Published in final edited form as:

J Immunol Methods. 2013 February 28; 388(0): 86–89. doi:10.1016/j.jim.2012.11.009.

Immunodetection and quantification of airborne (1–3)- β -D-glucan-carrying particles with the halogen immunoassay

Félix E. Rivera-Mariani*, Jana N. Mihalic, Ana M. Rule, and Patrick N. Breyse

Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States

Abstract

Fungal cell wall components, such as (1–3)- β -D-glucan, are known to be capable of activating the innate immune system and pose a respiratory health risk in different environments. Mass-based non-viable techniques commonly used for assessment of fungal exposures could be β -D-glucan-specific, but are limited to analysis of liquid extracts. The variable solubility of different β -D-glucans may underestimate β -D-glucan exposure and long sampling times required for mass-based methods make assessing short-term exposures difficult. In this study, we evaluated the utility of the halogen immunoassay (HIA), an immunoblotting technique previously used for allergens, to immunodetect and quantify β -D-glucan-carrying particles (BGCPs). The HIA was able to detect BGCPs without background staining when β -D-glucan standards and air samples collected at a poultry house during short sampling periods were evaluated. The image analysis protocol previously developed by our group for mouse allergen allowed simultaneous immunodetection and quantification of β -D-glucan-containing particles. Our results suggest that the HIA holds promise for quantifying β -D-glucan exposures. To our knowledge, this is the first time in which the HIA was used for non-allergenic compounds of microbial or fungal origins.

Keywords

Airborne; (1-3)- β -D-glucan; (1-3)- β -D-glucan-carrying particles; Halogen immunoassay

1. Introduction

Cell wall components of fungi, such as (1–3)- β -D-glucan (β -glucan), are known to be potent activators of the innate immune system (Noss et al., 2012). Exposure to β -glucan in indoor environments (e.g. home, occupational) can pose a respiratory health risk (Wouters et al., 2006; Tischer et al., 2011), and presence of airborne β -glucan-carrying particles (BGCPs) has been documented in the absence of obvious fungal contamination (Wu et al., 2010). Since airborne BGCPs are non-viable fungal components, relying only on culture methods could limit accurate assessment of fungal exposure.

Non-culture techniques commonly used to assess exposure to β -glucan-carrying particles include enzyme-linked immunosorbent assay (ELISA) or the modified *Limulus* amoebocyte lysate assay (LAL) (Lawniczek-Wakzyk and Gorny, 2010). Although these methods can be specific for (1–3)- β -D-glucans, there are major limitations including long sampling periods required to detect soluble (1–3)- β -D-glucans above the limit of detection, and that extracts

need to be prepared from the air sample prior to analysis. Different types of (1–3)- β -D-glucans with additional glycosidic linkages (i.e. 1–4, 1–6) could be present in the sample, contributing to structural and molecular modifications that affect solubility (Noss et al., 2012). This may prevent complete extraction of the β -glucans from the samples. High-performance liquid chromatography provides a quantitative alternative with high specificity, but requires highly expensive equipment and is time consuming. Direct detection and quantification of airborne BGCPs on the filter surface can overcome these limitations.

The halogen immunoassay (HIA) is an extract-independent method that combines immunoblotting, microscopy, and image analysis to detect and quantify airborne particles (Tovey et al., 2000).

In this study, we examined the utility of the halogen immunoassay to detect and quantify airborne β -glucan-carrying particles.

2. Methods

We first validated the application of HIA for the detection and quantification of β -glucans by analyzing known concentrations of β -glucan standards deposited on a filter. Once validated, we tested the method by sampling an environment known to have airborne β -glucan-carrying particles.

2.1. Halogen immunoassay with known concentrations of β -glucan

As an initial test of the HIA analysis for β -glucans, suspensions with known concentrations (750 μ g/ml and 75 μ g/ml) of particles from a standard (1–3)- β -D-glucan (curdlan) (Sigma Aldrich, St Louis, MO USA) were filtered onto 37-mm 0.8 μ m pore-size mixed-cellulose ester protein-binding membrane filters (MCE-PBMs) (Millipore Corp., Billerica, MA, USA). The MCE-PBMs with known concentrations of β -glucan particles were analyzed with the HIA as described below.

