Exogenous and endogenous triggers differentially stimulate Pigr expression and antibacterial secretory immunity in the murine respiratory tract

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Research Article

Keywords: secretory immunity, respiratory tract, polymeric immunoglobulin receptor, immune modulation, infection

Posted Date: August 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-759484/v1

Version of Record: A version of this preprint was published at Lung on November 26th, 2021. See the published version at https://doi.org/10.1007/s00408-021-00498-8.

Exogenous and endogenous triggers differentially stimulate *Pigr* expression and antibacterial secretory immunity in the murine respiratory tract

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Word count abstract: 213

Word count body of the manuscript: 2567

Numbers of tables and figures: 5 figures, 2 supplementary figures

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Abstract

Purpose

Transport of secretory immunoglobulin A (sIgA) through the airway epithelial cell barrier into the mucosal lumen by the polymeric immunoglobulin receptor (pIgR) is an important mechanism of respiratory mucosal host defense. Identification of immunomodulating substances that regulate secretory immunity might have therapeutic implications with regard to an improved immune exclusion.

Thus, we sought to analyze secretory immunity under homeostatic and immunomodulating conditions in different compartments of the murine upper and lower respiratory tract (URT&LRT).

Methods

Pigr gene expression in lung, trachea and nasal-associated lymphoid tissue (NALT) of germ-free mice, specific-pathogen-free mice, mice with an undefined microbiome as well as LPS- and IFN-γ-treated mice was determined by quantitative real-time RT-PCR. IgA levels in bronchoalveolar lavage (BAL), nasal lavage (NAL) and serum were determined by ELISA. LPS- and IFN-γ-treated mice were colonized with *Streptococcus pneumoniae* and bacterial CFUs were determined in URT and LRT.

Results

Respiratory *Pigr* expression and IgA levels were dependent on the degree of exposure to environmental microbial stimuli. While immunostimulation with LPS and IFN-γ differentially impact respiratory *Pigr* expression and sIgA in URT *vs*. LRT, only prophylactic IFN-γ treatment reduces nasal colonization with *S. pneumoniae*.

Conclusion

Airway-associated secretory immunity can be partly modulated by exposure to microbial ligands and proinflammatory stimuli. Prophylactic IFN-γ-treatment significantly improves antibacterial immunity in the URT.

Key words: secretory immunity, respiratory tract, polymeric immunoglobulin receptor, immune modulation, infection

Introduction

Airway epithelial cells (AECs) constitute the first line of defense against respiratory pathogens. They express transmembrane proteins, which form tight junctions that allow only small ions or water to traverse paracellularly [1]. Claudin and occludin are vital for epithelial defense [2] and altered claudin expression affects airway epithelial barrier function [3, 4]. Furthermore, AECs constitutively secrete antimicrobial proteins, complement factors and cytokines and rapidly mount antimicrobial immune responses upon inflammatory and infectious stimuli [5-9]. Importantly, AECs have a central function in antibody-mediated mucosal immunity. Multimeric IgA and IgM are actively transported through AECs via the polymeric immunoglobulin receptor (PIGR) and are secreted into the mucosal lumen as secretory immunoglobulins (sIgs) [10]. Especially sIgA is known to prevent pathogen adhesion, thus averting microbial infiltration [11]. Moreover, sIgA plays a crucial role in the regulation of *Streptococcus pneumoniae* nasal colonization in mice [12].

In this context, *Pigr*-deficiency manifests in susceptibility to mycobacterial respiratory infections [13] and development of a COPD-like phenotype driven by an altered lung microbiome and bacterial invasion of the airway epithelium [14]. The importance of sIgA for airway homeostasis is furthermore highlighted by the findings of sIgA deficiency in small airways of COPD patients, which is associated with persistent inflammation and airway wall remodeling [15]. Moreover, chronic airway diseases reduce PIGR-expression in the bronchial epithelium resulting in increased disease severity (COPD) and impaired sIgA-mediated mucosal defense (asthma) [16, 17]. While the key role of PIGR and secretory immunity for airway homeostasis is undisputable, knowledge on their expression and regulation in the airways is still fragmentary. Since targeted modulation of secretory immunity represents an interesting option to improve immune exclusion of respiratory pathogens, we here aimed to further dissect PIGR-mediated immunity in the airways with the specific focus on the applicability of exogenous and endogenous stimuli to regulate this aspect of humoral antimicrobial defense.

Methods

Mice

BALB/c and C57BL/6J mice (age: 11-46 weeks) were maintained in individually ventilated cages (IVCs) under specific pathogen-free (SPF) conditions at the Helmholtz Centre for Infection Research (HZI), Braunschweig. Germ-free mice (C57BL/6N, age: 10 weeks) were bred and maintained in isolators in a germ-free (GF) facility (HZI). C57BL/6J mice with an undefined microbiome (maintained in open cages, age: 10-18 weeks) were provided by Dirk Schlüter (Otto-von-Guericke-University [OvGU], Magdeburg). For pneumococcal colonization experiments female C57BL/6JRj mice (age: 12 weeks) were purchased from Janvier Labs (France) and maintained in IVCs under SPF conditions (OvGU).

Treatment with Immunomodulating Substances

BALB/c, C57BL/6J and C57BL/6JRj mice were treated intranasally (i.n.) with LPS (Sigma-Aldrich, Germany), (10µg/ 25µl PBS or solvent alone) or recombinant murine IFN-γ (Peprotech, Germany), (1µg/ 20µl ddH_2O with 5% BSA or solvent alone). BALB/c and C57BL/6J mice were sacrificed 1 or 2 days post treatment. Lung, trachea, NALT, BAL, NAL and serum were collected. Organs were used for RNA isolation and qPCR. Fluids were used for ELISA analysis. Blood was collected by cardiac puncture. BAL fluid was collected by flushing the lungs with 1ml PBS via the trachea. The nasopharynx was flushed with 1ml PBS via the trachea, NAL fluid was collected at the nostrils.

Pneumococcal Infection

Streptococcus pneumoniae serotype 19F (strain BHN100) [18] was provided by Birgitta Henriques-Normark (Karolinska Institutet, Stockholm). Bacteria were grown in Todd-Hewitt yeast (THY) medium as previously described [19]. LPS- and IFN-γ-treated C57BL/6JRj mice and control groups were infected i.n. with 10^8 *S. pneumoniae* 19F in 10µl PBS 48h after the first treatment. Mice were sacrificed 18h post infection and lung, trachea, NALT and nasopharynx were homogenized using a tissue homogenizer (KINEMATICA AG, Switzerland). Samples were plated onto Columbia blood agar plates (BD Diagnostic Systems, Germany) and incubated over night at 37°C, 5% CO_2. CFU were counted to determine the bacterial burden.

