CHARACTERIZATION OF THE INFLAMMATORY POTENTIAL OF BIOAEROSOLS BASED ON HUMAN WHOLE BLOOD CYTOKINE RESPONSE

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Introduction

Exposure to bioaerosols can induce pulmonary diseases and subjective symptoms in humans and animals. Some of these diseases are of inflammatory origin, such as organic dust toxic syndrome or chronic bronchitis. The inflammatory reaction in the respiratory system is caused by various agents of the bioaerosols like endotoxins, glucans or tannic acids. These agents activate different cells in the lung tissue, chiefly macrophages to excrete inflammatory mediators like IL-1ß and TNF-a which initiate and modulate the inflammation process [Holt, 1990; Rylander, 1994].

Currently risk assessment to bioaerosol exposure is based mainly on the determination of airborne endotoxin concentration by using the Limulus amebocyte lysate (LAL) assay. Endotoxins serve as a marker for disease risk [Rylander, 1997]. However, the value of the Limulus reaction to characterize the inflammation process in mammals is under discussion for various reasons: 1) the Limulus assay is based on the coagulation reaction of amebocytes of a crab and may not reflect the inflammatory reaction in mammals, 2) the Limulus assay is limited to the detection of endotoxins.

In this study we compared the activity of typical components of bioaerosols and bioaerosol samples from animal houses to coagulate Limulus Amebocytes (LAL assay) and to induce IL-1ß in human blood macrophages (human whole blood assay) to gain information about the problems described above.

Material and Methods

- Test procedures

The LAL assay QLC-1000 (Bio Whittaker, Walkersville, USA) was used to characterize the ability of different bacteria and bioaerosol samples from animal houses to agglutinate amebocytes of the horseshoe crab *Limulus polyphemus*. The ability of these substances to activate human blood macrophages was determined by using a human whole blood assay as described by Hartung and Wendel (1995) and Weigandt (2000). Bioaerosol samples (impinger fluid, washing fluid of exposed filters, exposed filters without any preparation) are incubated with diluted human whole blood from healthy donors. After contact with relevant contaminations monocytes (blood macrophages) release pro-inflammatory signal molecules such as interleukin-1ß (IL-1ß). IL-1ß release is quantified by ELISA measurement.

- Testing of bacterial strains

Strains of bacterial species, which were frequently isolated from the airborne state of animal houses [Dutkiewicz, 1978, Chai et al., 1997, Zucker et al., 2000] were investigated in both test systems. After cultivation the bacteria were harvested by using pyrogen-free water. These bacterial suspensions were adjusted to an optical density of 1.0 at 520 nm. From these suspensions serial dilutions were prepared (dilution factor 10). After that a heat inactivation (30 min at 80°C in a water-bath) was done. In both test systems that dilution was determined that caused a just positive reaction.

- Testing of bioaerosol samples from animal houses

Bioaerosol samples from different animal houses (poultry houses, pig stables, sheep barns) were collected by filtration (PGP-dust-sampling system, Ströhlein GmbH, Germany) and impingement (AGI-30 Impinger) [Brachmann et al., 1964].

AGI-30 impingers were operated for 20 min at an air flow rate of 12.5 l min^{-1}. Air samples were collected in 50 ml of pyrogen-free water. The crude impinger fluid was centrifuged at 2000g for 10 min and directly investigated in both test systems. The exposed filters of the dust sampling system were rocked in 50 ml of pyrogen-free water for 2h and than centrifuged at 2000g for 10 min. The activity of the washing fluid was determined in both test systems.

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 results of both test systems. The activity in the LAL assay was expressed in Endotoxin Units (EU), in the whole blood assay in Endotoxin Equivalent Units (EEU).

