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Acellular filtrate of a microbial-based cleaning product potentiates house dust mite allergic lung inflammation

Azam F. Tayabali[a,*], Yan Zhang[a], Jason H. Fine[b], Don Caldwell[b], Martha Navarro[b]

[a] Biotechnology Laboratory, Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, K1A 0K9, Canada

[b] Scientific Services Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, K1A 0K9, Canada

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ABSTRACT

Microbial-based cleaning products (MBCPs) contain bacteria and chemical constituents. They are used in consumer applications such as odor reduction, unclogging drains, and surface cleaning. To determine the capacity of a model MBCP to contribute to acute allergic lung inflammation, a two-week repeated exposure regimen was used. Mice were exposed by endotracheal instillation to saline alone, MBCP alone, house dust mites (HDM) alone, or sequentially (i.e., MBCP followed by HDM, HDM followed by MBCP, or HDM + MBCP followed by HDM). Both whole MBCP and acellular MBCP filtrate were investigated, and showed minimal differences in the endpoints examined. HDM exposure caused pulmonary perivascular inflammation, bronchiolar mucous cell metaplasia, elevated bronchoalveolar lavage fluid (BALF) eosinophils, and HDM-specific IgG1. For MBCP, notable changes were associated with sequential exposures. MBCP/HDM caused elevated T_H2 cytokines in BALF, and elevated neutrophils, eosinophils and IL-5 in peripheral blood. Co-administration of MBCP and HDM followed by HDM resulted in elevated blood and BALF eosinophils and HDM-specific IgE and IgG1. These results demonstrated that acellular MBCP filtrate, and not bacteria within MBCPs, potentiated the acute allergic inflammation to HDM. This methodology could be extended to investigate chronic allergic inflammation and inflammatory potential of other MBCPs and biotechnology products with complex compositions.

1. Introduction

Spray-type microbial-based cleaning products (MBCPs) are marketed internationally for use by consumers and smaller industries such as restaurants and recreational facilities. They have a wide range of applications, including cleaning a variety of surfaces, deodorizing pet or sports equipment, and removing oil and pet residue stains (Vandini et al., 2014; SpÖk and Klade, 2015). An important distinction between these and non-microbial products is that the living microorganisms and proprietary enzymes, surfactants and detergents collectively contribute to the efficacy of the product. This complex composition also creates uncertainties for regulators responsible for assessing the human health risk during manufacture or use by the consumer.

Spray MBCPs could pose a hazard from accidental inhalation exposures during product use, or after surface treatment where chemicals adsorbed to dust or other particles are aerosolized and subsequently inhaled (Poulsen et al., 2000). Furthermore, the prevalence of occupational allergy resulting from industrial enzyme exposure is an ongoing concern (Lindstedt et al., 2005). Currently, there are no studies investigating the respiratory hazard of MBCPs, but the potential for acute symptoms such as local airway inflammation or exacerbation of pre-existing respiratory conditions is possible.

Either the filterable formulation constituents or microbial components of MBCPs could contribute to the allergic response. Chemical and enzymatic cleaning agents are known to be occupational and domestic allergens. For example, disinfectants, detergents, and bacterial enzymes can act as sensitizers, and are capable of elevating the risk of asthma and rhinitis (Lindstedt et al., 2005; Clausen et al., 2000; Folletti et al., 2014; Rosenman, 2006). Alternatively, the microbes within the MBCPs may function as inducers of allergies. Although exposure to bacteria or substances derived from them most often result in a T_H1 type response (North et al., 2016), biomonitoring data and mouse exposure studies have demonstrated that bacteria or substances derived from them (e.g., cell wall constituents) can contribute to allergic asthma by functioning as sensitizers or adjuvants (Bernstein et al., 1999; Berstad et al., 2000; Huang et al., 2011; Kawai et al., 2002; Schröder et al., 2008). Moreover, *Bacillus* spores have been reported to induce hypersensitivity pneumonitis, which is also known as detergent workers disease or humidifier

* Corresponding author.
E-mail addresses: azam.tayabali@canada.ca (A.F. Tayabali), mzhang@ottawaheart.ca (Y. Zhang), jason.fine@canada.ca (J.H. Fine), don.caldwell@canada.ca (D. Caldwell), martha.navarro@canada.ca (M. Navarro).

