Chapter

Intratracheally Therapeutic Option for COPD: A Potential Usage of the Therapeutic Microbe for Delivering Specific Protein to the Lungs

Takashi Sato and Takeshi Shimosato

Abstract

Currently, inhaled therapy using corticosteroids and/or bronchodilators is the major established treatment for chronic obstructive pulmonary disease (COPD). The topic to be covered in this chapter is the recently developed experimental approach using biologically active molecules secreted by the live genetically modified lactic acid bacteria (gmLAB). The strategy to use gmLAB as a therapeutic/delivering tool targeting disease-specific active molecules/cites is proceeding. The role of inflammation and oxidative stress in COPD development is a valid target point. Heme oxygenase (HO)-1 as an anti-inflammatory and antioxidative stress molecule has been examined to attenuate the lung function decline and inflammation in the murine model of COPD. Recently, HO-1-secreting gmLAB as a tool for targeting inflammatory diseases has been developed and examined in several disease models including COPD. When administered intratracheally, the gmLAB showed migration to the peripheral lung and overexpression of anti-inflammatory/oxidative HO-1 in both lung and serum, protecting the lung from COPD development.

Keywords: chronic obstructive pulmonary disease, inhaled therapy, intratracheal therapy, anti-inflammatory therapy, antioxidative therapy, genetically modified lactic acid bacteria, heme oxygenase-1

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airway remodeling due to chronic inflammation and subsequent airflow limitation that should be considered most to be associated with chronic symptoms such as shortness of breath and dyspnea [1]. Inhaled bronchodilators of long-acting beta-2 agonist/muscarinic antagonist have been introduced to treat symptomatic COPD [2]. Recently, more focus on the inflammation as a background condition of COPD is growing attention to a therapeutic factor to be considered [3]. In this regard, an inhaled corticosteroid (ICS) has been involved in the standard therapy for moderate to severe COPD. However, using ICS raises the concern of an increased risk of pneumonia [4]. Thus, another class of anti-inflammatory therapeutic options would be awaited. In line with this concept, experimental anti-inflammatory therapy using heme oxygenase (HO)-1 administration or induction in murine lung disease model including emphysema has been reported with successful amelioration of disease progression [5, 6]. HO catalyzes the degradation of heme to biliverdin, carbon monoxide (CO), and iron [7]. Thus, the by-products of biliverdin and CO act as anti-inflammatory and antioxidative agents [8, 9]. The results showing that the serum levels of HO-1 in patients with COPD having significantly lower compared to those in healthy adults could support the benefits of HO-1 adminitration/induction in the lungs of COPD [10]. A recent report indicates that the HO-1 could regulate lung inflammatory/oxidtative stress status by modulating mitogen-activated protein kinase (MAPK) pathway especially for extracellular signal-regulated kinase (ERK) [11].

There are several ways of induction and/or upregulation of HO-1 in the lungs by 1) chemical induction using hemin or CoPP [10, 12] and 2) local/systemic administration of recombinant HO-1 [5, 6, 13].

Especially, the use of generally recognized as safe (GRAS) materials such as lactic acid bacteria (LAB) for producing/delivering the therapeutics for human diseases such as inflammatory bowel disease and colorectal cancer has been gaining growing attention [14–17]. In addition, exploring the conceptional use of GRAS materials for lung diseases has been planned and tried for an experimental COPD model [13, 18].

This chapter summarizes the detailed experimental approach of the intratracheal administration of GRAS microbes for producing/delivering therapeutics in the COPD model.

2. Usage of lactic acid bacteria for intratracheal administration

2.1 Construction of genetically modified lactic acid bacteria (LAB)

There have been various LABs constructed for specific target therapy and/or monitoring the LAB dynamics after administration in the animal/human body. Lactococcus (L.) lactis NZ9000 for nisin regulated target gene expression system (MoBiTec, Goettingen, Germany) was used for these purposes. The genetically modified *L. lactis* was grown under the anaerobic condition at 30°C in M17 broth (BD Difico^{TM}) overnight. The target gene expression was induced by adding 1.25 ng/mL of nisin (MoBiTec). Of these gmLABs, a green fluorescent protein (GFP)-fusion target gene expressing LAB enables researchers to monitor the levels of target gene expressions [19]. **Figure 1** shows the vector constructed for monitoring the time-dependent migration after nasally administering *Lactococcus lactis* that express/produce GFP over time.

The GFP-expressing *L. lactis* was cultured, and further time course was monitored for expression levels of GFP. Three hours after adding nisin (1.25 ng/mL), the cultured/induced GFP-expressing *L. lactis* was visualized under fluorescent microscope observation (**Figure 2**).

