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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 7.4TNF- 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-1 β > 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 immunoglobulins 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

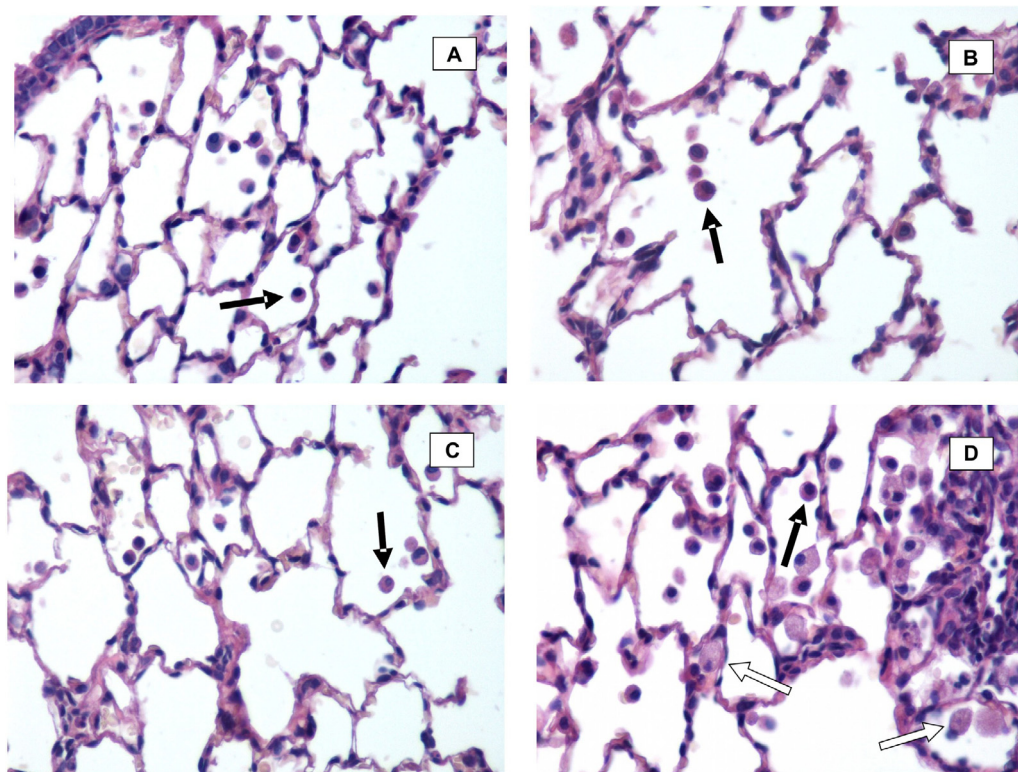


Fig. 1 – Histopathological examination of rat lungs. Representative lung sections were taken from PBS- (A), Fab- (B), IgY- (C) or OVA-treated rats (D). Lung sections were stained with haematoxylin and eosin dyes. Black arrows show normal alveolar macrophages, while white arrows indicate macrophages with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

bronchoalveolar lavage (BAL) fluid and in serum using a BioPlex Rat Cytokine kit (BioRad, CR). Airway tissue was examined for potential signs of inflammation by two experienced pathologists. The differential cell counts in BAL were determined using a light microscope (Nikon Eclipse E600, Japan) and the results were expressed as percentages of alveolar macrophages, lymphocytes and neutrophils.

Results

Rats across the groups developed comparable antibody response to inhaled proteins that is in agreement with documented airway mucosal reactivity to antigens (Brandtzaeg et al., 1996). Relatively high IgG responses to the corresponding antigens, allowing their detection at a dilution of 1:1000, suggest that the daily administration of Fab, IgY, or OVA for four weeks was successful and long enough to develop an appreciable immune response (Fig. 1).

Although the pro-inflammatory properties of antibody-antigen complexes make the IgG antibodies of local immunopathological importance, no marked adverse tissue changes were found in the lung slices of exposed rats. Histological examinations of lungs allowed also observing alveolar macrophages. While their amounts were comparable among animals, the inhalation of OVA-induced

macrophages with foamy cytoplasm, suggesting a reaction to an exogenous agent.

The results of differential cell counts in BAL indicated that the majority of cells identified in BAL were macrophages (up to 98%), mostly with a small number of neutrophils, and lymphocytes (data not shown). Solely in the case of OVA-treated animals, the number of neutrophils was increased (>3%) and the ratio of lymphocytes to alveolar macrophages was shifted to higher values (>15%) for animals in this group. The elevated percentages in these counts can be considered borderline markers of lung inflammation.

The initial stages of potential inflammation of lung tissue were traced by the detection of pro-inflammatory cytokines, IL-1 β , IL-6, TNF- α , IL-18, MIP-1 α , RANTES, GM-CSF and GRO/KC. In BAL fluid, only TNF- α and GRO/KC of all assayed inflammation indicators were increased after the exposure to OVA and IgY, when compared to PBS-treated animals. To monitor a systemic inflammatory response of exposed animals, the levels of cytokines released into free circulation were measured in serum. None of the assayed inflammation indicators was elevated in the response to IgY exposure. Cytokines IL-1 β and GM-CSF were markedly increased in serum (compared to PBS control) after animal inhalation of Fab and OVA, respectively. In addition, the treatment with Fab also increased serum concentrations of IL-18, although to a lesser extent (Fig. 2).

