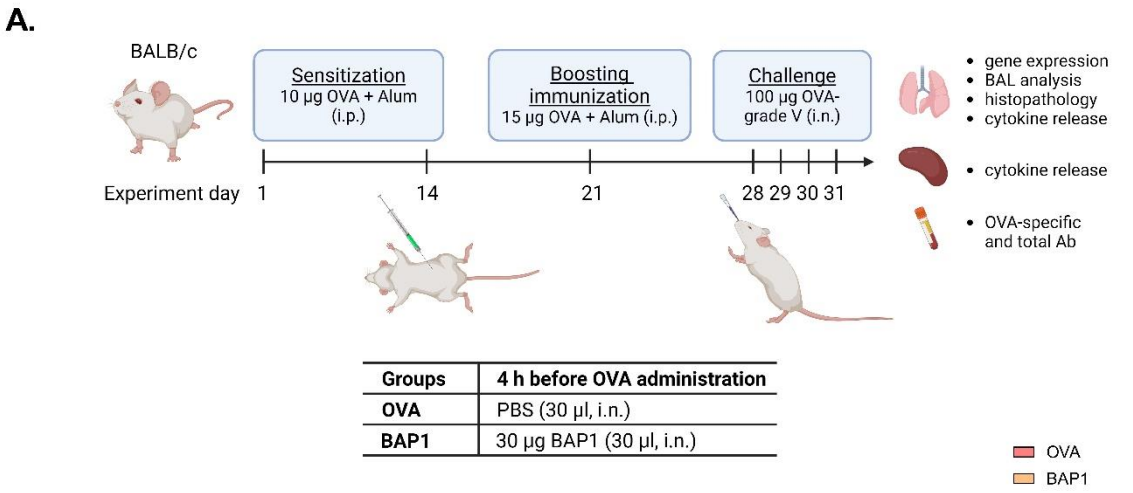
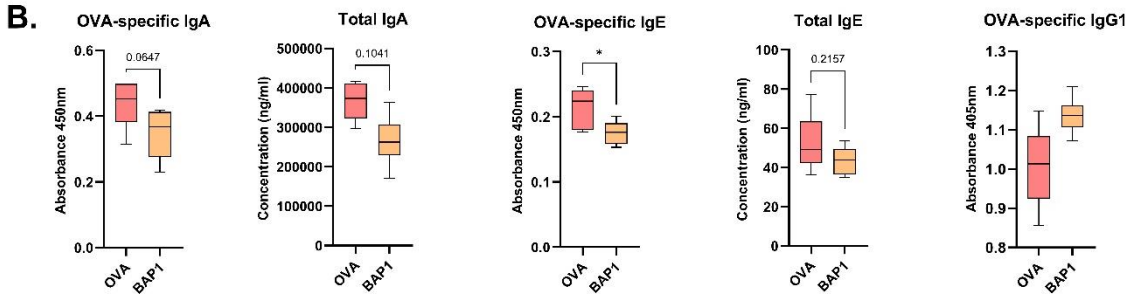
with PBS or BAP1. An unpaired t-test was performed and significant differences between PBS- and BAP1-treated mice were calculated (* $p \leq 0.05$).

3.4. BAP1 treatment reduces OVA-specific IgE levels in mice with allergic airway inflammation

Given the established immunomodulatory potential of BAP1, we tested the ability of BAP1 to alleviate allergy symptoms in a mouse model of allergic airway inflammation. Experimental design was performed according to our previous study with 3 i.p. OVA-injections and 4 i.n. challenges. BAP1 was administered intranasally to mice 4 h prior to each OVA sensitization and challenge (Figure 3A) [11]. The levels of total IgA and IgE antibodies in the sera were not notably affected by BAP1 treatment, but we observed a trend towards a reduction in the OVA-specific IgA production in sera. Moreover, PS administration dampened Th2 systemic humoral sensitization, as the OVA-specific IgE level was significantly reduced. We observed a significant increase in the production of OVA-specific IgG1 antibodies in serum and no changes in OVA-specific IgG2a (Figure 3B, Supplementary Figure 2). At the same time, a significant reduction in the level of Th2-related IL-5 cytokine, and a tendency to smaller production of IL-10, IL-13, and IFN-γ was detected in splenocytes stimulated by OVA (Figure 3C).