2.2 Airway migration of nasally administered *L. lactis*

GFP-expressing *L. lactis* were nasally administered to the anesthetized mice. A total of 50 μL of saline containing 1.0 × 10^9 of *L. lactis* was dropped into the nares and migrated to the lungs through stable nasal breathing.

DOI: http://dx.doi.org/10.5772/intechopen.106491

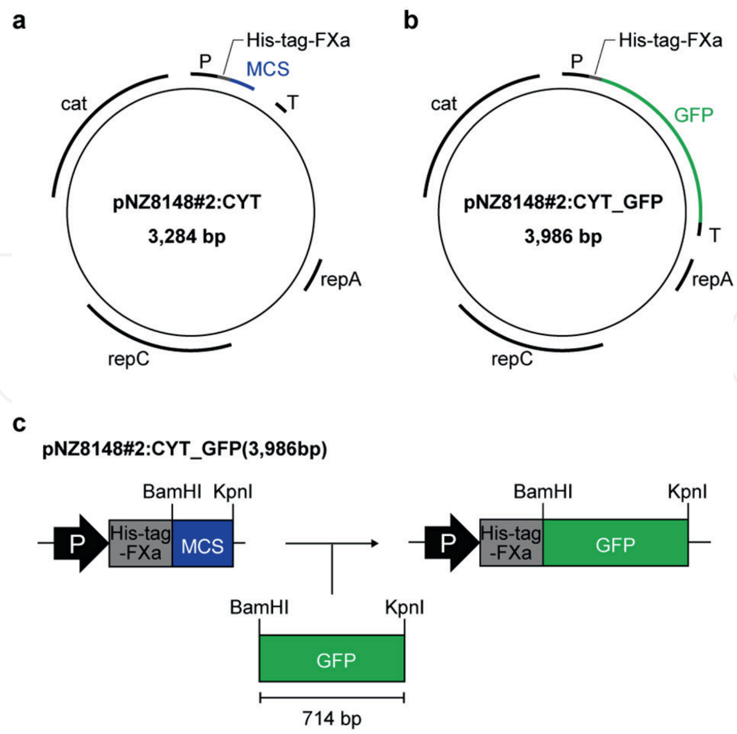

Figure 1.
Construction of GFP-expressing vector incorporated into the LAB. (a) lactococcal plasmid pNZ8148#2:CYT. (b) A green fluorescent protein (GFP) expression vector (pNZ8148#2:CYT_GFP). (c) Vector map of the pNZ8148#2:CYT_GFP. Notes: P = nisin A promotor; His-tag = hexahistidine tag; FXa = Factor Xa recognition site; MCS = multiple cloning site; T = terminator; rep = replication gene; and cat = chloramphenicol acetyltrasferase gene.

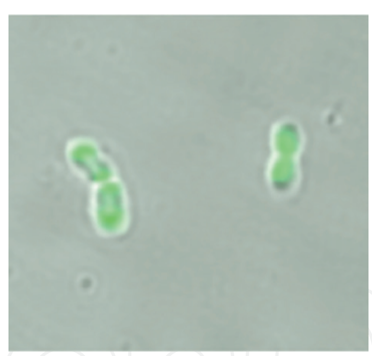

Figure 2.
Image of GFP-expressing Lactococcus lactis after induction with nisin. Genetically modified Lactococcus (L.) lactis were cultured and further induced with nisin for expression of a specific protein. The high-power field image of L. lactis showing diplococci morphology with a green signal derived from the GFP expression vector-incorporated system was visualized using a fluorescent microscope (BZ-X800; Keyence, Japan).

As shown in **Figure 3**, visualized GFP signal was time-dependently moved from the central lesion to the peripheral lesion of the lungs. Finally, the GFP signal was cleared from the lungs 96 hr after administration. Notably, at the same time of 96 hr, there was still an apparent GFP signal in the trachea, indicating 1) the high affinity of *L. lactis* for tracheal epithelium and 2) the potential usage of *L. lactis* as a carrier of airway mucosal vaccination.

2.3 Systemic effect of nasally administered *L. lactis*

Potential systemic influences after administering *L. lactis* would be body temperature, body weight, and eating behavior. Of these, time-course analysis of percent