2.2. Halogen immunoassay

The particles on the MCE-PBMs were immobilized by sealing with strips of optically-clear ABgene™QPCR adhesive film (Thermo Scientific, Waltham, MA USA). Air bubbles were removed by applying pressure with a small round object. Sealed MCE-PBMs were cut into 5-mm squares before being analyzed with the HIA as previously described (Rivera-Mariani et al., 2012), but using mouse monoclonal IgG anti-(1–3)- β -D-glucan (Biosupplies Australia Pty Ltd, La Trobe Uni, Bundoora AU) and biotinylated goat polyclonal anti-mouse IgG (R&D Systems, Minneapolis, MN USA). Previous studies have demonstrated no cross-reactivity of this anti-(1–3)- β -D-glucan antibody with other β -D-glucans containing different (i.e. 1–4 or 1–3;1–4) glycosidic linkage (Meikle et al., 1991). Briefly, β -D-glucans were eluted overnight (800 μ l of borate buffer 4 °C) from the immobilized particles. After washing three times with phosphate buffer saline containing 0.05% Tween 20 (PBS-T), the elution was stopped with PBS-3% bovine serum albumin (PBS-BSA) (Sigma Aldrich). The MCE-PBMs were washed as before prior to incubating with 400 μ l of monoclonal anti-(1–3)- β -D-glucan antibody (1:1000 in PBS-BSA) for 1 h at room temperature (RT). After washing, 400 μ l of biotinylated anti-mouse IgG (1:1000 in PBS-BSA) was added and incubated for 1 h at RT. Unbound biotinylated anti-mouse IgG was washed prior to adding 400 μ l streptavidin-alkaline phosphatase (1:400 in PBS-BSA) (KPL Inc., Gaithersburg, MD USA). The MCE-PBMs were developed by incubating with 400 μ l of phosphatase substrate (5-bromo-4-chloro-3'-indolylphosphate/nitro-blue tetrazolium) (KPL) for 20 min before stopping the reaction with ice-cold deionized water. The developed MCE-PBMs were kept at 4 °C for a maximum of three days before performing microscopy and image analysis (Rivera-Mariani et al., 2012).

2.3. Air samples

Samples were collected at a poultry farm facility at the University of Delaware. Two samples were collected on two different days (total of 4 sequential samples). Poultry farm samples were used for this study because they are known to be a source of airborne β -glucan (Rylander and Carvalheiro, 2006). Samples were collected on 37-mm 0.8 μ m pore size MCE-PBM without respect to size since we do not know the size distribution of β -glucan-carrying particles. Samples were collected using BGI 400 sampling pumps (BGI Inc., Waltham, MA, USA) at a flow rate of 2 l/min. Flow rates were calibrated before and after the sample collection. The samplers were located outside the poultry barn approximately 2 m from the barn exhaust and 1.5 m above ground level. An initial air sample was collected for 2 h, after which a second sample was collected for the same sampling time. The air sample filters were returned to the laboratory, sealed, and kept in a dessicator at room temperature until analyzed by HIA as performed with MCE-PBMs with known concentrations of standard β -D-glucan particles. The approximate time the samples were kept in the dessicator was seven days. Filter blanks were manipulated similar to filters with air samples.

2.4. Concentration of haloed β -D-glucan-carrying-particles (BGCPs) in air samples

The concentration of BGCPs/filter was calculated using Eq. (1) (Adhikari et al., 2003):

$$\text{BGCPs/filter} = (N_f/A_f)A_t \quad (1)$$

where N_f is the average particles per field, A_t is the area of the MCE-PBM (962 mm²) filter, and A_f is the area of the microscopic field at 200 $\times$ (0.2954 mm²). To calculate BGCPs/m³, the following formula was used (Eq. (2))

$$\text{BGCPs/m}^3 = \text{BGCPs/filter} / (Q \times t) \quad (2)$$

where Q is the flow rate (2 $\times$ 10⁻³ m³/m) and t is the sampling time (120 min). The limit of detection, based on 1 particle on 10 fields (0.1 BGCPs/field), was determined to be 0.4 BGCPs/m³ for a 120 min sample.

3. Results

In the initial experiment with the known spiked concentrations of (1–3)- β -glucan standard (Fig. 1A), we determined that the with antibody combinations selected and the image analysis protocol (Rivera-Mariani et al., 2012) we were able to detect β -glucan in both filters spiked with known concentrations. At this stage we were only interested in detecting the particle, without regarding the size of the halo around the BGCPs. Quantitative HIA results for spiked β -glucan samples in terms of BGCPs/ml are presented in Fig. 2A. We detected 483 $\pm$ 25 BGCPs/ml and 125 $\pm$ 19 (error bars correspond to triplicates of the HIA) in the 750 μ g/ml and 75 μ g/ml standard solutions, respectively. Furthermore, there was no background staining as shown in the image corresponding to the blank (Fig. 1A). These results indicate that the HIA can detect β -glucan particles.

