CHARACTERIZATION OF THE INFLAMMATORY POTENTIAL OF BIOAEROSOLS FROM A DUCK FATTENING UNIT BY USING A LIMULUS AMEBOCYTE LYSATE ASSAY AND HUMAN WHOLE BLOOD CYTOKINE RESPONSE

Bert-Andree Zucker, Petra Scharf, Cornelia Kersten

Institute of Animal and Environmental Hygiene, Freie Universität Berlin, Germany

Key words: bioaerosols, whole blood assay, cytokine response

Introduction

Inhalation of bioaerosols from animal houses can induce acute inflammatory reactions in the respiratory tract. Determination of the concentration of airborne endotoxins is widely used to characterize this risk (Danuser and Moon, 1999; Douwes et al., 2003). However, besides endotoxins also other inflammation inducing substances existing in bioaerosols, e.g. glucans. In this study the activity of bioaerosol samples from a duck fattening unit to coagulate Limulus Amebocytes (LAL-assay) and to induce the proinflammatory cytokine IL-1ß in human blood (whole blood assay) was investigated.

Material and methods

- Description of the duck fattening unit

The fattening unit (92m x 36m x 6m) housed 15.000 Peking ducks. The facility was forced ventilated. Straw was used as bedding material.

- Sampling of endotoxins and inflammation inducing substances

For the investigation in the LAL-assay and the whole blood assay bioaerosols were sampled by using AGI-30 Impingers (AGI = All Glass Impinger) and the PGP-Dust sampling system (Ströhlein GmbH, Germany).

Concentrations of endotoxins in the impinger fluid and in the washing fluid of the filters were determined by using the LAL-assay QCL-1000 (BioWhittaker, Walkersville, Maryland, USA) as described by Zucker et al. (2000). The activity in the LAL-assay was expressed in Endotoxin Units (EU). The inflammation-inducing potential of the bioaerosol samples was characterized by using a semi-quantitative system of the human whole blood assay as described in detail by Zucker (2004). Bioaerosol samples (impinger fluid, washing fluid of exposed filters) were incubated with diluted human whole blood from healthy donors for 22 h at 37°C. After centrifugation the concentration of the proinflammatory cytokine IL-1ß was determined in the supernatant by using Endogen®Matched Antibody Pairs for human IL-1ß

(Product # M421BE and M420BB, Perbio Science Deutschland GmbH) as recommended by the manufacturer.

- Sampling of airborne bacteria and airborne fungi

To obtain further information about the bioaerosol in the duck fattening unit the concentration of total airborne aerobic bacteria (AGI-30 Impinger, Standard I Agar, 37°C for 48 h), airborne aerobic gram-negative bacteria (6-stage Andersen Sampler, MacConkey 3 agar, 24 h at 37°C and 24 h at 22°C) and airborne fungi (6-stage Andersen Sampler, DG 18 agar, 48 h at 30°C and 72 h at 22°C) was investigated. Further the species of isolated airborne gram-negative bacteria were identified by using the api 20 E and the api 20 NE system (Bio Merieux, Marcy-I'Etoile, France).

Results and discussion

In both test systems the activity of the bioaerosol samples was related to the activity of the same Control Standard Endotoxin (*E. coli* 0113:H10; BioWhittaker, USA). Therefore it was possible to compare the activity in both test systems. The activity detected in the whole blood assay correlated well with the endotoxic activity found in the LAL-assay (Spearmen's Rho = 0,902; n = 17; Table 1). In the duck fattening unit the bioaerosols were sampled under constant conditions. Special situations which can cause peak concentrations of bioaerosols such as litter down or veterinary treatment were strictly excluded from the investigation. Under these conditions one can expect a relatively stable relationship between endotoxins and other inflammation inducing substances in the bioaerosol. That may explain the high correlation between the endotoxic activity in the LAL-assay and the activity in the whole blood assay.