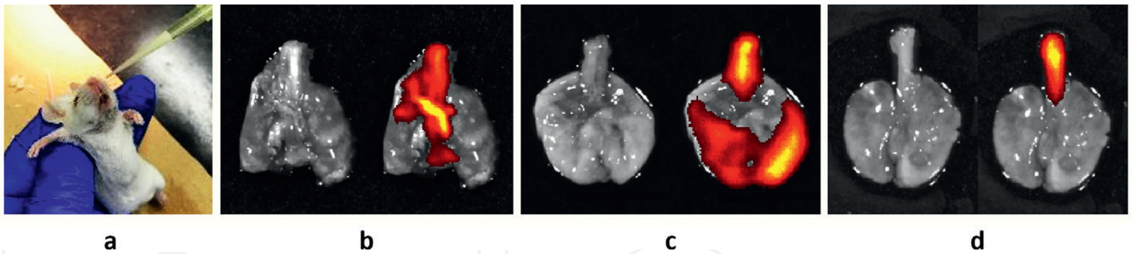

Figure 3.
Time-course analysis of ex vivo fluorescence images of removed lungs after administering GFP-expressing L. lactis. *(a) Mice (8–9 weeks of age) administered nasally with 1.0 × 10^9 of GFP-expressing* L. lactis *under anesthetized with pentobarbital sodium (30 mg/kg) were euthanized at an indicated timepoint of (b) 24 hr, (c) 48 hr, and (d) 96 hr. The removed lungs were observed under IVIS (In Vivo Imaging System, Perkin-Elmer) with (right panel) or without (left panel) fluorescence excitation. GFP signal visualized in right panel at each time point appeared in the central lesion (trachea and hilar area of the lungs) at 24 hr (b), moved to the peripheral lesion at 48 hr (c), and cleared from the lungs at 96 hr (d).*

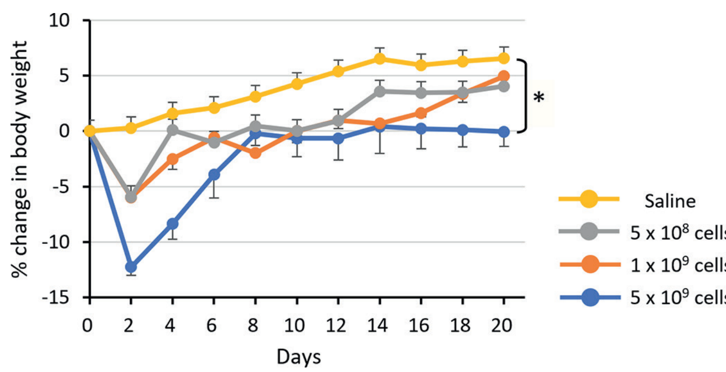

Figure 4.
*Change in body weight after nasal administration of L. lactis. Time-course analysis of percent change in body weight in mice (8–9 weeks of age) administered nasally with 0, 5 × 10^8, 1 × 10^9, or 5 × 10^9 of L. lactis. Results showed that a significant body weight loss was observed in mice treated with 5 × 10^9 of L. lactis. The calculated area under the curve of body weight from 3 to 4 mice per group indicated a statistically significant body weight loss in 5 × 10^9 of the L. lactis group compared with the saline group. * $p < 0.05$. Adapted from reference [13].*

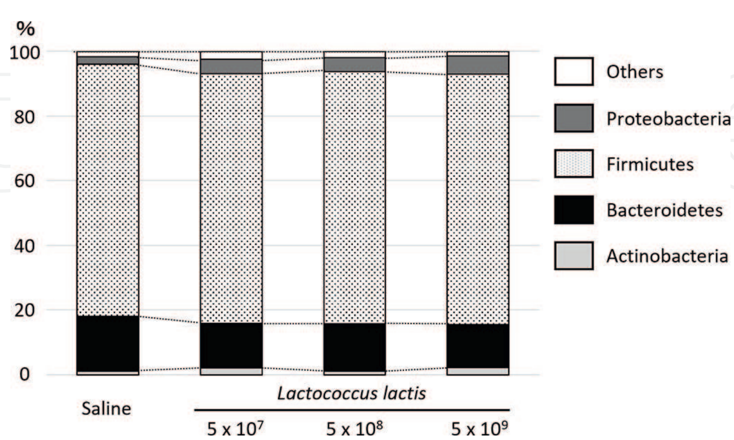

Figure 5.
Analysis of lung microbiota 14 days after nasal administration of L. lactis. Mice (8–9 weeks of age) administered nasally with 0, 5 × 10^7, 5 × 10^8, or 5 × 10^9 of L. lactis were euthanized and collected bronchoalveolar lavage (BAL) fluids 14 days after administration. The analysis of the 16S rRNA gene (V3-V4 region) was amplified and subjected to next-generation sequencing (3 mice per group).

change in body weight showed the safety concern of mice (8–9 weeks of age) administered nasally with over 1 × 10^9 of *L. lactis*. As shown in **Figure 4**, the calculated area under the curve of body weight from 3 to 4 mice per group indicated a statistically significant body weight loss in 5 × 10^9 of the *L. lactis* group compared with the saline group. Based on these results, the optimized amount of nasal administration of *L. lactis* was set to less than 1 × 10^9 per body at one time.

