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Organic components of airborne dust influence the magnitude and kinetics of dendritic cell activation

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ABSTRACT

Bioaerosol exposure in highly contaminated occupational settings is associated with an increased risk of disease. Yet, few determinants allow for accurate prediction of the immunopathogenic potential of complex bioaerosols. Since dendritic cells are instrumental to the initiation of immunopathological reactions, we studied how dendritic cell activation was modified in response to individual agents, combined microbial agents, or air sample eluates from highly contaminated environmental settings. We found that combinations of agents accelerated and enhanced the activation of *in vitro*-generated murine bone marrow-derived dendritic cell cultures, when compared to individual agents. We also determined that endotoxins are not sufficient to predict the potential of air samples to induce bone marrow-derived dendritic cell activation, especially when endotoxin levels are low. Importantly, bone marrow-derived dendritic cell activation stratified samples from three environmental settings (swine barns, dairy barns, and wastewater treatment plants) according to their air quality status. As a whole, these results support the notion that the interplay between bioaerosol components impacts on their ability to activate dendritic cells and that bone marrow-derived dendritic cell cultures are promising tools to study the immunomodulatory impact of air samples and their components.

1. Introduction

Environments heavily contaminated with bioaerosols are associated with increased prevalence of adverse symptoms and diseases (Greskevitch et al., 2007; May et al., 2012). Bioaerosols are aerosolized particles that may be composed of microorganisms and fragments of plants, animals and soil. The diversity of contemporary activities leading to the generation of bioaerosol is increasing, and the nature of the immunopathogenic stimuli likely differs between occupational settings. For instance, swine confinement buildings (Nehme et al., 2008), dairy barns (Blais Lecours et al., 2012) and wastewater treatment plants (Bauer et al., 2002) display different concentrations of airborne organic dust (Kirkhorn and Garry, 2000), bacteria (Blais-Lecours et al., 2015), archaea (Nehme et al., 2009), fungi (Eduard et al., 2012) and endotoxins (Mayeux, 1997). Although general patterns of bioaerosols are found among different occupational settings, predicting their propensity to elicit immunopathological responses remains a challenge.

The nature of the interactions between components found in bioaerosols *versus* their impact on human health remains misunderstood. Several studies have attempted to correlate single parameters, such as endotoxins or total organic dust content, with the occurrence of symptoms (Donham et al., 2000; Vogelzang et al., 1997). Together, these studies failed to agree on an existing and significant correlation (May et al., 2012). This lack of consensus has led, among others, to the concept that combination of bioaerosol components can have additive, synergistic, or even antagonistic effects (Liebers et al., 2008). Yet, these hypotheses still need to be confirmed.

Dendritic cells (DCs) are located at the interface of the host and his environment and they play a central role in the initiation of immune reactions (Vermaelen and Pauwels, 2005). These cells express a wide variety of pattern-recognition receptors and are highly sensitive to a wide array of immunogenic components (Banchereau and Steinman, 1998). Upon encounter with various agents including endotoxins, peptidoglycans, zymosan, poly-(I:C), Pam_3Cys-Ser-$(Lys)_4$, polyuridylic acid, CpG and β-D glucans (Dearman et al., 2009), DCs initiate an activation process in order to fulfill their role of professional antigen presenting cells. This process, which is replicated *in vitro* by murine

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bone marrow-derived dendritic cell (BMDC) cultures, involves the cell surface upregulation of the major histocompatibility complex-II (MHC II) and co-stimulatory molecules in order to efficiently prime cells of the acquired arm of the immune system. DCs also represent sources of pro-inflammatory cytokines, and consequently play key functions in the amplification of the innate and acquired immune responses (Banchereau and Steinman, 1998).

In line with these functions, the intensity of immune responses was repeatedly shown to correlate with the level of DC activation *in vivo* (Blais Lecours et al., 2011; Marsolais et al., 2009). In addition to the magnitude of the DC responses, other parameters could also influence the nature of the reactions triggered by foreign particles. For instance, the kinetics of DC activation can influence the polarity of immunity with early-activated DCs having a T_H1-polarizing phenotype, while late-activated DCs being biased towards T_H2 polarity (Langenkamp et al., 2000). Although antigenic load can influence the magnitude and kinetics of DC activation (Langenkamp et al., 2000), the stimulatory impact of complex antigens, as they are found in bioaerosols, remains mischaracterized.

The primary goal of this study was to determine if the complexity of antigenic triggers influenced the kinetics and the magnitude of BMDC activation *in vitro*. We also investigated which parameter accounted for BMDC activation by air sample eluates (ASE) obtained from three distinct occupational settings. We found that combinations of different stimuli accelerated BMDC activation and enhanced the absolute level of active BMDCs. Moreover, air samples from three different working environments displayed a differential potency to induce BMDC activation, which was consistent with their respective air quality status.