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lung (Reynolds, 1988).

Thus, there is sufficient evidence to suggest that cleaning agents and/or microbial constituents have the capacity to elicit or contribute to the allergic response. The aim of this study was to determine if whole MBCP or its acellular formulation filtrate could induce or potentiate the early allergic response. To address this, an acute (two week) mouse exposure model of allergic airway inflammation was used, which has been well-characterized using a known respiratory allergen, the house dust mite (HDM; *Dermatophagoides farina*) (Cates et al., 2004; Piyadasa et al., 2016; Bazett et al., 2016), which is a relevant allergen when considering residential MBCP usage. MBCP exposures with and without HDM were investigated by measuring indicators of inflammation, airway remodeling, and induction of HDM-specific immunoglobulins. The data presented here demonstrate the capacity of MBCP components to contribute to the allergic inflammatory response caused by HDM.

2. Materials and methods

2.1. MBCP characterization and preparation of MBCP filtrate

A liquid MBCP was selected based on its application in spray format and our initial analysis of contents. The product is used for pet odor reduction and could potentially result in a substantial respiratory exposure during application. It was described by the manufacturer to contain non-pathogenic bacteria, natural enzymes and mild cleansers. The microbial content was assessed by spread plating serial dilutions and incubating at 28 °C for 24 h on Trypticase Soy Agar (TSA) with or without 5% sheep blood (37 °C). Colonies were enumerated and processed for identification as outlined by the MIDI-Sherlock® fatty acid methyl ester (FAME) microbial identification system (MIDI Inc., Newark, DE). FAME profiles from isolated colonies were compared to both the RTCLIN6 clinical library and a previously developed custom in-house library consisting of FAME profiles from domestic microbial strains approved for biotechnology applications in Canada. Acellular product filtrates were prepared by passing the contents through a 0.22 µm pore size hydrophilic polyethersulfone (PES) membrane. Lack of microbial growth was confirmed by streaking the filtrate onto TSA.

2.2. Animals used in this study

All procedures involving animals were approved by the Health Canada Animal Care Committee and overseen by a board certified veterinarian. Female BALB/c mice between 18 and 20 g (8 weeks old) were purchased from Charles River Laboratories Inc. (Saint-Constant, QC), and acclimated for at least 1 week prior to experimentation. Animals were housed in cages under a 12-h light/dark cycle at a constant room temperature (20 ± 2 °C) and relative humidity of 50–70%. The commercial pellet diet and reverse osmosis (RO) water were available *ad libitum*.

2.3. Experimental design and animal exposure

The inflammatory effects of exposure to either whole MBCP or its filtrate were investigated using a modification of a procedure used for HDM–induced inflammation in allergic airway disease (Cates et al., 2004; Piyadasa et al., 2016; Bazett et al., 2016). Animal exposure schedules are shown in Fig. 1. Six mice were used for each two-week treatment with the following: (1) saline alone (2) HDM alone (3) MBCP alone (4) MBCP (week 1) followed by HDM (week 2) referred to as MBCP/HDM (5) HDM (week 1) followed by MBCP (week 2) referred to as HDM/MBCP, and (6) HDM mixed with MBCP (week 1) followed by HDM (week 2) referred to as HDM + MBCP/HDM.