In all samples the inflammation-inducing potential was overestimated by the LAL-assay (from factor 1.4 up to factor 8.2). That was unexpected since the whole blood assay is not limited to the detection of endotoxins. Also cell components of gram-positive bacteria, which are dominating the airborne bacterial flora in animal houses (see also Table 2), are able to activate different cell systems including epithelial cells, alveolar macrophages and monocytes (Larsson et al.,1999). But compared to endotoxins their potential to induce cytokines in the human whole blood assay is limited (Fennrich et al., 1998; Zucker, 2004).

Different reasons could be possible for this overestimation. First it should be considered that the calculation of the inflammation inducing potential in the whole blood assay was based on a semi-quantitative method. The true result obtained by this method is actually somewhere

between two serial dilution. This will lead to an underestimation of the true inflammation inducing potential of the sample. Second it should be considered that the LAL-assay is not able to distinguish between Endotoxins of different bacterial origins. For instance heat inactivated suspensions of *E. coli* and *Pseudomonas* spp. expressed similar activities in the LAL-assay. However in the whole blood assay *Pseudomonas* spp. were clearly less potent to activate macrophages compared to *E. coli* (up to factor 100) (Zucker, 2004). Similar results were reported from Fennrich et al. (1998) comparing chemically isolated endotoxins from different bacterial species in both test systems. This could explain the overestimation of the inflammation inducing potential in the bioaerosol samples by the LAL-assay since *Pseudomonas* spp. could regularly isolated from the airborne state of the duck fattening unit. 26% of all isolated airborne gram-negative bacteria were identified as *Pseudomonadaceae*. Compared to this degree of overestimation the additional inflammation inducing potential of gram-positive bacteria in the bioaerosol samples seems to be negligible. Furthermore the role of fungal ß-glucans in bioaerosols needs further investigation. Several investigations have indicated that ß-glucans can both induce and suppress TNF-α release from alveolar macrophages. Hoffman et al. (1993) and Olson et al. (1996) have shown that maximal TNF-α release occurred with moderate concentrations of ß-glucan, whereas higher concentrations of ß-glucan caused apparent suppression of the TNF-α activity released. Therefore it is also possible that exposure to bioaerosols which contain high concentrations of airborne fungi (= high concentration of airborne ß-glucan) will lead to a reduced production of proinflammatory cytokines due to the immunomodulatory activity of ß-glucans. High concentrations of airborne fungi are found especially in animal houses using bedding material like straw.

Conclusion

The results presented here indicate that the whole blood assay can be used besides the LAL-assay as an additional method to characterize the inflammation inducing potential of bioaerosols. However, a larger study is needed to investigate thoroughly how well the whole blood assay reflects the risk of exposure to airborne inflammation inducing substances.

Table 1: Activity of bioaerosol samples from a duck fattening unit in a LAL-assay and a whole blood assay

Nr.	Sampling methods	Activity in the LAL-assay (EU/m³)	Activity in the whole blood assay (EEU/m³)	(EU/m³) : (EEU/m³)*
1	I	3480	1000	3.8
2	I	436	150	2.9
3	I	1064	400	2.7
4	I	190	100	1.9
5	I	1064	300	3.5
6	I	638	200	3.2
7	I	9870	1200	8.2
8	I	1294	300	4.3
9	I	3880	1200	3.2
10	I	2940	1200	2.4
11	I	1240	300	4.1
12	I	1210	600	2.0
13	F	6500	1587	4.1
14	F	1190	816	1.5
15	F	196	40	4.9
16	F	1081	473	2.3
17	F	6721	3747	1.8

Nr. = sample number, I = impingement, F = filtration, EU = Endotoxin Unit, EEU = Endotoxin Equivalent Unit
* = activity in the LAL-assay divided by activity in the whole blood assay

Table 2: Concentration of airborne bacteria and airborne fungi in the duck fattening unit

Parameter	n	Median (cfu/m³)	Minimum (cfu/m³)	Maximum (cfu/m³)
Total airborne aerobic bacteria	12	3.8×10^5	1.1×10^4	1.7×10^6
Airborne aerobic gram-negative bacteria	12	5.2×10^1	7.4×10^0	1.8×10^2
Airborne fungi	12	6.7×10^3	2.0×10^3	2.6×10^4

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