2.4 Local effects of nasally administered *L. lactis*

Another concern after nasally administering *L. lactis* would be a potential alteration of lung microbiota. As shown in **Figure 5**, intratracheal administration of up to 5 × 10^9 of *L. lactis* would show no statistical significance in 1) Bacteroidetes to Firmicutes ratio and 2) the composition of the microbiota belonging to Bacteroidetes or Firmicutes compared with those observed in control (saline) group.

3. Usage of lactic acid bacteria for COPD model

3.1 Construction of genetically modified *L. lactis* secreting anti-inflammatory/antioxidative stress protein HO-1

To explore the anti-inflammatory therapeutic option other than corticosteroids in COPD, HO-1 was focused on because of its low serum level shown in patients with COPD [10]. The newly constructed HO-1 secreting *L. lactis* (**Figure 6**) was examined by oral administration in a dextran sulfate sodium-induced murine colitis model [17]. Since the favorable alleviation of disease symptoms was observed in this model, a further trial was planned for lung diseases by exploring another delivery method of intratracheal administration. **Figure 6** shows the vector constructed for the HO-1 secreting *L. lactis* NZ9000 system.

3.2 HO-1 production in the lungs after nasally administering HO-1 *L. lactis*

HO-1 secreting *L. lactis* were nasally administered to the anesthetized mice. A total of 50 μL of saline containing 1.0 × 10^9 of *L. lactis* was migrated to the lungs through stable nasal breathing. Production of HO-1 derived from HO-1 *L. lactis* was confirmed by immunoblotting using anti-His antibody and anti-HO-1 antibody in lung homogenates (**Figure 7a**). Through the pulmonary trafficking of HO-1 *L. lactis*, serum HO-1 levels were significantly increased (**Figure 7b**).

3.3 Effect of nasally administered HO-1 secreting *L. lactis* in murine emphysema model

HO-1-secreting *L. lactis* were nasally administered to the anesthetized mice 48 hr before instillation with porcine pancreatic elastase (PPE) (**Figure 8**). A total of 50 μL of saline containing 1.0 × 10^9 of *L. lactis* was dropped into the nares and migrated to the lungs through stable nasal breathing.

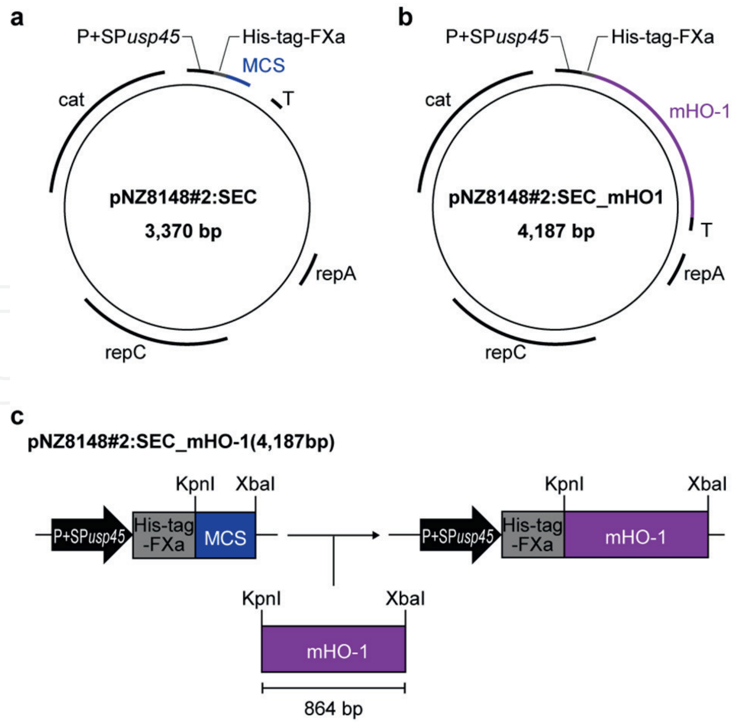

Figure 6.

Construction of HO-1-expressing vector incorporated into L. lactis. (a) A lactococcal plasmid pNZ8148#2:SEC. (b) A heme oxygenase-1 (HO-1) expression vector (pNZ8148#2:SEC_mHO1). (c) Vector map of the pNZ8148#2:SEC_mHO1. Notes: P = nisin A promotor; SP_{USP45} *= sequence of the signal peptide from the USP45 protein; His-tag = hexahistidine tag; FXa = Factor Xa recognition site; MCS = multiple cloning site; T = terminator; rep = replication gene; and cat = chloramphenicol acetyltrasferase gene.*