Quantitative Real-Time RT-PCR (qPCR)

RNA was isolated from lung, trachea, NALT and MLE-15 cells using RNeasy Plus Mini Kit (QIAGEN, Germany). cDNA was synthesized from 1µg of RNA using Oligo dT Primers (Thermo Fisher Scientific, USA), Random-Primers (Thermo Fisher Scientific, USA), dNTP-Mix (10 mM) and SuperScript™ III Reverse Transcriptase (Thermo Fisher Scientific, USA). QPCR was performed using the SensiFAST™ SYBR® No-ROX Kit (Bioline, USA). Temperature profile: 95°C for 2min, 40 cycles at 95°C for 5s, 60°C for 10s and 72°C for 5s. ß-Actin (*Actb*) served as reference gene. Primer sequences: *Pigr* forward: 5'-GTGCCCGAAACTGGATCACC-3', *Pigr* reverse: 5'-TGGAGACCCCTGAAAAGACAGT-3', *Actb* forward: 5'-ACACCCGCCACCAGTTCG-3', *Actb* reverse: 5'-GTCACCCACATAGGAGTCCTTC-3', *Cldn-7* forward: 5'-AGCGAAGAAGGCCCGAATAG-3', *Cldn-7* reverse: 5'-AGGTCCAAACTCGTACTTAACG-3', *Cldn-18* forward: 5'-GACACCAGATGACAGCAACTTC-3', *Cldn-18* reverse: 5'-TTCATCGTCTTCTGTGCGGG-3', *IgJ* forward: 5'-GCATGTGTACCCGAGTTACC-3', *IgJ* reverse: 5'-TTCAAAGGGACAACAATTCGG-3', *CD19* forward: 5'-CCTGGGCATCTTGCTAGTGA-3', *CD19* reverse: 5'-CGGAACATCTCCCCACTATCC-3'. Expression of target genes in relation to reference gene was determined using the $2^{-\Delta\Delta CT}$ method.

Enzyme-Linked Immunosorbent Assay (ELISA)

Relative IgA levels in BAL, NAL and serum were determined by ELISA using a monoclonal rat anti-mouse IgA capture antibody (Southern Biotech, USA) in combination with a polyclonal rabbit anti-mouse IgA secondary antibody (Abcam, UK) and a polyclonal swine anti-rabbit, HRPO-linked detection antibody (Dako, UK).

In Vitro Stimulation

MLE-15 cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA) supplemented with 4.5g/l glucose, 10% FBS (Biowest, USA) and 1% penicillin/streptomycin (Gibco, USA). $3x10^5$ cells were seeded in 12-well plates and incubated in 1ml medium over night at 37°C, 5% CO_2. Cells were washed using 1ml DMEM (4.5g/l glucose, no additives). Recombinant murine IFN-γ (Peprotech, Germany) was diluted in DMEM (w/o additives) and added to the cells. After 24h supernatants were removed, RNA was isolated from the cells and used for qPCR.

Statistical Analysis

Statistical analyses were performed either by two-tailed, unpaired t-test (Gaussian distribution, two groups), Mann-Whitney test (no Gaussian distribution, two groups), one-way ANOVA (Gaussian distribution, more than two groups) or Kruskal-Wallis test (no Gaussian distribution, more than two groups) using GraphPad Prism software (GraphPad Software Inc., USA, Version 5.04).

Results

In order to determine differences in *Pigr* gene expression in the upper (URT) and lower respiratory tract (LRT) and its dependency on genetic background and sex, we initially compared *Pigr* expression patterns in commonly used mouse strains and different sexes. BALB/c (Fig. 1a) as well as C57BL/6J mice (Fig. 1b) exhibit highest *Pigr* expression in the trachea, followed by nasal-associated lymphoid tissue (NALT) and lung. While pulmonary *Pigr* expression levels were relatively constant, higher variations were detected in trachea and NALT. Moreover, comparative analyses of *Pigr* expression in BALB/c *vs.* C57BL/6J mice (Fig. S1a) and male vs. female C57BL/6J mice (Fig. S1b) revealed no significant differences. Together, these data demonstrate marked tissue-specific differences in *Pigr* gene expression, which were however independent of genetic background and sex.

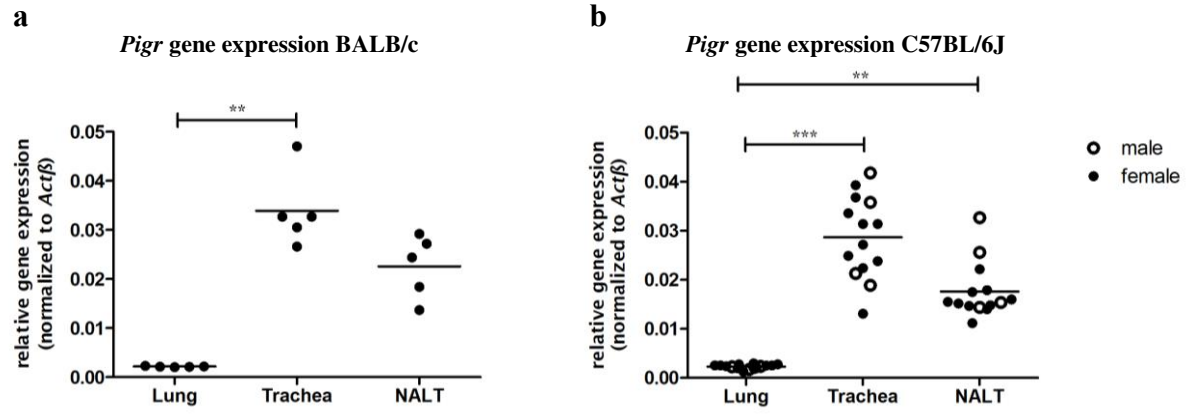

Fig. 1 Basal *Pigr* expression in the airways RNA from lung tissue, trachea and nasal-associated lymphoid tissue (NALT) of (a) BALB/c mice and (b) C57BL/6J mice was isolated and reversely transcribed into cDNA. *Pigr* expression was assessed by qPCR. *Actb* served as reference gene (data for individual mice are graphed; mean is indicated by horizontal line; ** for $p \leq 0.01$; *** for $p \leq 0.001$).

To determine, whether *Pigr* expression and secretory immunity in the airways were influenced by microbial exposure we compared germ-free (GF) mice (no microbial exposition), SPF mice (IVCs, exposition to a limited microbial flora) and mice with an undefined microbiome (open cage maintenance, highest degree of exposition to airborne microorganisms). While similar *Pigr* expression levels were observed in the LRT of all three experimental groups, we detected significantly lower *Pigr* expression in the NALT of mice with an undefined microbiome compared to SPF mice (Fig. 2a). In contrast to the unaltered (lung, trachea) or even reduced (NALT) *Pigr* expression levels in mice with an undefined microbiome, we detected significantly increased IgA concentration in the LRT (Fig. 2b) and URT (Fig. 2c) in this group, which was associated with a systemic IgA increase (Fig. 2d).