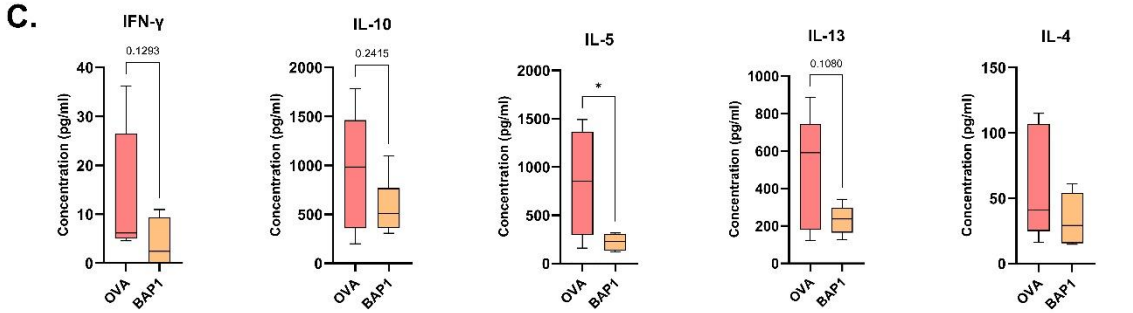


Figure 3. Systemic response after i.n. administration of BAP1 in the mouse model of allergic airway inflammation. **A.** Experimental design for BAP1 treatment, OVA sensitization and challenge. **B.** OVA-specific and total IgA, IgE, and IgG serum antibodies tested by ELISA. **C.** Cytokines and chemokines production in PBS- and BAP1-treated mice in response to restimulation with an allergen (OVA) measured in splenocyte cultures by Luminex. An unpaired t-test was performed and significant differences between PBS- and BAP1-treated mice were calculated (* $p \leq 0.05$).

3.5. BAP1 reduces airway inflammation and decreases the expression of the Il10 gene

Directly in the lung tissue, i.n. BAP1 treatment was able to reduce eosinophil and macrophage numbers without affecting lymphocyte or neutrophil cell populations (Figure 4A). Evaluation of the total and OVA-specific IgA levels in BALF showed no differences between the studied groups (Figure 4B).

However, histological analysis of the lung tissue showed a reduction in leukocyte infiltration in the perivascular, peribronchiolar, and alveolar spaces, and a reduction in mucus-producing cells in the airways of BAP1-treated mice (summarized in the histopathological evaluation in Figure 4C). Similar to the cytokine recall response in splenocyte cultures, BAP1 treatment significantly downregulated the OVA-induced IL-5 and IL-10 levels and decreased the IL-4 and IL-13 production in lung cell cultures (Figure 4D).