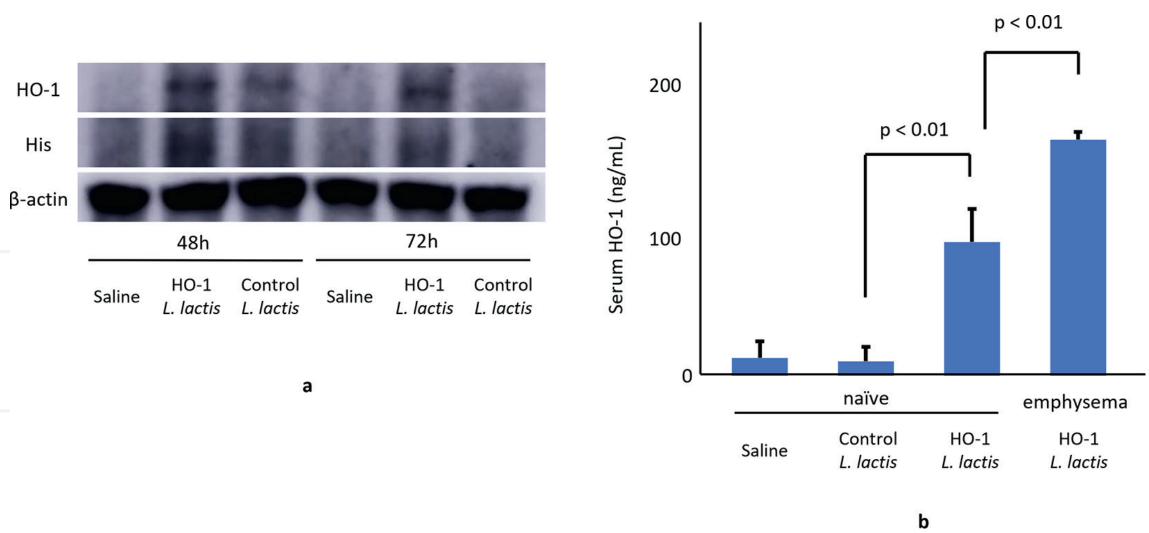

Figure 7.

Systemic and local HO-1 production after nasal administration of HO-1 L. lactis. Mice (8–9 weeks of age) administered nasally with HO-1 L. lactis were subjected to assess the local (lung) and systemic (serum) HO-1 levels 48 or 72 hr after administration. (a) The lung homogenates from naïve mice receiving either control or HO-1 L. lactis were assessed by immunoblotting. The representative result showed that the nisin-induced HO-1 was confirmed. Adapted from reference [13]. (b) Serum HO-1 levels were assessed using ELISA (MK125, TAKARA Bio Inc., Japan) 48 hr after administration. Results from 5 to 6 mice/group showed a significant increase in HO-1 in both naïve and emphysema models receiving HO-1 L. lactis compared with those receiving control L. lactis.

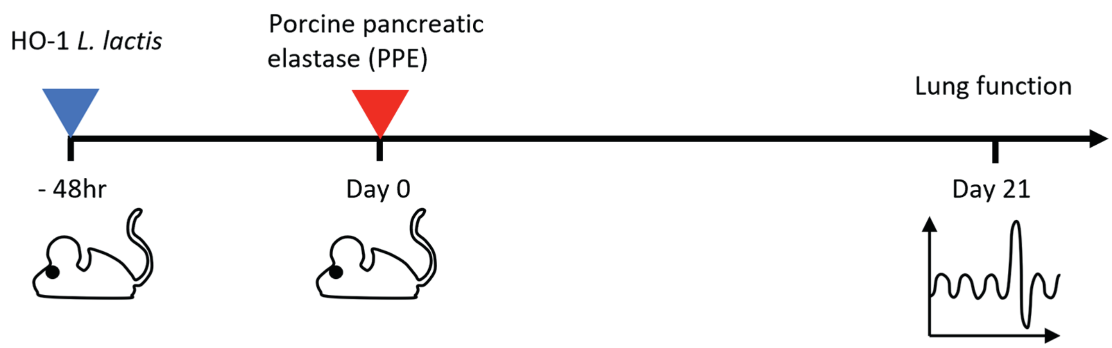

Figure 8.
Protocol of the prophylactic use of HO-1 L. lactis in emphysema model. HO-1 L. lactis was administered 48 hr before instillation of 1 unit of porcine pancreatic elastase (PPE; Elastin Products Co., Inc., USA) in 50 μL of saline. The mice treated with PPE showed progressive destruction of the alveolar structure, leading to emphysematous morphologic deterioration up to day 21.

On day 21, after PPE instillation, the mice developing pulmonary emphysema were evaluated by pulmonary function test using the flexiVent system (emka TECHNOLOGIES Japan).