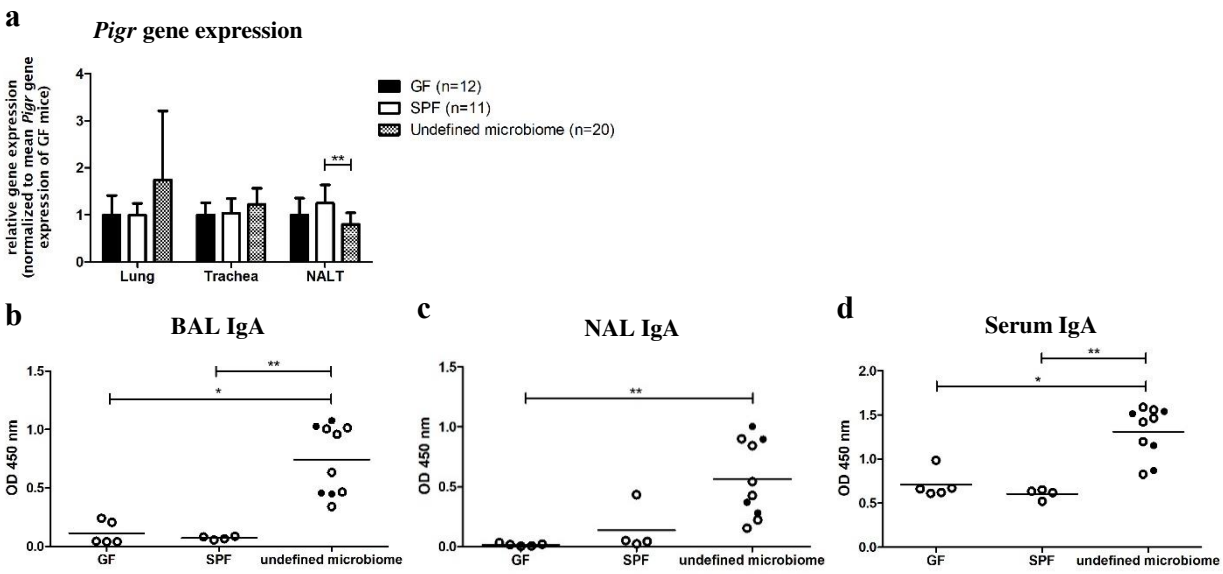

Fig. 2 *Pigr* expression and secretory immunity in differentially colonized mice RNA from lung tissue, trachea and NALT of specific pathogen-free (SPF), germ-free (GF) and mice with an undefined microbiome was isolated, reversely transcribed into cDNA and qPCR analysis was performed (● indicates female, ○ indicates male). (a) *Pigr* expression normalized to *Actb*. Normalized gene expression values of each organ were divided by the mean gene expression of the GF mice for the respective organ (mean expression values ± SD are graphed). IgA levels were determined in (b) bronchoalveolar lavage (BAL), (c) nasal lavage (NAL) and (d) serum by semi-quantitative ELISA (data for individual mice are graphed; mean is indicated by horizontal line; * for $p \leq 0.05$; ** for $p \leq 0.01$).

Since in the intestine IgA production and *Pigr* expression are induced by microbial components [20-22] we investigated whether intranasal (i.n.) treatment of mice with LPS would affect airway *Pigr* expression and IgA levels. While *Pigr* expression in nose and trachea was not affected, we detected significantly increased expression in lung tissue 48h after LPS treatment (Fig. 3a). Interestingly and in discordance with the increased pulmonary *Pigr* expression IgA levels in BAL and NAL fluid significantly decreased after LPS treatment. The amount of IgA in serum was however not affected (Fig. 3b). We thus speculated that LPS altered the barrier function of AECs, resulting in a decreased epithelial leakage. Therefore, we analyzed *Cldn* gene expression in lung and NALT. However, no significant differences in lung or NALT became apparent upon LPS treatment (Fig. 3c, 3d).

