophages, eosinophils, and bronchial epithelial cells (data not shown). These findings were consistent with prior reports (10), and these cells contain the 5-LO/FLAP/LTC4S pathway and generate LTC4 (16, 23). Among them, the mast cell is the most potent IgE-mediated LTC4-synthesizing cell in allergic airway inflammation and expresses MRP1 in human and murine allergic airway disease. However, eosinophils, macrophages, and bronchial epithelial cells are also important sources of CysLTs. We would like to perform in vitro experiments for CysLT export from eosinophils, macrophages, and...
bronchial epithelial cells of mrp1−/− mice in a future project. In this study, we focused on the involvement of MRP1 in IgE-dependent CysLT export from mast cells and confirmed that MRP1 plays an important role in the IgE-dependent release of CysLTs from mast cells by using murine BMMCs from mrp1−/− mice. However, decreases in CysLT levels in BALF from mrp1−/− mice in vivo were <50% compared with those in mrp1+/+ mice, although the difference was statistically significant (P = 0.0082). We speculate that residual CysLT production in mrp1−/− mice may be due to another export pump and/or derived from eosinophils, macrophages, and bronchial epithelial cells. However, it is thought that MRP1 is at least one of the important transporters on mast cells for LTC4 export in the pathogenesis of allergic airway inflammation, although other transporters may exist, because differences in data between mrp1−/− and mrp1+/+ mice were statistically significant.

There are a few interesting reports of studies that investigated the association between MRP1 and anti-asthma drugs for patients with bronchial asthma. Bandi et al. (1) incubated the human airway epithelial cell line Calu-1 with budesonide, an efficacy of therapy in the management of asthma. Bandi et al. (1) incubated the human airway epithelial cell line Calu-1 with budesonide, an integrated asthma corticosteroid, and revealed that treatment with budesonide significantly inhibits MRP1 expression and activity in Calu-1 cells. MRP1 has been screened for genetic variations, and several mutations have been identified in the MRP1 gene in the human population (21). Montelukast is a selective CysLT1 receptor antagonist that is clinically used as an anti-asthma drug. Interestingly, genetic variations in MRP1 are associated with variability in montelukast response in patients with asthma (21). These previous studies suggest that MRP1 polymorphism may be useful as a predictive marker for the efficacy of therapy in the management of asthma.

In conclusion, our study revealed that airway inflammation and goblet cell hyperplasia after OVA exposure were reduced in mrp1−/− mice compared with mrp1+/+ mice. Levels of CysLTs, antigen-specific IgE, IL-4, and IL-13 in BALF from OVA-exposed mrp1−/− mice were significantly lower than those from OVA-exposed mrp1+/+ mice. Export of IgE-dependent CysLTs from murine BMMCs was mediated by MRP1. On the basis of these findings, MRP1 expressed on mast cells functions as a CysLT export pump in the development of allergic airway disease. These findings also suggest the possibility that MRP1 may be one of the important therapeutic targets and provide new insights for understanding its role in allergic asthma.

ACKNOWLEDGMENTS

We thank Dr. Takeo Ohmura, Dr. Toshio Kumasaka, Dr. Motomi Zemba, Dr. Ri Cui, Dr. Tao Gu, Dr. Rina Ohashi, and Dr. Ken Tajima for excellent support.

GRANT

This study was supported by Grants-in-Aid for Scientific Research No. 18790551 (to F. Takahashi) and No. 14770279 (to M. Zemba).

REFERENCES

25. Robbiani DF, Finch RA, Jager D, Muller WA, Sartorelli AC, Randolph GJ. The leukotriene C(4) transporter MRPI regulates CCL19 (MIP-3beta, 

Downloaded from http://ajplung.physiology.org/ by 10.220.33.6 on April 9, 2017