3.3.1 Systemic effect of nasally administered HO-1 secreting L. lactis

Mice pretreated with 1.0×10^9 of HO-1 *L. lactis* showed a significant increase in body weight compared with those pretreated with control *L. lactis* or only saline

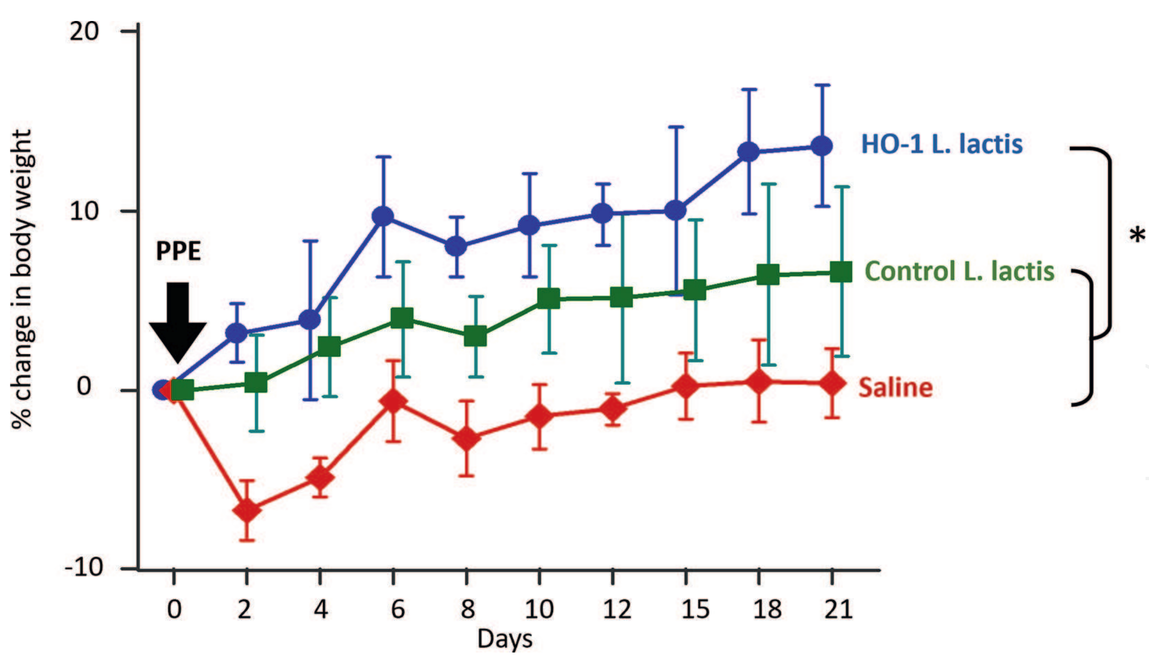

Figure 9.
*Effect of nasal administration of L. lactis on PPE-induced weight loss. Time-course analysis of percent change in body weight after PPE instillation (Day 0) in mice pretreated nasally with 1×10^9 of either HO-1 L. lactis or control L. lactis (Day -2). A significant body weight loss observed in mice pretreated with saline (vehicle only) was not reproduced in mice pretreated with HO-1 L. lactis. The calculated area under the curve of body weight from 5 to 6 mice per group indicated a statistically significant improvement in body weight loss in the HO-1 L. lactis group compared with the control L. lactis or saline group. * $p < 0.05$. Adapted from reference [13].*