Images of the consecutive (same day) air samples collected outside the poultry facility (Fig. 1B) were analyzed. Quantitative HIA results (concentrations of BGCPs/m³) for pairs of samples collected over two days are presented in Fig. 2B (day 1) and 2C (day 2). The consecutive samples in the 1st day had similar concentrations of BGCPs/m³ (178 $\pm$ 77 and 175 $\pm$ 40 BGCPs/m³) (error bars correspond to triplicates of the HIA); however, the consecutive samples in the second day were more variable (359 $\pm$ 13 and 523 $\pm$ 42). Similar to the HIA with the known β -glucan spiked concentrations, there was no background staining as shown in the blanks analyzed in both processed in days (Fig. 1B).

4. Discussion

In this pilot study, we evaluated the utility of the HIA to immunodetect and quantify BGCPs deposited on a filter membrane in two different settings: with a (1–3)-D- β -glucan standard (curdlan) and in air samples collected in an environment known to contain high concentrations of BGCPs. We also examined if the image analysis protocol previously developed by our group for mouse allergens (Rivera-Mariani et al., 2012) could be applied to immunodetect and quantify β -glucan. Our results show that the HIA and image analysis methodologies used in our study could be used to specifically immunodetect and quantify BGCPs.

The HIA was initially used to immunodetect indoor allergens, such as those from pets and insects (O'Meara et al., 1998; De Lucca et al., 1999). Further development of the assay allowed its application to outdoor airborne allergens, such as pollen and fungi, an even to detect allergens originating from fungal fragments and previously undocumented fungal allergens (Green et al., 2005). By using a similar immunoblotting methodology applied in previous studies with the HIA and with an image analysis protocol developed by our group (Rivera-Mariani et al., 2012), we were able to immunodetect and quantify BGCPs when standard (1–3)- β -D-glucan particles deposited on a filter surface and on air samples collected in an agricultural setting with a short sampling duration. Furthermore, we showed that the antibody combinations, with the corresponding washing steps selected for our pilot study allowed detection of β -glucans without background staining.

This paper presents a proof of concept demonstrating that HIA can be used to quantify BGCPs. The HIA provides a new exposure assessment method for β -glucan health risk assessments. This paper is not intended to provide a compressive assessment of β -glucan exposures in poultry facilities. We chose sampling conditions known to yield BGCPs. Furthermore, we chose to use two antibodies (monoclonal mouse IgG anti-beta glucan and a biotinylated goat anti-mouse IgG) and a conjugate (streptavidin-alkaline phosphatase) in order to maximize the detection of BGCPs. It could be argued that this may have increased the possibility of cross-reactivity with other β -glucans and false positive particle detection. We could have biotinylated or conjugated with alkaline phosphatase the anti- β -glucan antibody. This could have eliminated one or two incubation steps in the HIA. Due to the limited quantity of the anti- β -D-glucan antibody, we were not able to perform preliminary experiments to attempt to biotinylate or conjugate with alkaline phosphatase the anti- β -glucan antibody. Nevertheless, the combinations of antibodies used in this study seem to specifically detect (1–3)- β -D-glucan without background staining. In addition, previous studies have documented the specificity of the anti-(1–3)- β -glucan used in this study (Meikle et al., 1991). Another observation that may raise questions is that when the HIA was performed with standard β -glucan particles (curdlan), the BGCPs detected with the HIA did not follow a 10:1 ratio similar to the mass concentration tested (750:75 μ g/ml). This is not surprising because not all β -D-glucan particles have similar size (Fig. 1A) and equal mass. Size of the β -D-glucan particles may also affect the size of the halo, which could be examined in further studies with additional microscopical protocols (e.g. fluorescent/confocal microscopy) (Green et al., 2009).

In summary, we show in this pilot study that the HIA not only can be used for airborne allergen-carrying particles, but also for non-allergenic particles of microbial origins. To our knowledge, this is the first study in which the HIA was used to detect non-allergenic compounds from microbial or fungal origins.

Acknowledgments

We acknowledge the contributions of Dr. Alan Scot and his graduate students of the department of Microbiology and Molecular Immunology at the Johns Hopkins School of Public Health for allowing us to use the microscope facility. We are also grateful of Dr. Euan Tovey, Research Leader at the Woolcock Institute of Medical Research (Sydney, Australia), for his generosity in providing the adhesive to fix the membranes, and Dr Donald Sparks, and Shannon Carter at the University of Delaware for allowing us to collect air samples in their facilities. This study was supported by NIESH (T32 ES 07141-28, PO1 ES 018176, P50 ES 015903) and U.S. EPA (RD8345101).

