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Original Research Article

Chicken immunoglobulins for prophylaxis: Effect of inhaled antibodies on inflammatory parameters in rat airways

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Abbreviations:
BSA, bovine serum albumin
BAL, bronchoalveolar lavage
CF, cystic fibrosis
DL, detection limit
GM-CSF, granulocyte macrophage-colony stimulating factor
IgY, chicken yolk antibody
IL, interleukin
OVA, ovalbumin
PBS, sodium phosphate buffered isotonic saline pH 7.4
TNF-α, tumor necrosis factor α

ABSTRACT

The prophylaxis against microbial airway infections of cystic fibrosis (CF) patients is an emerging application of chicken yolk antibody (IgY), however, no data on the effect of inhaled IgY have been published yet. Rats were daily (for 28 days) exposed to an aerosol of IgY, ovalbumin (OVA), Fab fragment of IgY, or PBS and their serum, bronchoalveolar lavage (BAL) and lung tissue were examined for inflammation signs. There were no marked changes in lung parenchyma, except for an elevated number of alveolar macrophages in the OVA-exposed group. While the administration of OVA or IgY aerosols slightly increased levels of cytokine TNF-α and GRO/KC in BAL fluid, a marked elevation of GM-CSF in serum was observed after the OVA inhalation. The administration of Fab induced expression of IL-1β > IL-18 in serum, in contrast no effect exerted by IgY. Our results suggest that the aerosolized IgY did not cause any deleterious effects in rat lungs.

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Introduction

Immunoglobulins prepared from the chicken egg yolks (IgY) of immunized hens have been recognized as a suitable alternative to mammalian antibodies derived from blood. The large scale production of IgY (~100 mg/yolk) makes these antibodies an excellent tool for passive immunization (Hodek and Stiborova, 2003; Hodek et al., 2013). Administered antibodies can help in the prevention of viral and microbial infections and neutralization of toxins. The prophylactic use of IgY against bacteria (often antibiotic-resistant) causing infections of airways might provide a life-saving treatment for cystic fibrosis (CF) patients suffering from repeated lung infections (*Staphylococcus* sp. or *Pseudomonas* sp.) resulting in tissue damage and loss of the lung function. While there are some efforts to prevent the infection of CF patients with *Pseudomonas* sp. by gargling crude yolk extracts of eggs laid by hens immunized with the microbe (Nilsson et al., 2007), the inhalation of highly purified IgY directed against virulence factors of bacteria should provide more efficient protection. This ''direct'' prophylactic approach may profit also from the unique properties of yolk immunoglobulins: IgY, in contrast to other antibodies (even humanized ones), should not cause detrimental inflammation processes in lung tissue upon antigen binding, because of their inability to fix complement and a failure of the Fc-receptor to mediate cell-cell interactions (e.g. antibody-dependent cell-mediated cytotoxicity). Currently in the literature there are no data available regarding the inhaled IgY.

The present work was therefore undertaken to assess the impact of the IgY lung exposure on histological changes of airways, shift in cell counts, and the cytokine induction, as early signs of allergy or chronic inflammation.

Materials and methods

Chicken IgY antibodies were prepared from egg yolks as described by Hodek et al. (2013). Fab fragments of IgY were purified from the papain digest of yolk immunglobulins using DEAE-ion exchange chromatography (Akita and Nakai, 1993).

The animal study was conducted in accordance with the Regulations for the Care and Use of Laboratory Animals (311/1997, Ministry of Agriculture, Czech Republic) and efforts were made to minimize the number of animals and any discomfort to them. Wistar rats were exposed daily for 10 min to the stream of nebulized PBS containing in total either 10 mg IgY, or 5 mg Fab fragment, or 20 mg OVA (OVA), or plain PBS (two animals per each group). The aerosol of tested proteins was generated by a compressor-operated PARI (Germany) nebulizer commonly used by CF patients to deliver medication to the lower respiratory tract. After 4 weeks the inhalation experiment was terminated. The levels of antibodies developed against the inhaled proteins were determined in sera by ELISA. Pro-inflammatory cytokines were assayed in

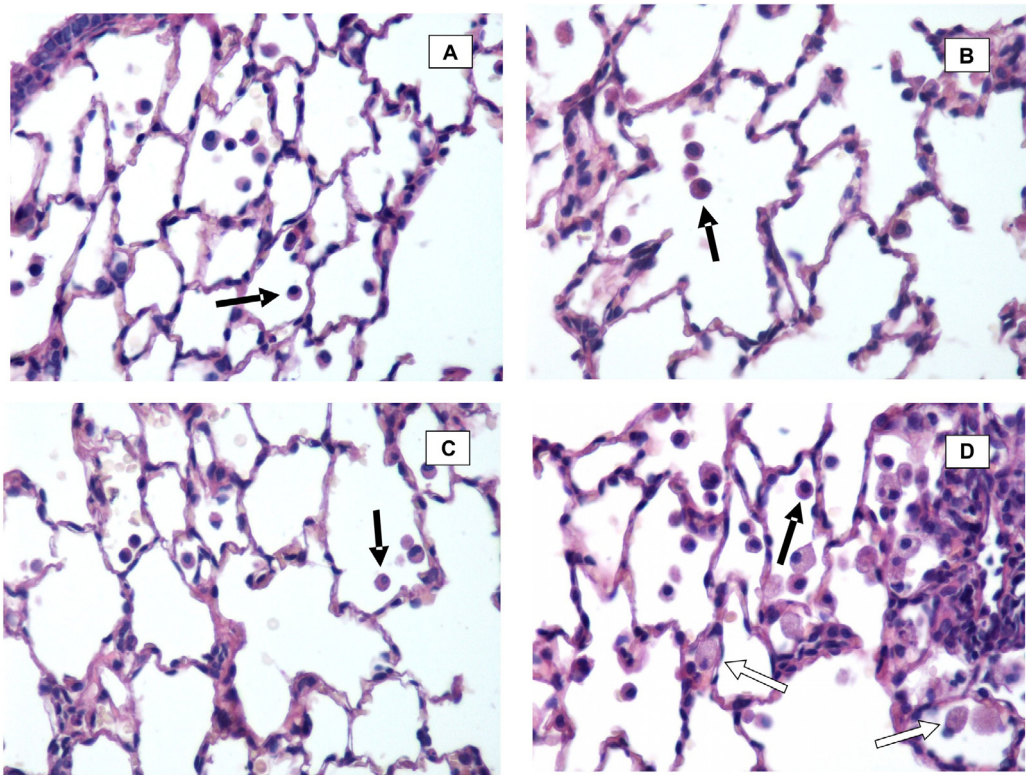

Fig. 1 – Histopathological examination of rat lungs. Representative lung sections were taken from PBS- (A), Fab- (B), IgY- (C) or OVA-treated rats (D). Lung sections were stained with haematoxylin and eosin dyes. Black arrows show normal alveolar macrophages, while white arrows indicate macrophages with foamy cytoplasm (as in D). Sections were evaluated under light microscopy, original magnification 400×.