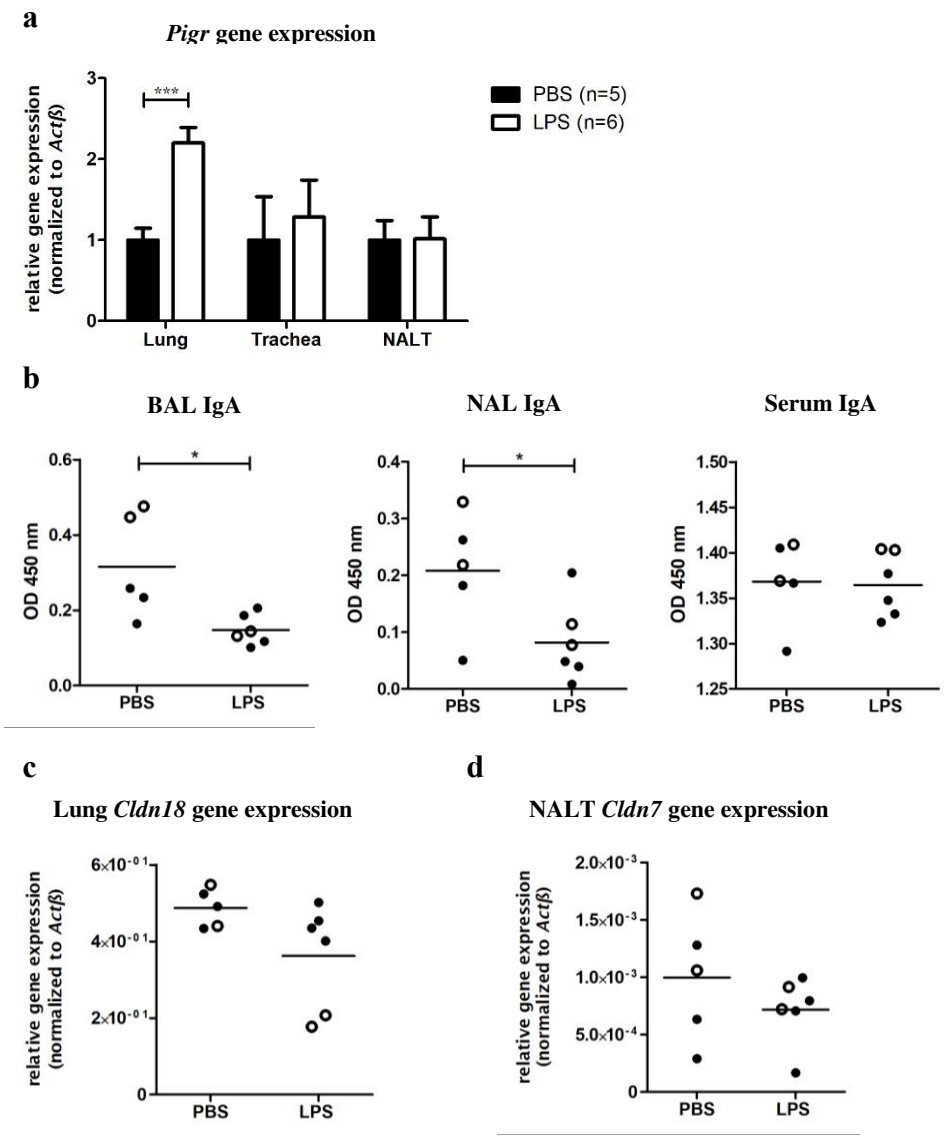

Fig. 3 Effect of LPS treatment on airway secretory immunity and epithelial barrier function BALB/c mice were treated i.n. with 10µg of LPS or solvent alone. 48h post treatment RNA from lung tissue, trachea and NALT was isolated, reversely transcribed into cDNA and qPCR analysis was performed (● indicates female, ○ indicates male). (a) *Pigr* expression normalized to *Actb*. Normalized *Pigr* expression values of each organ were divided by the mean *Pigr* expression of the PBS-treated mice for the respective organ (mean expression values ± SD are graphed). (b) IgA levels were determined in BAL, NAL and serum by semi-quantitative ELISA. (c) *Cldn18* expression normalized to *Actb* and (d) *Cldn7* expression normalized to *Actb* (cumulative data from two experiments; data for individual mice are graphed; mean is indicated by horizontal line; * for $p \leq 0.05$; *** for $p \leq 0.001$).

Next to LPS, Interferon-γ (IFN-γ) was shown to regulate human *PIGR* gene expression [23] and we confirmed the *Pigr*-inducing potential of this cytokine in murine AECs (Fig. S2). To assess possible effects of IFN-γ on PIGR-mediated secretory immunity *in vivo*, we analyzed airway secretory immunity in IFN-γ treated mice. While IFN-γ did not affect *Pigr* expression after 48h (Fig. 4a), the amount of pulmonary IgA increased after IFN-γ treatment. IgA levels in NAL and serum were however unaffected (Fig. 4b). We tested whether epithelial leakage might underlie the increased IgA levels by measuring *Cldn* gene expression in lung and NALT. *Cldn* gene expression was however not affected by single IFN-γ treatment (Fig. 4c, 4d). To investigate whether increased pulmonary IgA might arise from sIg-producing B cells which were induced by IFN-γ, we determined gene expression of the joining chain (*IgJ*) of multimeric IgA and IgM in lung and NALT. Nevertheless, airway *IgJ* expression was unaltered upon cytokine treatment (Fig. 4e, 4f), indicating that increased airway IgA levels are most likely not due to an IFN-γ-mediated increase of sIg-producing B cells.

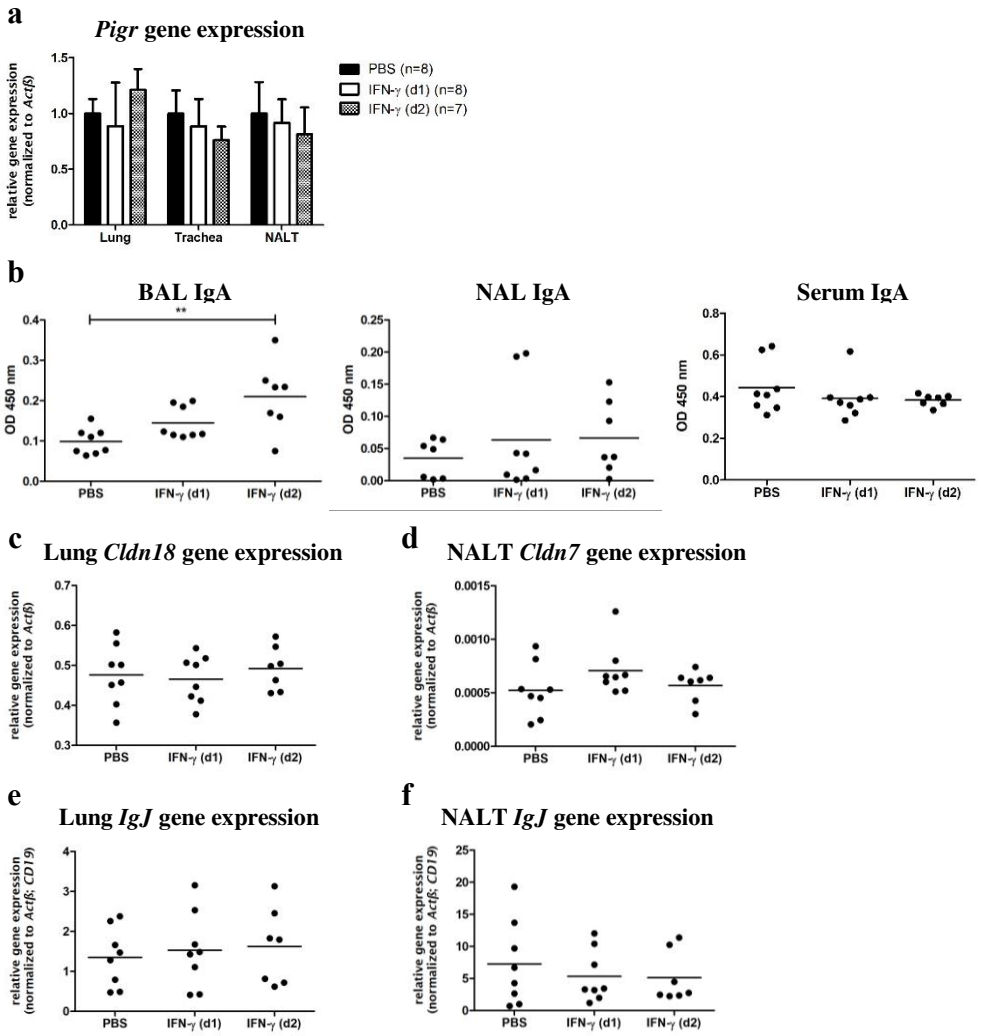

Fig. 4 Effect of IFN-γ treatment on secretory immunity, epithelial barrier function and B cells BALB/c and C57BL/6J mice were treated i.n. with 1µg of IFN-γ or solvent alone. Lung, trachea and NALT were removed on d1 or d2 post treatment, RNA was isolated, reversely transcribed into cDNA and qPCR analysis was performed. (a) *Pigr* expression normalized to *Actb*. Normalized *Pigr* expression values of each organ were divided by the mean *Pigr* expression of the PBS-treated group for the respective organ (mean expression values ± SD are graphed). (b) IgA levels were determined in BAL, NAL and serum by semi-quantitative ELISA. (c) *Cldn18* expression normalized to *Actb*, (d) *Cldn7* expression normalized to *Actb* and (e) + (f) *IgJ* gene expression normalized to *Actb* and *CD19* (cumulative data from two experiments; data for individual mice are graphed; mean is indicated by horizontal line; ** for $p \leq 0.01$).