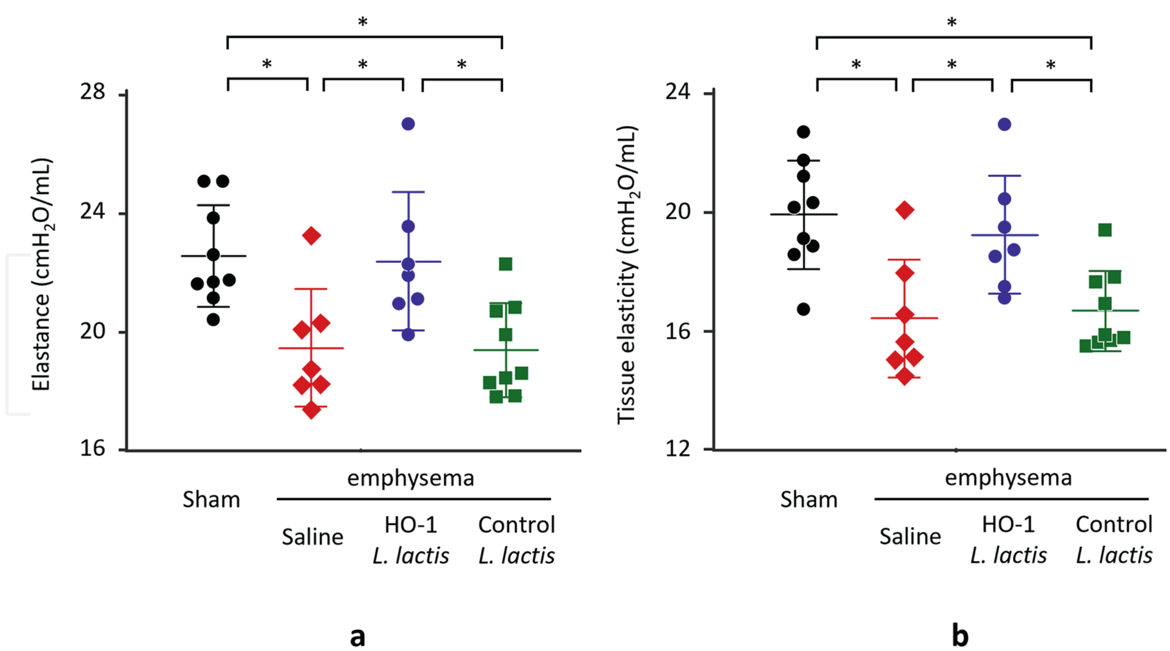

Figure 10.
Local effect of nasally administered HO-1 L. lactis on PPE-induced emphysema mice assessed by in vivo lung function measurements. Mice were treated as described in ***Figure 8****. In vivo lung function tests were performed under anesthesia using a flexiVent system on day 21. The results of lung function measurements of (a) Elastance and (b) Tissue elasticity were shown. Bars indicate the mean ± SD. * $p < 0.05$. Adapted from reference [13].*

($p < 0.05$) (**Figure 9**). Thus, nasal administration of HO-1 *L. lactis* reduced the physiological deterioration caused by PPE.

3.3.2 Local effect of nasally administered HO-1 secreting L. lactis

In human clinical trials, the efficacy of candidate drugs for COPD should be primarily assessed by inhibiting lung function deterioration [20]. Therefore, in vivo lung function measurements of mice receiving with or without HO-1 *L. lactis* before emphysema development were assessed using a highly sensitive and reproducible flexiVent system for small animal [21]. The characteristic of an emphysematous lung is reduced elasticity reflecting the hyperinflation and decreased elastic recoil [21]. Consistent with this lung morphologic deterioration, “elastance” (determined by single-frequency forced oscillation technique) and “tissue elasticity” (defined by a small amplitude broadband oscillation technique) were significantly decreased in PPE-induced emphysema mice pretreated with either saline or control *L. lactis*. Fortunately, however, the mice pretreated with HO-1 *L. lactis* showed satisfactory suppression of PPE-induced lung function deterioration (**Figure 10**).

4. Conclusions

This chapter summarizes the potential therapeutics of gmLAB and its application for lung diseases, including COPD. LAB has been widely used as probiotics for health, and to maximize its beneficial effects, gmLAB has been developed. Among several gmLABs, the use of *L. lactis* has been favored because of 1) its generally recognized as safe status, 2) its absence of endotoxins, 3) its easy manipulating property, and 4) its low cost and easy administration. When applied for lung diseases, direct delivery of the therapeutics (gmLAB) to the lungs by intratracheal administration would be favored in terms of efficacy and safety concerns. In addition, the successful attenuation of disease progression in the murine emphysema model by local administration of anti-inflammatory gmLAB would support a further human clinical trial.

Acknowledgements

Authors thank Drs. Kentaro Nakashima and Kentaro Yumoto (Yokohama City University, Japan) and Drs. Suguru Shigemori and Fu Namai (Shinshu University, Japan) for their significant contribution to the project. The project was funded by the Japan Society for the Promotion of Science (JSPS) KAKENHI, grant numbers JP15K09224, JP18K19935, JP19KK0208, and JP22K08578 to Takashi Sato.

Conflict of interest

The authors declare no conflict of interest.

Author details

Takashi Sato* and Takeshi Shimosato
Institute for Biomedical Sciences, Shinshu University, Nagano, Japan

*Address all correspondence to: satotak@shinshu-u.ac.jp

References

[1] James AL, Wenzel S. Clinical relevance of airway remodelling in airway diseases. The European Respiratory Journal. 2007;**30**(1):134-155. DOI: 10.1183/09031936.00146905

[2] Page C, Cazzola M. Bifunctional drugs for the treatment of asthma and chronic obstructive pulmonary disease. The European Respiratory Journal. 2014;**44**(2):475-482. DOI: 10.1183/09031936.00003814

[3] King PT. Inflammation in chronic obstructive pulmonary disease and its role in cardiovascular disease and lung cancer. Clinical and Translational Medicine. 2015;**4**(1):68. DOI: 10.1186/s40169-015-0068-z

[4] Wang CY, Lai CC, Yang WC, Lin CC, Chen L, Wang HC, et al. The association between inhaled corticosteroid and pneumonia in COPD patients: The improvement of patients' life quality with COPD in Taiwan (IMPACT) study. International Journal of Chronic Obstructive Pulmonary Disease. 2016;**11**:2775-2783. DOI: 10.2147/COPD.S116750