For endotracheal instillation, mice were anesthetized with isoflurane (4-5% for induction, and 1-3% for maintenance). The animals were treated with a final volume of 25 µL of saline with either *Dermatophagoides pteronyssimus* (HDM; 25 µg as used by (Bazett et al., 2016)) (Greer Laboratories, Lenoir, NC) or MBCP (10 µg). The 10 µg dose was chosen since it corresponds to 2×10^4 cfu, which did not cause notable toxicity in a previous mouse exposure study with *Bacillus* species (Tayabali et al., 2011), which are used in the formulation of most MBCPs (SpÖk and Klade, 2015). Control animals were treated with vehicle alone (0.9% saline). The doses were administered by instillation using a syringe-style nebulizer (Microsprayer™, Penn Century, Philadelphia, PA) through the trachea into the lungs. Treated mice typically recovered from anaesthesia and dosing within 2 min.

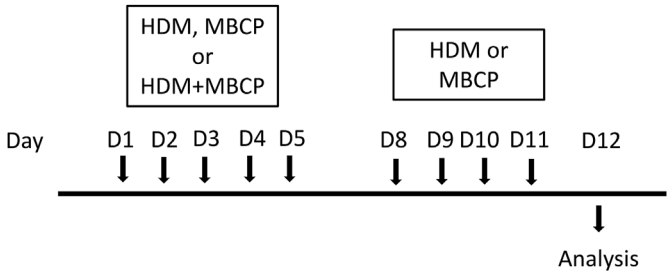

Fig. 1. Exposure schedule for administration of MBCP and HDM. The schedule followed was for 2 weeks as shown for days (D) 1–5 in the first week followed by D8-11 in the second week, and sample collection and analysis on D12.

2.4. Blood analysis

On Day 12, treated mice were anesthetized with isoflurane as described above, and euthanized by exsanguination. For this, 0.5–1.0 mL of blood was collected by cardiac puncture and transferred to tubes containing ethylenediaminetetraacetic acid (EDTA). Hematological analysis was performed using an automated haematology analyzer (Sysmex XT-2000iV-1, Kobe, Japan), and the remaining plasma was stored at -80 °C for pro-inflammatory cytokine analyses and immunoglobulin IgG1 and IgE measurements. Cytokines were measured according to the manufacturer's instructions (Bio-Plex, Biorad, Hercules, CA). A panel of 10 cytokines were investigated: IL-4, IL-5, IL-10, IL-13, Eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon (IFN)-γ, keratinocyte chemoattractant (KC), regulated on activation normal T cell expressed and secreted (RANTES), and tumor necrosis factor (TNF)-α. Data were analyzed by Bio-Plex Manager 6.0 software. For measurement of HDM-specific IgG1 and IgE, reagents from ELISA Ready-SET-Go! IgG1 and IgE kits (eBioscience, San Diego, CA) were used and a modification of the procedure by Bazett and colleagues was followed (Bazett et al., 2016). Briefly, HDM (2.5 µg/well) was coated into wells of a 96-well plate. Following blocking with 5% fetal bovine serum in PBS, undiluted plasma was incubated in the wells overnight at 4 °C. Following PBS washes, wells were incubated with horse-radish peroxidase (HRP) conjugated anti-mouse IgG1 or IgE polyclonal antibody for 1 h at 37 °C. Colorimetric detection of antibody conjugation was done with tetramethylbenzidine (TMB) substrate solution and measurement with a multi-well spectrophotometer (Molecular Devices, CA) at optical densities of 450 nm and 570 nm. Standard curves were generated by coating wells with known concentrations of IgG1 or IgE, binding with HRP-conjugated anti-mouse IgG1 or IgE, and detection with TMB.

2.5. Bronchoalveolar fluid (BALF) collection and analysis

Lung lavage fluid was collected by securing a 22-gauge plastic cannula (Instech Laboratories, Inc., Plymouth Meeting, PA) in the trachea using surgical thread. A 3-way stopcock (Harvard Apparatus, Holliston, MA) with two syringes was secured on the cannula. One syringe was used to inject sterile saline into the bronchioalveolar compartment, while the other syringe was used for BALF recovery.

For the cell population analysis, cells were retrieved by