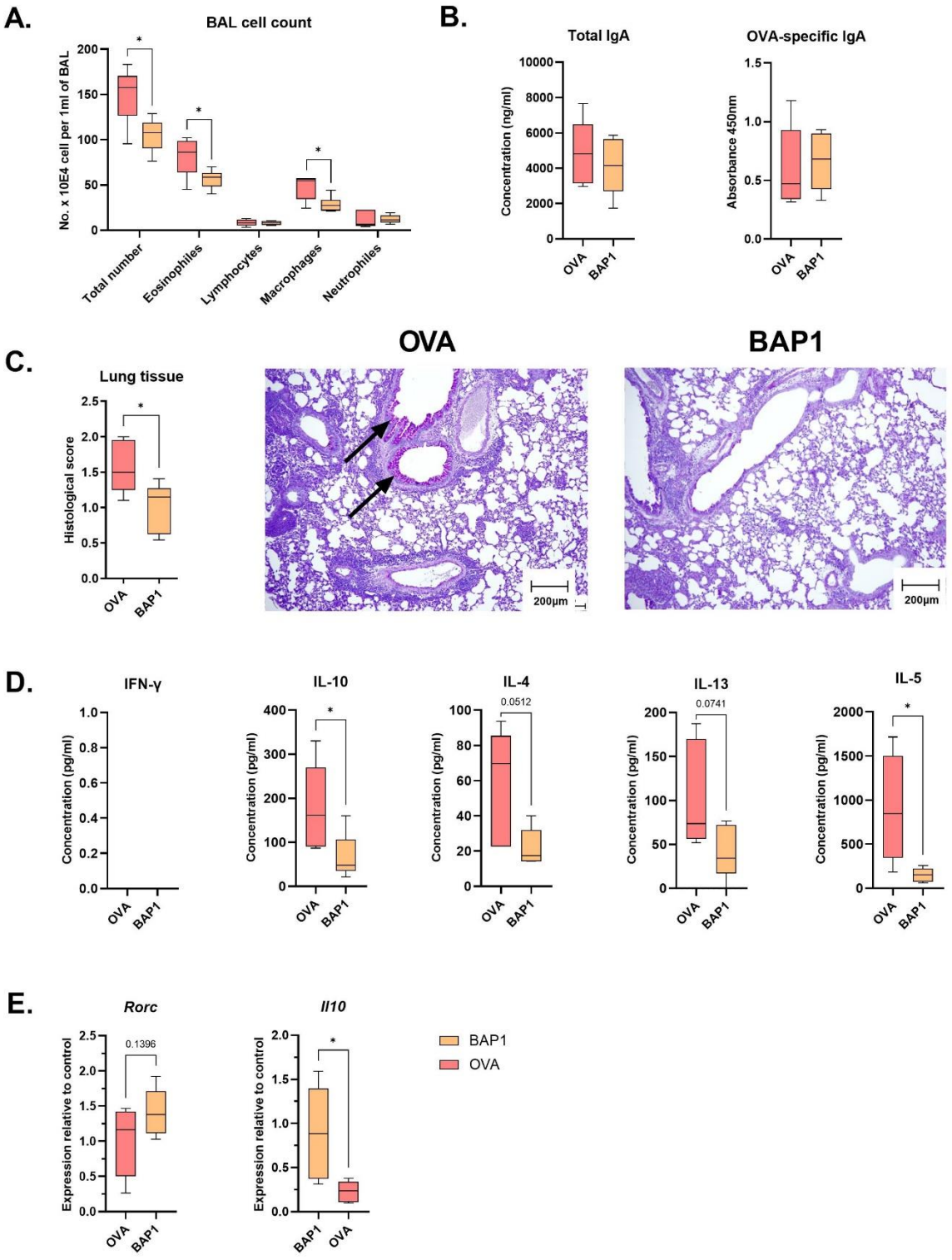


Figure 4. Lung-related response to BAP1 in mice with allergic airway inflammation. **A.** Cell count in BAL. **B.** Total and OVA-specific IgA BALF antibodies tested by ELISA. **C.** Representative histopathological section of lungs from OVA-sensitized mice treated with PBS or BAP1, stained with Periodic Acid-Schiff. **D.** Cytokines and chemokines production of BAP1-treated and OVA-control mice restimulated with an allergen (OVA) measured in lung cell cultures by Luminex and expressed as pg/ml. **E.** Changes in lung

gene expression measured in BAP1- and PBS-treated OVA-allergy mice. An unpaired t-test was performed and significant differences between OVA-allergy mice treated with PBS and BAP1 were calculated (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Evaluation of lung gene expression showed a tendency to increase *Rorc* levels, which is consistent with our findings in GF mice (Figure 4E). The analysis revealed a significant inhibition of *Il10* gene expression (Figure 4E). In addition, investigation of the remaining genes, including *Gata3*, *Tbx21*, and *Foxp3,* showed no changes between tested groups of mice (Supplementary Figure 3).

Discussion

In our recent study, we evaluated the role of surface molecules isolated from the *Bifidobacterium adolescentis* CCDM 368 strain using *in vitro* and *ex vivo* studies [17]. We showed that surface PS, BAP1, is an immunoregulatory molecule that is well-recognized and transferred by epithelial and immune cells. Its ability to restore the Th1/Th2 balance (disturbed in allergy) was highly desirable, as demonstrated by the induction of IFN-γ and the inhibition of IL-13 and IL-5 production in OVA-sensitized splenocyte cultures. However, to fully understand the exact nature of the molecule, it is necessary to know not only its function but also its structure. Thus, we determined the repeating unit of the BAP1, which turned out to be a unique, non-branched PS consisting of glucose, rhamnose, and galactose residues with a molecular mass of approximately 9.99 x 10^6 [17].