2. Materials and methods

2.1. BMDC culture and stimulation

Bone marrow cells were harvested from the hind limbs of C57BL/6J mice and cultured in presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) in order to generate large amounts of immature BMDCs, as described (Inaba et al., 1992; Lutz et al., 1999), with minor modifications (supplementary methods). Bone marrow cells were seeded into Petri dishes at a concentration of 5×10^6 cells. After 7 days of culture with GM-CSF, the non-adherent cells were seeded in a 24-well plate at a concentration of 3×10^5 cells per well. These enriched immature BMDC cultures were then incubated with microbial components (see supplementary methods for details) or with ASE, for up to 24 h. Supernatants were collected and stored at −80 °C and cells were harvested using trypsin-EDTA.

2.2. Flow cytometric analyses

BMDCs were labeled using the following commercially-available reagents: PE anti-CD11c (N418; Biolegend, San Diego, CA), PE-Cy7 anti-CD11b (M1/70; BD Bioscience, San Jose, CA), Pacific Blue anti-MHC II (M5/114.15.2; Biolegend), biotin anti-CD86 (GL1; BD pharmingen, San Diego, CA). Streptavidin-Alexa Fluor 700 (Invivogen) was used as a second step reagent. Data were collected with FACS Diva-driven LSR Fortessa (Becton Dickinson) and analyzed with the FlowJo software (Tree star, Inc., San Carlos, CA). After positive selection of the whole $CD11c^+$ $CD11b^+$ BMDC population, the frequency of $CD86^{hi}$ $MHCII^{hi}$ BMDCs was used as a surrogate for activation (Fig. 1A). Fluorescence minus one (FMO) controls were performed at every experiment. When multiple experiments were pooled, data were normalized to an internal control (100 ng/ml endotoxins, Figs. 1, 4, 5). Time-course experiments (Fig. 2) show raw frequencies of $CD86^{hi}$ MHC II^{hi} BMDCs out of the total viable BMDC pool.

2.3. Enzyme-linked immunosorbent assay (ELISA)

TNF was quantified in supernatants from BMDC cultures using the Mouse TNF-α ELISA MAX™ Standard (Biolegend).

2.4. Collection and processing of air samples

Samples from 9 swine barns, 3 dairy barns and 3 wastewater treatment plants were collected with the SASS® 3100 Dry Air Sampler (Research International, Monroe, WA) at 300 l per minute (lpm) for 10 min. The electret filters were eluted with phosphate-buffer saline (PBS) (pH 7.4) with the SASS® 3010 Particle Extractor (Research International) in order to obtain ASE an equivalent of 600 l of sampled air per ml. This procedure allowed efficient recovery of endotoxins in a medium compatible with cell culture (Fig. S1). One aliquot was centrifuged at 21000 ×*g* for 10 min and the pellet was used for total DNA extraction. Other aliquots were kept at −20 °C until further use.

2.5. Endotoxin and total organic dust quantification

The kinetic chromogenic limulus amebocyte lysate (LAL) assay (LONZA, Walkersville, MD) was used to quantify endotoxins according to the manufacturer's instruction. A β-1,3-glucan blocker was used to insure specificity for endotoxins (LONZA). Total organic dust was quantified on field with the DustTrak™ DRX Aerosol Monitor Model 8534 (TSI, St Paul, MN).

2.6. Total DNA extraction

Powerlyzer Powersoil DNA extraction kit (MO BIO, Carlsbad, CA) was used to obtain total DNA from air samples using the manufacturer's protocol. A bead-beating step was performed with the Mixer Mill MM300 (Retsch, Haan, Germany) at a speed of 20 Hz for 20 min after a heating step at 70 °C for 10 min. DNA extracts were stored at −20 °C until analyses.

2.7. Quantitative real-time polymerase chain reaction (qPCR)

PCR quantifications were done using CFX-96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). Data were analyzed using the Bio-Rad CFX Manager software (Version 3.1). Total bacterial 16S rRNA gene quantification was performed using primers (RP 16S rDNA and FP 16S rDNA) and probe (probe 16S rDNA), as described (Bach et al., 2002), using iQ Supermix (Bio-rad). The amplification program was the following: one step at 95 °C for 3 min, followed by 40 cycles at 95 °C for 20 s and 62 °C for 60 s before fluorescence acquisition. A ten-fold dilution of *Escherichia coli* DNA was used for standard curve and results were converted to total bacteria 16S rRNA gene. Total achaeal 16S rRNA gene quantification was performed using A751F and A976R (Baker et al., 2003; Reysenbach and Pace, 1995) primers as described (Blais Lecours et al., 2012). A ten-fold dilution of the genomic DNA of *Methanobrevibacter smithii* (MBS) was also used as a standard curve. Quantification of *mtaB1* gene of *Methanosphaera stadtmanae* (MSS) was performed using primers as described (Blais Lecours et al., 2014). The standard curve consisted in ten-fold MSS DNA serial dilutions. The quantification of the *NifH* gene of MBS was performed using primers and probe as previously described (Johnston et al., 2010). A ten-fold MBS genomic DNA dilution was used as standard curve.

2.8. Statistical analyses

The variables of combined stimulations were analyzed using a one-way analysis of variance (ANOVA) to compare groups. The normality assumption was verified with the Shapiro-Wilk test on residuals from the statistical model. The Brown and Forsythe's variation of Levene's test statistic was used to verify the homogeneity of variances. The