Results and Discussion

- Reactivity of bacteria

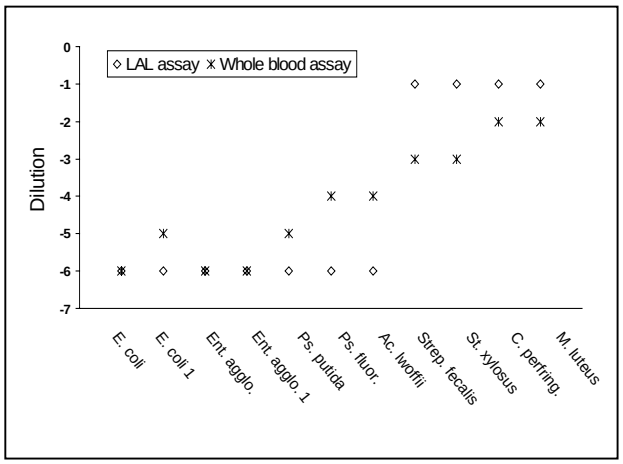
The reactivity of the different bacterial strains in both test systems is shown in figure 1. There are considerable differences between the ability of different gram-negative bacteria to induce limulus amebocyte coagulation and monocyte activation. For instance, the LAL assay overrates the inflammatory activity of *Pseudomonadaceae* and *Neisseriaceae* compared to *Enterobacteriaceae*. Similar results were found by Fennrich et al. (1998) for purified endotoxins from these bacteria. Further "Non-LAL-reactive material", such as components of gram-positive bacteria, are also able to induce monocyte activation suggesting that such components contribute to inflammation in the respiratory tract following bioaerosol exposure. However, these substances exhibited only a very weak activity in the LAL assay.

- Reactivity of bioaerosol samples

The activity detected in the whole blood assay correlated well with the endotoxic activity found in the LAL assay (figure 2). However, in some samples the inflammation-inducing potential was overestimated by the LAL assay (up to factor 8) suggesting a considerable amount of endotoxins originating from "non-*Enterobacteriaceae*" in the bioaerosol. In other samples the inflammation-inducing potential was underestimated by the LAL assay

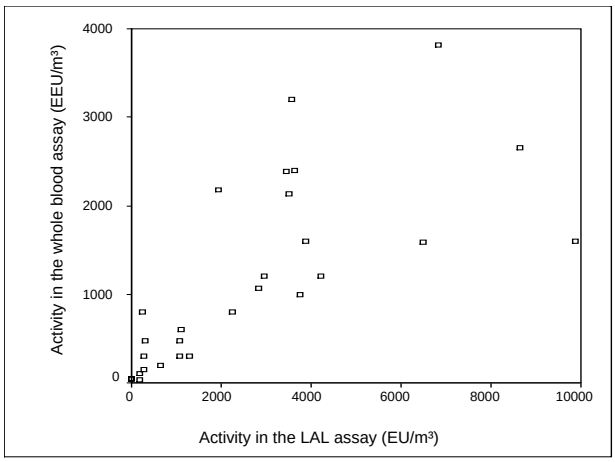
(up to the factor 4) indicating a considerable amount of "non-LAL-reactive material" in the bioaerosol.

Figure 1: Reactivity of different bacteria in the LAL- and whole blood assay



After cultivation the bacteria were harvested by using pyrogen-free water. These bacterial suspensions were adjusted to an optical density of 1.0 at 520 nm. From these suspensions serial dilutions were prepared (dilution factor 10). After that a heat inactivation (30 min at 80° C) was done. In both test systems that dilution was determined that caused a just positive reaction. (Ent. agglom = Enterobacter agglomerans, Ps. = Pseudomonas, St. = Staphylococcus, Sr. = Streptococcus, C. perfring. = Clostridium perfringens, M. = Micrococcus).

Figure 3: Reactivity of bioaerosol samples in the LAL- and whole blood assay



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). The activity in the LAL assay was expressed in Endotoxin Units (EU), in the whole blood assay in Endotoxin Equivalent Units (EEU).

Conclusion

The determination of the potential of bioaerosols to activate macrophages by using whole blood models could offer new perspectives in exposure and risk assessment. This currently has two major potential advantages over the LAL assay:

1. Simultaneously detection of a broad spectrum of inflammation-inducing substances
2. Detection of these substances by using a system that is relevant to the exposed species. The whole blood assay mimics the reaction that air contaminations would cause in the exposed species.

Further experiments will be necessary to examine in more detail how well this method reflects the risk of bioaerosol exposure and to optimize the methodology of the whole blood assay.

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