Here, to expand our knowledge about the BAP1 role in the immune response modulation, we tested it in a naïve system of airways in GF mice to observe the molecule-specific responses (Figure 1A). We noticed an increase in total IgA serum levels in BAP1-treated GF mice which is consistent with the previously published data (Figure 1B)[20,21]. Enhanced levels of this immunoglobulin are crucial for balanced microbial colonization and induction of immune tolerance against introduced microbiota or beneficial bacteria-derived antigens [18,22]. Further evaluation of splenocyte response showed no differences in cytokine and chemokine production after treatment of GF mice with BAP1 (Figure 1C). The neutral effect of tested PS favors our work, since it indicates, that BAP1 doesn't cause a systemic inflammatory response of the naïve immune system.

For the next step, we decided to focus on the immune response directly in the lungs of GF mice, as it is one of the main organs affected by airway allergy inflammation. Evaluation of cell count in BALF showed no differences between the PS-treated and control groups, however, the total IgA level was significantly increased (Figure 2A and B). Similarly, increased levels of total IgA in GF mice lungs have been shown upon bacterial PS administration in our previous study [18]. Also, the PAS staining indicated no evidence of inflammatory signs in the lung tissue (Figure 2C). Interestingly, we observed a significant decrease in CCL2 (C-C Motif Chemokine Ligand 2) protein levels in lung cells supernatants (Figure 2D). This chemokine is known to be upregulated in airway allergy, leading to the recruitment of macrophages and basophils, thereby, inducing the development of inflammation [23]. Activation of CCL2 is mediated via Th2 cytokines, indicating, that our PS may have an inhibitory effect on the production of cytokines such as IL-4 [24]. We also observed a trend towards decreased levels of CCL11/eotaxin, which recruits eosinophils, key factors in type 2 eosinophilic asthma (Figure 2D) [25]. Finally, we complemented our analysis in GF mice by investigating the expression of transcription factors *Tbx21*, *Gata3*, *Foxp3*, and *Rorc*, which are responsible for the activation of four different pathways – Th1, Th2, Treg, and Th17 respectively. The results pointed to the possible role of BAP1 in activating the Th17 and Th1

pathways, as we observed an increased level of *Rorc* gene expression and a tendency to increase the *Tbx21*, which in turn could restore the balance of the immune responses in allergic diseases (Figure 2E).

Considering the data obtained in GF mice, and the previously described *ex vivo* anti-allergic potential, we continued the BAP1 studies *in vivo* using the mouse model of allergic airway inflammation to OVA. The available research describing the impact of beneficial bacteria on the development of respiratory allergic diseases has focused mainly on the oral administration of whole bacteria. For instance, Cavalcanti et al. (2024) described the anti-allergy potential of *Lacticaseibacillus paracasei* on the OVA-induced inflammation in BALB/c mice [26]. Results showed an inhibitory effect of the investigated bacteria to reduce nasal and lung tissue inflammation and decrease BALF eosinophils number and OVA-specific IgE in sera. Also, the strain mitigated Th2-related cytokines and TGF-β production, while increasing IL-10 levels. A similar effect in alleviating airway allergy was observed for other orally delivered lactic acid bacteria strains [27–31]. However, only a few publications focus on the i.n. administration of bacteria or bacterial antigens [11,18,32,33]. This attempt allows to achieve a stronger local response to the treatment. One of the advantages of i.n. probiotic administration is that bacteria do not pass through the digestive system, and thus are not exposed to the digestive enzymes. Spacova et al. investigated the i.n. application of *Lactobacillus rhamnosus* GG to birch pollen-induced BALB/cOlaHsd. The results showed that bacteria treatment reduced inflammation and eosinophil infiltration in the lungs and inhibited Th2-related IL-5, IL-13, and IL-10 production [33]. Previously, we tested i.n. administration of EPS from *Lacticaseibacillus rhamnosus* LOCK900 to OVA-allergy mice and observed the alleviation of allergy symptoms through inhibition of Th2-related cytokine responses and decreased number of eosinophils [34]. Therefore, based on available data and our own experience, in this study, we focused on i.n. administration of the BAP1 molecule as the most effective route to modulate the immune response in a mouse model of airway allergy inflammation.