References

- Adhikari A, Martuzevicius D, Reponen T, Grinshpun SA, Cho SH, Sivasubramani SK, Zhong W, Levin L, Kelley AL, Clair HG, Lemasters G. Performance of the button personal inhalable sampler for the measurement of outdoor aeroallergens. *Atmos Environ*. 2003; 37:4723.
- De Lucca SD, Taylor DJ, O'Meara TJ, Jones AS, Tovey ER. Measurement and characterization of cockroach allergens detected during normal domestic activity. *J Allergy Clin Immunol*. 1999; 104:672. [PubMed: 10482845]
- Green BJ, Sercombe JK, Tovey ER. Fungal fragments and undocumented conidia function as new aeroallergen sources. *J Allergy Clin Immunol*. 2005; 115:1043. [PubMed: 15867864]
- Green BJ, Tovey ER, Beezhold DH, Perzanowski MS, Acosta LM, Divjan AI, Chew GL. Surveillance of fungal allergic sensitization using the fluorescent halogen immunoassay. *J Mycol Med*. 2009; 19:253. [PubMed: 20495612]
- Lawniczek-Wakzyk A, Gorny RL. Endotoxins and β -glucans as markers of microbiological contamination – characteristics, detection, and environmental exposure. *Ann Agric Environ Med*. 2010; 17:193. [PubMed: 21186760]
- Meikle PJ, Bonig I, Hoogenraad NJ, Clarke AE, Stone BA. The location of (1 $\rightarrow$ 3)- β -glucans in the walls of pollen tubes of *Nicotiana glauca* using a (1 $\rightarrow$ 3)- β -glucan-specific monoclonal antibody. *Planta*. 1991; 185:1.
- Noss I, Doekes G, Thorne PS, Heederik DJ, Wouters IM. Comparison of the potency of a variety of beta-glucans to induce cytokine production in human whole blood. *Innate Immun*. 2012
- O'Meara TJ, De Lucca S, Sporik R, Graham A, Tovey E. Detection of inhaled cat allergen. *Lancet*. 1998; 351:1488. [PubMed: 9605809]
- Rivera-Mariani FE, Matsui EC, Breyse PN. Performance of the halogen immunoassay to assess airborne mouse allergen-containing particles in a laboratory animal facility. *J Expo Sci Environ Epidemiol*. 2012
- Rylander R, Carvalheiro M. Airways inflammation among workers in poultry houses. *Int Arch Occup Environ Health*. 2006; 79:487. [PubMed: 16395591]
- Tischer C, Gehring U, Chen CM, Kerkhof M, Koppelman G, Sausenthaler S, Herbarth O, Schaaf B, Lehmann I, Kramer U, Berdel D, von Berg A, Bauer CP, Koletzko S, Wichmann HE, Brunekreef B, Heinrich J. Respiratory health in children, and indoor exposure to (1,3)-beta-D-glucan, EPS mould components and endotoxin. *Eur Respir J*. 2011; 37:1050. [PubMed: 20817706]
- Tovey ER, Taylor DJM, Graham AH, O'Meara TJ, Lovborg U, Jones A, Sporik R. New immunodiagnostic system. *Aerobiologia*. 2000; 16:113.
- Wouters IM, Spaan S, Douwes J, Doekes G, Heederik D. Overview of personal occupational exposure levels to inhalable dust, endotoxin, β (1 $\rightarrow$ 3)-glucan and fungal extracellular polysaccharides in the waste management chain. *Ann Occup Hyg*. 2006; 50:39. [PubMed: 16141253]
- Wu FF, Wu MW, Chang CF, Lai SM, Pierse N, Crane J, Siebers R. Endotoxin and β -(1,3)-glucan levels in automobiles: a pilot study. *Ann Agric Environ Med*. 2010; 17:327. [PubMed: 21186778]

Fig. 1. Images of the HIA with known mass-concentration of a β -D-glucan standard (A) and air samples (B). With the β -D-glucan standard, suspensions of 750 $\mu\text{g/mL}$ and 75 $\mu\text{g/mL}$ were prepared and filtered through mixed-cellulose-ester filters and the HIA performed. With air samples, two samples were collected on two different days and β -D-glucan-carrying particles (purple halo particles) detected with the HIA.

Fig. 2. Concentrations of BGCPs (β -D-glucan-carrying particles) on filters spiked with known concentration of a β -D-glucan standard (A) and on air samples collected outside a poultry farm (B and C) were immunodetected with the HIA. ImageJ® was used to simultaneously detect and quantify BGCPs.