We finally tested whether modulation of airway secretory immunity by LPS- and IFN-γ-treatment would ultimately affect antimicrobial defense. To this end, mice were i.n. treated with a single dose of LPS or IFN-γ. Two days post treatment mice were inoculated with a colonizing strain of *S. pneumoniae* serotype 19F and airway bacterial burden was assessed (Fig. 5a). As expected, no pneumococci were detected in the lung tissue. While LPS treatment did not affect pneumococcal colonization (Fig. 5b), IFN-γ treatment led to significantly decreased nasal bacterial burden (Fig. 5c). These results demonstrate that at least in the URT mucosal immunity can be augmented by prophylactic IFN-γ treatment.

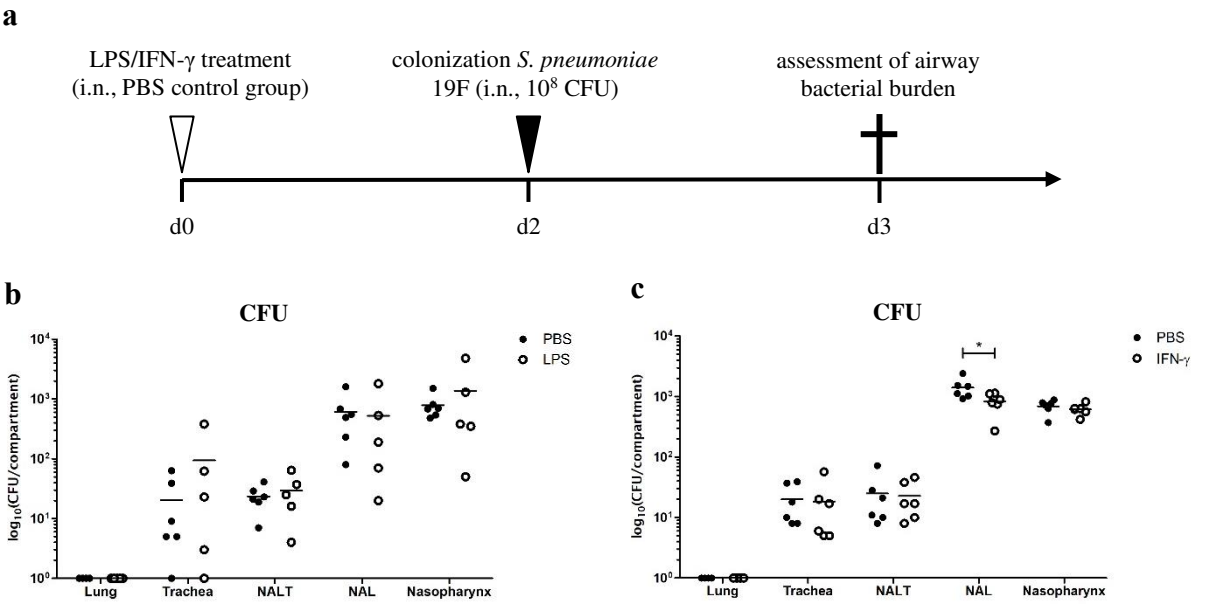

Fig. 5 Effect of LPS or IFN-γ treatment on pneumococcal colonization *in vivo* (a) Schematic representation of the experimental setup. (b) C57BL/6JRj mice were treated i.n. with 10µg of LPS, control mice received PBS only. (c) C57BL/6JRj mice were treated i.n. with 1µg of IFN-γ, control mice received PBS only. 2 days post treatment all mice were i.n. infected with 10^8 CFU of *S. pneumoniae* 19F and airway bacterial burden was assessed 18h post infection (individual data from one experiment are graphed; mean is indicated by horizontal line; * for $p \leq 0.05$).

Supplement

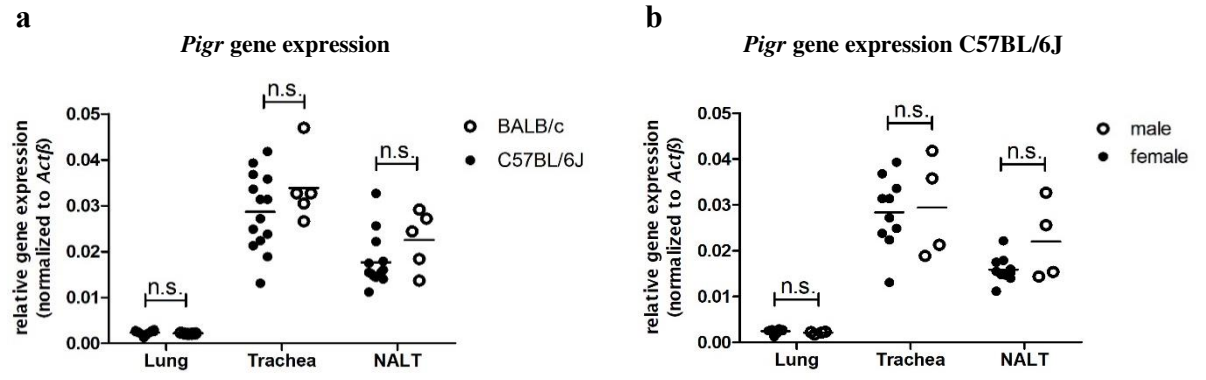

Fig. S1 Influence of genetic background and sex on *Pigr* expression (a) BALB/c vs. C57BL/6J mice and (b) male vs. female C57BL/6J mice. RNA from lung tissue, trachea and NALT of BALB/c and C57BL/6J mice was isolated and reversely transcribed into cDNA. *Pigr* expression was assessed by qPCR. *Actb* served as reference gene (data for individual mice are graphed; mean is indicated by horizontal line; n.s.: not significant).

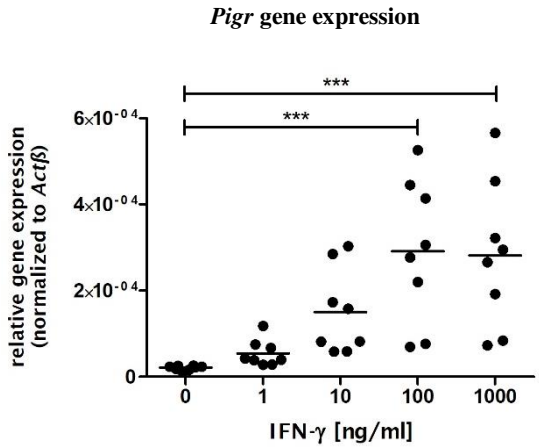

Fig. S2 *Pigr* expression in IFN-γ treated MLE-15 cells Murine lung epithelial (MLE-15) cells were treated with 1, 10, 100 or 1000ng of IFN-γ in 1ml medium or medium alone for 24h. RNA was isolated and reversely transcribed into cDNA. *Pigr* expression was assessed by qPCR analysis. *Actb* was used as reference gene (cumulative data from two experiments; data for individual cell culture wells are graphed and mean is indicated by horizontal line; *** for $p \leq 0.001$).