[5] Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, Choi AM. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. The Journal of Clinical Investigation. 1999;**103**(7):1047-1054. DOI: 10.1172/JCI5342

[6] Shinohara T, Kaneko T, Nagashima Y, Ueda A, Tagawa A, Ishigatsubo Y. Adenovirus-mediated transfer and overexpression of heme oxygenase 1 cDNA in lungs attenuates elastase-induced pulmonary emphysema in mice. Human Gene Therapy. 2005;**16**(3):318-327. DOI: 10.1089/hum.2005.16.318

[7] Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proceedings of the National Academy of Sciences of the United States of America. 1968;**61**(2):748-755. DOI: 10.1073/pnas.61.2.748

[8] Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nature Medicine. 2000;**6**(4):422-428. DOI: 10.1038/74680

[9] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;**235**(4792):1043-1046. DOI: 10.1126/science.3029864

[10] Sato T, Takeno M, Honma K, Yamauchi H, Saito Y, Sasaki T, et al. Heme oxygenase-1, a potential biomarker of chronic silicosis, attenuates silica-induced lung injury. American Journal of Respiratory and Critical Care Medicine. 2006;**174**(8):906-914. DOI: 10.1164/rccm.200508-1237OC

[11] Nakashima K, Sato T, Shigemori S, Shimosato T, Shinkai M, Kaneko T. Regulatory role of heme oxygenase-1 in silica-induced lung injury. Respiratory Research. 2018;**19**(1):144. DOI: 10.1186/s12931-018-0852-6

[12] Minamoto K, Harada H, Lama VN, Fedarau MA, Pinsky DJ. Reciprocal regulation of airway rejection by the inducible gas-forming enzymes heme oxygenase and nitric oxide synthase.

The Journal of Experimental Medicine. 2005;**202**(2):283-294. DOI: 10.1084/jem.20050377

[13] Yumoto K, Sato T, Nakashima K, Namai F, Shigemori S, Shimosato T, et al. Nasally administered lactococcus lactis secreting heme oxygenase-1 attenuates murine emphysema. Antioxidants (Basel). 2020;**9**(11): 283-294. DOI: 10.3390/antiox9111049

[14] Ciacma K, Wieckiewicz J, Kedracka-Krok S, Kurtyka M, Stec M, Siedlar M, et al. Secretion of tumoricidal human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by recombinant Lactococcus lactis: Optimization of in vitro synthesis conditions. Microbial Cell Factories. 2018;**17**(1):177. DOI: 10.1186/s12934-018-1028-2

[15] Namai F, Shigemori S, Ogita T, Sato T, Shimosato T. Microbial therapeutics for acute colitis based on genetically modified Lactococcus lactis hypersecreting IL-1Ra in mice. Experimental & Molecular Medicine. 2020;**52**(9):1627-1636. DOI: 10.1038/s12276-020-00507-5

[16] Shigemori S, Shimosato T. Applications of genetically modified immunobiotics with high immunoregulatory capacity for treatment of inflammatory bowel diseases. Frontiers in Immunology. 2017;**8**:22. DOI: 10.3389/fimmu.2017.00022

[17] Shigemori S, Watanabe T, Kudoh K, Ihara M, Nigar S, Yamamoto Y, et al. Oral delivery of Lactococcus lactis that secretes bioactive heme oxygenase-1 alleviates development of acute colitis in mice. Microbial Cell Factories. 2015;**14**:189. DOI: 10.1186/s12934-015-0378-2

[18] Sato T, Shimosato T. Development of a New Treatment Modality for Lung Diseases That Uses Innovative Fine Droplet Drying (fdd) Technology Offering Inhalable Nano/microparticles-incorporated Therapeutic Agents. New York: Science Impact Ltd; 2019. DOI: 10.21820/23987073.2019.3.56

[19] Shigemori S, Namai F, Yamamoto Y, Nigar S, Sato T, Ogita T, et al. Genetically modified Lactococcus lactis producing a green fluorescent protein-bovine lactoferrin fusion protein suppresses proinflammatory cytokine expression in lipopolysaccharide-stimulated RAW 264.7 cells. Journal of Dairy Science. 2017;**100**(9):7007-7015. DOI: 10.3168/jds.2017-12872

[20] Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: From lung function to biomarkers. The European Respiratory Journal. 2008;**31**(2):416-469. DOI: 10.1183/09031936.00099306

[21] Vanoirbeek JA, Rinaldi M, De Vooght V, Haenen S, Bobic S, Gayan-Ramirez G, et al. Noninvasive and invasive pulmonary function in mouse models of obstructive and restrictive respiratory diseases. American Journal of Respiratory Cell and Molecular Biology. 2010;**42**(1):96-104. DOI: 10.1165/rcmb.2008-0487OC