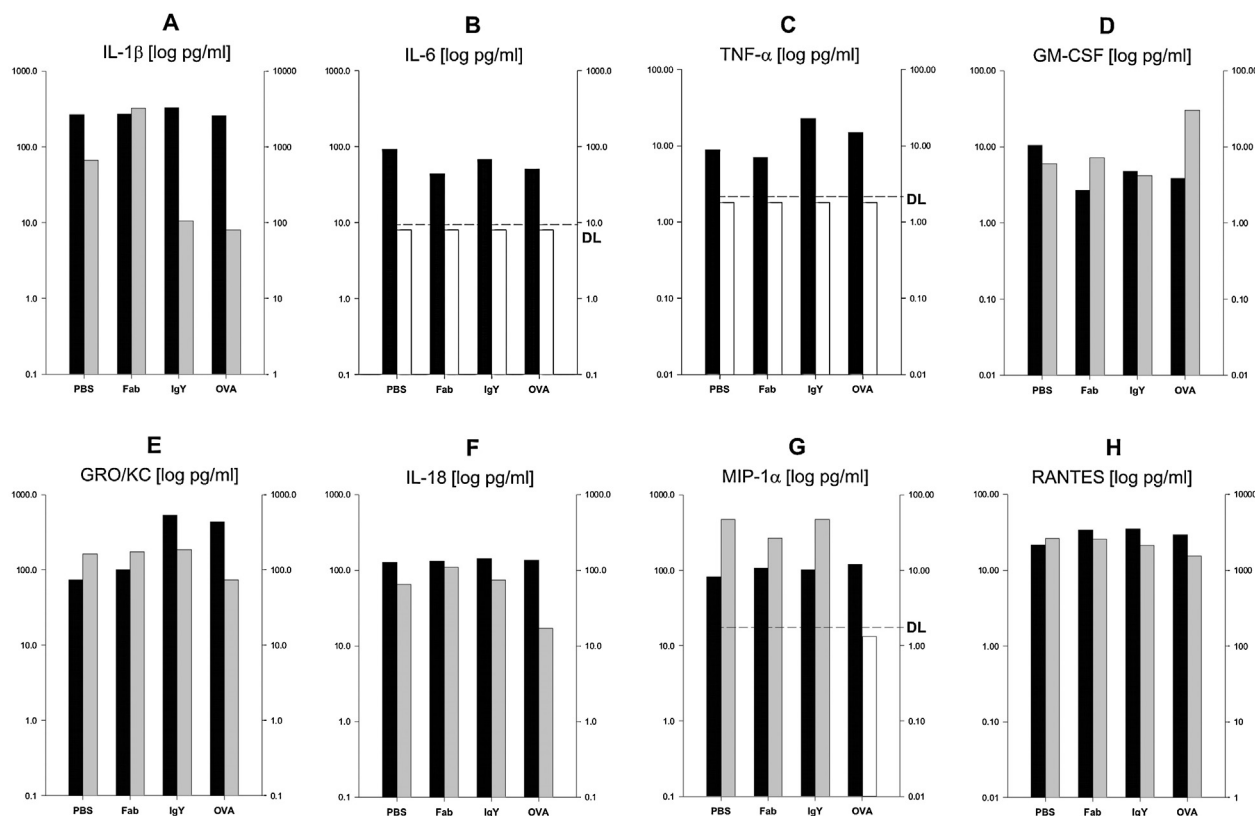


Fig. 2 – Concentrations of pro-inflammatory cytokines (panels A–H) in bronchoalveolar lavage fluid, BALF (black bars), and serum (grey bars) from PBS-, Fab-, IgY- or OVA-treated rats. The sensitivities of BioPlex assays were <3.31 pg/ml, <8.11 pg/ml, <11.90 pg/ml, <1.86 pg/ml, <1.43 pg/ml, <2.17 pg/ml, <0.31 pg/ml and <1.56 pg/ml for IL-1 β , IL-6, IL-18, MIP-1 α , RANTES, TNF- α , GM-CSF and GRO/KC, respectively. Bars in white represent values below the detection limit (DL). Results are presented as means for each group of animals; each sample was determined by two independent analyses.

Discussion

To get a rough estimate whether the IgY inhalation is detrimental to mammals we designed a pilot experiment with a limited number of animals extensively exposed to aerosols of this chicken immunoglobulin. In addition to IgY, OVA and Fab fragment of IgY were included in the study. Ovalbumin was selected for inhalations as a possible contaminant of IgY fractions. The prepared Fab fragment was employed for testing, since it is assumed to be less immunogenic than IgY (Sarnesto et al., 1983). Amounts of IgY and Fab were chosen to allowed exposing animals to comparable doses of reactive immunoglobulin as far as the content of antigen-binding domain is concerned. The concentration of OVA used for the rat inhalation was adopted from the experiments described by Zhu et al. (2007). They, however, sensitized rats by subcutaneous injections of OVA prior being exposed to aerosolized OVA to induce adverse effects of OVA on bronchial epithelia. That might explain only marginal histological changes of airway tissue of animals exposed to OVA found by us.

Unexpected properties of Fab in respect to the cytokine production were confirmed in our experiments with cell lines (unpublished data), when the formation of IL-1 β was induced in human airway epithelial cell line NuLi-1 incubated with Fab. At present, the reason why Fab triggered the induction of cytokine IL-1 β in rat airways and epithelial cell lines is unclear.

Although the current study was carried out with a limited number of animals to draw statistically significant conclusions, our data clearly show no detrimental changes in the lung of rats exposed to IgY detectable on cellular and molecular levels. Thus, the exposure of rats to IgY does not seem to act as a trigger of inflammatory diseases. Marginal effects occurred upon exposure to OVA and Fab. As OVA induced the level of GM-CSF, elevated count of neutrophils and induced macrophages with foamy cytoplasm, possible traces of OVA in IgY preparations should be taken under consideration.

The reported data provide a rational basis for further studies with aerosolized IgY, namely for long-term experiments focused on IgY prophylaxis of CF-induced animals against *Pseudomonas* sp.

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