Discussion

Previous studies on airway *Pigr* gene and PIGR protein expression mainly employed *in vitro* approaches [24, 25] or utilized tissue from patients with chronic respiratory diseases [16, 26-28]. Murine studies were either analyzing respiratory *Pigr* expression in the context of interleukin treatment [29, 30] or exposition to pathogen-associated molecules (Cholera toxin, amoeba lysates) [31]. These studies revealed that PIGR/*Pigr* expression is considerably influenced by exogenous and endogenous stimuli present in the airway microenvironment.

To our knowledge, we are first to report compartment-specific and sex-independent differences in basal airway *Pigr* gene expression levels *in vivo*. We observed that BALB/c as well as C57BL/6J mice showed highest *Pigr* expression in trachea, followed by NALT and lung. We speculate, that this originates from inherent differences in microbial density. Due to their anatomical localization, trachea and nasal cavities are more frequently exposed to microbial stimuli compared to the lung [32]. Most likely, a higher abundance of microbial ligands in the murine URT provides more signals triggering *Pigr* gene expression compared to the LRT, as well.

This in turn might prevent bacterial spread from URT to LRT contributing to the relatively low bacterial density in the lung. However, as we assessed whole-tissue *Pigr* gene expression, we cannot fully exclude the possibility that signal dilution effects (*e.g.* from leukocytes) affect compartment-specific *Pigr* expression in our analyses.

Commensal intestinal bacteria induce the production of IgA in mice [20]. Furthermore, it is known that lymphocyte numbers in nasal mucosa are dependent on housing conditions and exposure to microbial stimuli [33]. While those findings clearly highlight the impact of the microbiota on lymphocyte-associated mucosal immunity, the relationship between airway-associated secretory immunity and the level of microbial exposition is largely unknown. Our experiments revealed that *Pigr* expression was significantly lower in NALT of mice with an undefined microbiome (highest microbial exposure) compared to mice maintained under SPF conditions. Interestingly, this finding was linked to higher airway and systemic IgA levels in these mice. As fecal IgA levels depend on the composition of the intestinal microflora [34], it is conceivable that a similar effect might be present in murine airways.

The fact that *Pigr* expression in NALT is reduced while IgA levels are increased seems to be contradictory. However, we did not analyze *Pigr* expression exclusively in stromal cells but in whole tissue. Since it was shown that the microbial environment shapes cell composition in the mucosa, it is possible that an accumulation of leukocytes in mice with a high microbial exposure reduces the overall *Pigr* signal.

Previous studies revealed that human and murine intestinal epithelial cells exhibit increased *Pigr*/*PIGR* gene expression after LPS stimulation *in vitro* [22, 35, 36]. In line with this, we detected significantly increased pulmonary *Pigr* expression after LPS treatment. However, we also found that LPS did not alter overall *Pigr* expression in trachea or NALT. This might arise from the fact that bacterial colonization - and therefore exposure to *e.g.* LPS - is more pronounced in the URT [32], resulting in a lower sensitivity of URT airway stromal cells to LPS. In contrast to this result, airway IgA levels were decreased in LPS-treated mice, while systemic IgA levels were unaffected. We hypothesized that decreased IgA levels resulted from decreased epithelial leakage and tested this by determining *Cldn18* and *Cldn7* expression. Claudins are major proteins that maintain epithelial barrier function and altered claudin expression results in altered AEC barrier function in the LRT (*Cldn18*) and URT (*Cldn7*) [2-4]. However, *Cldn18* and *Cldn7* expression was not affected by LPS, which disconfirmed our hypothesis. Since IgA binds LPS [37], it is possible that the administered LPS was already bound to IgA in the mucosal lumen. This might reduce the amount of detectable IgA, as the ELISA detects free IgA molecules with the highest functionality.

As LPS, IFN-γ induces *PIGR* expression in human epithelial cells [23, 38, 39], however its effect on *Pigr* and secretory immunity *in vivo* has not been addressed before. Despite no effect on airway *Pigr* expression, IFN-γ treatment increased pulmonary IgA levels. As *Cldn* expression was unaltered, we hypothesized that increased IgA concentrations after IFN-γ treatment might arise from mucosal B cells. Yet, airway *IgJ* expression was not affected, which suggests that activated B cells are most likely not the cause of increased airway IgA levels following IFN-γ stimulation. As mentioned before, we analyzed *Pigr* gene expression in tissues and not exclusively in stromal cells. It is known that intradermal IFN-γ injection stimulates intradermal lymphocyte migration in rats [40]. Thus, it is conceivable that i.n. IFN-γ treatment leads to the accumulation (and activation) of lymphocytes in the airways as well, which might reduce net *Pigr* expression.

As IgA is crucial for antimicrobial defense [15, 41-43], we investigated whether altered IgA levels upon LPS- and IFN-γ-treatment correlated with altered antimicrobial immunity. We have chosen *S. pneumoniae* for experimental colonization of mice as it is one of the most relevant respiratory pathogens [44] and IgA is vital for antagonizing pneumococcal colonization and infection *in vivo* and *in vitro* [12, 45-47]. Indeed, prophylactic IFN-γ-treatment significantly reduced nasal pneumococcal counts indicating improved antibacterial immunity. Since IFN-γ triggers antibacterial activity in pulmonary macrophages [48] and macrophages are present in the murine NALT [49], it is conceivable that IFN-γ induces antibacterial activity in these cells as well. IFN-γ triggers the production of antibacterial molecules (e.g. β-defensins) [50]. Future studies will clarify whether IFN-γ-stimulated production of these antibacterial factors underlies the improved mucosal immunity in the URT.

In conclusion, our study demonstrates that *Pigr* and secretory immunity in URT and LRT are regulated by endogenous as well as exogenous stimuli. Further studies are needed to elucidate the underlying molecular frameworks as well as possible avenues for *e.g.* prophylactic enhancement of airway mucosal immunity in infection-prone individuals.

Declarations

Funding

This work was funded by the federal state Saxony-Anhalt and the European Structural and Investment Funds (ESF, 2014–2020), project number P4410032038.

Availability of data and material

All data of this manuscript can be made available by the corresponding author.

Code availability

Not applicable

Authors' contributions

Conceptualization: Alexander Pausder, Dunja Bruder and Julia D. Boehme; Methodology: Alexander Pausder, Jennifer Fricke; Formal analysis and investigation: Alexander Pausder, Jennifer Fricke; Writing - original draft preparation: Alexander Pausder; Writing - review and editing: Dunja Bruder, Julia D. Boehme; Funding acquisition: Dunja Bruder, Julia D. Boehme; Resources: Klaus Schughart, Jens Schreiber, Till Strowig; Supervision: Dunja Bruder, Julia D. Boehme.

Ethics approval

The experiments were approved by the federal ethical bodies and were carried out in accordance with the guidelines of the Saxony-Anhalt State Administration Office and the guidelines of the Lower Saxony State Office for Consumer Protection and Food Safety.

Consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of interest

None

References

1. Gumbiner B (1987) Structure, biochemistry, and assembly of epithelial tight junctions. *The American journal of physiology* 253 (6 Pt 1), C749-58. DOI: 10.1152/ajpcell.1987.253.6.C749
2. Tsukita S, Furuse M (1999) Occludin and claudins in tight-junction strands: leading or supporting players? *Trends in Cell Biology* 9 (7), S. 268–273. DOI: 10.1016/S0962-8924(99)01578-0
3. LaFemina MJ, Sutherland KM, Bentley T et al. (2014) Claudin-18 deficiency results in alveolar barrier dysfunction and impaired alveologenesis in mice. American journal of respiratory cell and molecular biology 51 (4), S. 550–558. DOI: 10.1165/rcmb.2013-0456OC
4. Clarke TB, Francella N, Huegel A et al. (2011) Invasive bacterial pathogens exploit TLR-mediated downregulation of tight junction components to facilitate translocation across the epithelium. Cell host & microbe 9 (5), S. 404–414. DOI: 10.1016/j.chom.2011.04.012
5. Mills PR, Davies RJ, Devalia JL (1999) Airway epithelial cells, cytokines, and pollutants. American journal of respiratory and critical care medicine 160 (5 Pt 2), S38-43. DOI: 10.1164/ajrccm.160.supplement_1.11
6. Bals R (2000) Epithelial antimicrobial peptides in host defense against infection. Respiratory research 1 (3), S. 141–150. DOI: 10.1186/rr25
7. Varsano S, Frolkis I, Rashkovsky L et al. (1996) Protection of human nasal respiratory epithelium from complement-mediated lysis by cell-membrane regulators of complement activation. American journal of respiratory cell and molecular biology 15 (6), S. 731–737. DOI: 10.1165/ajrcmb.15.6.8969267
8. Kulkarni HS, Liszewski MK, Brody SL et al. (2018) The complement system in the airway epithelium: An overlooked host defense mechanism and therapeutic target? The Journal of allergy and clinical immunology 141 (5), 1582-1586.e1. DOI: 10.1016/j.jaci.2017.11.046
9. Stegemann-Koniszewski S, Jeron A, Gereke M et al. (2016) Alveolar Type II Epithelial Cells Contribute to the Anti-Influenza A Virus Response in the Lung by Integrating Pathogen- and Microenvironment-Derived Signals. mBio 7 (3). DOI: 10.1128/mBio.00276-16
10. Phalipon A, Corthésy B (2003) Novel functions of the polymeric Ig receptor: well beyond transport of immunoglobulins. Trends in Immunology 24 (2), S. 55–58. DOI: 10.1016/S1471-4906(02)00031-5
11. Pilette C, Ouadrhiri Y, Godding V et al. (2001) Lung mucosal immunity: immunoglobulin-A revisited. The European respiratory journal 18 (3), S. 571–588. DOI: 10.1183/09031936.01.00228801
12. Fukuyama Y, King JD, Kataoka K et al. (2010) Secretory-IgA antibodies play an important role in the immunity to Streptococcus pneumoniae. Journal of immunology (Baltimore, Md. : 1950) 185 (3), S. 1755–1762. DOI: 10.4049/jimmunol.1000831
13. Tjärnlund A, Rodríguez A, Cardona P et al. (2006) Polymeric IgR knockout mice are more susceptible to mycobacterial infections in the respiratory tract than wild-type mice. International immunology 18 (5), S. 807–816. DOI: 10.1093/intimm/dxl017
14. Richmond BW, Brucker RM, Han W et al. (2016) Airway bacteria drive a progressive COPD-like phenotype in mice with polymeric immunoglobulin receptor deficiency. Nature communications 7, S. 11240. DOI: 10.1038/ncomms11240
15. Polosukhin VV, Richmond BW, Du R et al. (2017) Secretory IgA Deficiency in Individual Small Airways Is Associated with Persistent Inflammation and Remodeling. American journal of respiratory and critical care medicine 195 (8), S. 1010–1021. DOI: 10.1164/rccm.201604-0759OC
16. Gohy ST, Detry BR, Lecocq M et al. (2014) Polymeric immunoglobulin receptor down-regulation in chronic obstructive pulmonary disease. Persistence in the cultured epithelium and role of transforming growth factor-β. American journal of respiratory and critical care medicine 190 (5), S. 509–521. DOI: 10.1164/rccm.201311-1971OC
17. Ladjemi MZ, Gras D, Dupasquier S et al. (2018) Bronchial Epithelial IgA Secretion Is Impaired in Asthma. Role of IL-4/IL-13. American journal of respiratory and critical care medicine 197 (11), S. 1396–1409. DOI: 10.1164/rccm.201703-0561OC
18. Sandgren A, Albiger B, Orihuela CJ et al. (2005) Virulence in mice of pneumococcal clonal types with known invasive disease potential in humans. The Journal of infectious diseases 192 (5), S. 791–800. DOI: 10.1086/432513
19. Stegemann-Koniszewski S, Gereke M, Orrskog S et al. (2013) TLR7 contributes to the rapid progression but not to the overall fatal outcome of secondary pneumococcal disease following influenza A virus infection. Journal of innate immunity 5 (1), S. 84–96. DOI: 10.1159/000345112

20. Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science (New York, N.Y.) 303 (5664), S. 1662–1665. DOI: 10.1126/science.1091334
21. Hooper LV, Wong MH, Thelin A et al. (2001) Molecular analysis of commensal host-microbial relationships in the intestine. Science (New York, N.Y.) 291 (5505), S. 881–884. DOI: 10.1126/science.291.5505.881
22. Bruno MEC, Frantz AL, Rogier EW et al. (2011) Regulation of the polymeric immunoglobulin receptor by the classical and alternative NF-κB pathways in intestinal epithelial cells. Mucosal immunology 4 (4), S. 468–478. DOI: 10.1038/mi.2011.8
23. Piskurich JF, France JA, Tamer CM et al. (1993) Interferon-γ induces polymeric immunoglobulin receptor mrna in human intestinal epithelial cells by a protein synthesis dependent mechanism. Molecular Immunology 30 (4), S. 413–421. DOI: 10.1016/0161-5890(93)90071-I
24. Khattar NH, Lele SM, Kaetzel CS (2005) Down-regulation of the polymeric immunoglobulin receptor in non-small cell lung carcinoma: correlation with dysregulated expression of the transcription factors USF and AP2. Journal of biomedical science 12 (1), S. 65–77. DOI: 10.1007/s11373-004-8185-5
25. Loman S, Jansen HM, Out TA et al. (1999) Interleukin-4 and interferon-gamma synergistically increase secretory component gene expression, but are additive in stimulating secretory immunoglobulin A release by Calu-3 airway epithelial cells. Immunology 96 (4), S. 537–543. DOI: 10.1046/j.1365-2567.1999.00731.x
26. Polosukhin VV, Cates JM, Lawson WE et al. (2011) Bronchial secretory immunoglobulin a deficiency correlates with airway inflammation and progression of chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine 184 (3), S. 317–327. DOI: 10.1164/rccm.201010-1629OC
27. Pilette C, Godding V, Kiss R et al. (2001) Reduced epithelial expression of secretory component in small airways correlates with airflow obstruction in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine 163 (1), S. 185–194. DOI: 10.1164/ajrccm.163.1.9912137
28. Hupin C, Rombaux P, Bowen H et al. (2013) Downregulation of polymeric immunoglobulin receptor and secretory IgA antibodies in eosinophilic upper airway diseases. Allergy 68 (12), S. 1589–1597. DOI: 10.1111/all.12274
29. Lewis CC, Aronow B, Hutton J et al. (2009) Unique and overlapping gene expression patterns driven by IL-4 and IL-13 in the mouse lung. The Journal of allergy and clinical immunology 123 (4), 795-804.e8. DOI: 10.1016/j.jaci.2009.01.003
30. Jaffar Z, Ferrini ME, Herritt LA et al. (2009) Cutting edge: lung mucosal Th17-mediated responses induce polymeric Ig receptor expression by the airway epithelium and elevate secretory IgA levels. Journal of immunology (Baltimore, Md. : 1950) 182 (8), S. 4507–4511. DOI: 10.4049/jimmunol.0900237
31. Carrasco-Yepez M, Campos-Rodriguez R, Lopez-Reyes I et al. (2014) Intranasal coadministration of Cholera toxin with amoeba lysates modulates the secretion of IgA and IgG antibodies, production of cytokines and expression of pIgR in the nasal cavity of mice in the model of Naegleria fowleri meningoencephalitis. Experimental parasitology 145 Suppl, S84-92. DOI: 10.1016/j.exppara.2014.04.002
32. Man WH, Steenhuijsen-Piters WAA de, Bogaert D (2017) The microbiota of the respiratory tract: gatekeeper to respiratory health. Nature reviews. Microbiology 15 (5), S. 259–270. DOI: 10.1038/nrmicro.2017.14
33. Ichimiya I, Kawauchi H, Tanaka T et al. (1991) Distribution of immunocompetent cells in normal nasal mucosa: comparisons among germ-free, specific pathogen-free, and conventional mice. The Annals of otology, rhinology, and laryngology 100 (8), S. 638–642. DOI: 10.1177/000348949110000807
34. Ohashi Y, Hiraguchi M, Ushida K (2006) The composition of intestinal bacteria affects the level of luminal IgA. Bioscience, biotechnology, and biochemistry 70 (12), S. 3031–3035. DOI: 10.1271/bbb.60164
35. Schneeman TA, Bruno MEC, Schjerven H et al. (2005) Regulation of the polymeric Ig receptor by signaling through TLRs 3 and 4: linking innate and adaptive immune responses. Journal of immunology (Baltimore, Md. : 1950) 175 (1), S. 376–384. DOI: 10.4049/jimmunol.175.1.376
36. Moon C, VanDussen KL, Miyoshi H et al. (2014) Development of a primary mouse intestinal epithelial cell monolayer culture system to evaluate factors that modulate IgA transcytosis. Mucosal immunology 7 (4), S. 818–828. DOI: 10.1038/mi.2013.98
37. Lüllau E, Heyse S, Vogel H et al. (1996) Antigen binding properties of purified immunoglobulin A and reconstituted secretory immunoglobulin A antibodies. The Journal of biological chemistry 271 (27), S. 16300–16309. DOI: 10.1074/jbc.271.27.16300
38. Nihei Y, Maruyama K, Endo Y et al. (1996) Secretory component (polymeric immunoglobulin receptor) expression on human keratinocytes by stimulation with interferon-γ and differences in response. Journal of Dermatological Science 11 (3), S. 214–222. DOI: 10.1016/0923-1811(95)00444-0

39. Loman S, Radl J, Jansen HM et al. (1997) Vectorial transcytosis of dimeric IgA by the Calu-3 human lung epithelial cell line: upregulation by IFN-gamma. The American journal of physiology 272 (5 Pt 1), L951-8. DOI: 10.1152/ajplung.1997.272.5.L951
40. Issekutz TB, Stoltz JM, vd Meide P (1988) Lymphocyte recruitment in delayed-type hypersensitivity. The role of IFN-gamma. Journal of immunology (Baltimore, Md. : 1950) 140 (9), S. 2989–2993
41. Cunningham-Rundles C (2001) Physiology of IgA and IgA deficiency. Journal of clinical immunology 21 (5), S. 303–309. DOI: 10.1023/A:1012241117984
42. Edwards E, Razvi S, Cunningham-Rundles C (2004) IgA deficiency: clinical correlates and responses to pneumococcal vaccine. Clinical immunology (Orlando, Fla.) 111 (1), S. 93–97. DOI: 10.1016/j.clim.2003.12.005
43. Janzi M, Kull I, Sjöberg R et al. (2009) Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. Clinical immunology (Orlando, Fla.) 133 (1), S. 78–85. DOI: 10.1016/j.clim.2009.05.014
44. Catterall JR (1999) Streptococcus pneumoniae. Thorax 54 (10), S. 929–937. DOI: 10.1136/thx.54.10.929
45. Sun K, Johansen F, Eckmann L et al. (2004) An important role for polymeric Ig receptor-mediated transport of IgA in protection against Streptococcus pneumoniae nasopharyngeal carriage. Journal of immunology (Baltimore, Md. : 1950) 173 (7), S. 4576–4581. DOI: 10.4049/jimmunol.173.7.4576
46. Sestini P, Nencioni L, Villa L et al. (1988) IgA-driven antibacterial activity against Streptococcus pneumoniae by mouse lung lymphocytes. The American review of respiratory disease 137 (1), S. 138–143. DOI: 10.1164/ajrccm/137.1.138
47. Fasching CE, Grossman T, Corthésy B et al. (2007) Impact of the molecular form of immunoglobulin A on functional activity in defense against Streptococcus pneumoniae. Infection and immunity 75 (4), S. 1801–1810. DOI: 10.1128/IAI.01758-06
48. Jensen WA, Rose RM, Burke RH Jr. et al. (1988) Cytokine activation of antibacterial activity in human pulmonary macrophages: Comparison of recombinant interferon-γ and granulocyte-macrophage colony-stimulating factor. Cellular Immunology 117 (2), S. 369–377. DOI: 10.1016/0008-8749(88)90126-8
49. Heritage PL, Underdown BJ, Arsenault AL et al. (1997) Comparison of murine nasal-associated lymphoid tissue and Peyer's patches. American journal of respiratory and critical care medicine 156 (4 Pt 1), S. 1256–1262. DOI: 10.1164/ajrccm.156.4.97-03017
50. Albanesi C, Fairchild HR, Madonna S et al. (2007) IL-4 and IL-13 negatively regulate TNF-alpha- and IFN-gamma-induced beta-defensin expression through STAT-6, suppressor of cytokine signaling (SOCS)-1, and SOCS-3. Journal of immunology (Baltimore, Md. : 1950) 179 (2), S. 984–992. DOI: 10.4049/jimmunol.179.2.984

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