Next, we evaluated the systemic response to BAP1 treatment. Investigation of serum levels of total and allergen-specific IgA and IgE showed the ability of BAP1 to inhibit the production of both immunoglobulins with the significant reduction of OVA-specific IgE (Figure 3B). We observed a similar trend in our previous studies regarding L900/2 and L900/3 PSs isolated from *Lactobacillus rhamnosus* LOCK 0900, where tested PS significantly reduced allergen-specific IgE and showed a tendency to mitigate specific IgA levels [34,35]. Investigation of splenocyte culture restimulated with OVA showed a role of BAP1 in the inhibition of the Th2-related cytokines, responsible for the development of allergy inflammation and the recruitment of IgE antibodies. That included a significant inhibition of IL-5 and a tendency to decrease IL-4 and IL-13 cytokines (Figure 3C).

Research focusing on the impact of probiotic bacteria on the development of allergies indicates the beneficial effect of lactic acid bacteria in reducing cell infiltration thus alleviating cell inflammation and tissue damage [11,27,30,31]. Further investigation in the lung has yielded promising results in favor of the anti-allergic potential of BAP1. Histopathology evaluation of the lung tissue showed significantly reduced inflammation in BAP1-treated mice compared to OVA mice, accompanied by a reduced number of cells counted in BALF. Particularly, we saw a decrease in macrophages as well as eosinophils, crucial for IgE-mediated inflammation (Figure 4A and C). Reduced eosinophilia upon probiotic-treatment is well described in the literature and is associated with anti-allergic properties of the strain in the airway tract [36,37]. Also, postbiotic impact on cell infiltration was described in the literature. PS isolated from *Bifidobacterium longum* subsp. *longum* 35624™ significantly inhibited eosinophil infiltration in BAL of OVA-sensitized mice [38].

Further, BAP1 treatment downregulated the Th2 allergic cytokine response locally in lung cells and systemically in splenocytes (Figure 4B and D). Generally, the available research mostly agrees that probiotics' mechanism of restoring disrupted Th1/Th2 balance is based on the increased production of IFN-γ or IL-10 [39]. Interestingly, in both systemic and local responses, the level of IL-10 was significantly lower upon BAP1 treatment in comparison to OVA mice. At first, studies focusing on IL-10 associated this cytokine only with Th1/Th2 functionalities. However, current knowledge underlines the complexity of this molecule and shows a broad range of cells that can produce IL-10 for different anti-, pro-inflammatory, or regulatory purposes [40,41]. A great number of studies show the protective role of IL-10 in the development of asthma diseases and the role of probiotics in increasing this cytokine. [26,30,31,42]. However, there is evidence that it can also be responsible for increasing airway inflammation. Polukort et al. described IL-10-dependent mast cell activation affecting the IgE-mediated food allergy development in OVA-sensitized BALB/c mice [43]. Wu et al., 2016 have shown that administration of *L. rhamnosus* GG before or after the induction of OVA inflammation reduced allergy symptoms while decreasing IL-10 cytokine levels, in both serum and BALF. This proves that the alleviation of allergy can be associated with mitigated levels of IL-10 [27]. Interestingly, examination of *Rorc* gene indicated an increased expression, as in GF mice administered with BAP1, however, the effect was statistically insignificant (Figure 4E). The proper control of the *Rorc* gene is crucial for the prevention of allergy development. Abdel-Gadir et al., described the influence of Clostridiales species on food allergy in mice. In addition to describing the bacterial suppressive effect on allergy inflammation, they investigated its signaling. The results highlighted the role of ROR-γ Treg cells in disease alleviation [44]. The described process is a turnover in current knowledge, since, until now, the ROR-γ molecule was mainly associated with the allergy occurrence. We are aware that the mechanism differs between food

and airway hyperresponsiveness, but this example shows that there's still much to be discovered about the mechanisms that underlie the beneficial effect of bacteria in alleviating allergies.

It is worth noticing that in our studies, we decided to go further and evaluate the effect of bacterial components instead of the whole microorganism. Through this attempt, we could overcome the difficulties connected to the use of a live microorganism such as the transfer of antibiotic resistance genes or bacteriemia [15,45]. Moreover, PSs are heat resistant, thus more stable molecules, and in comparison to the bacteria cells, it is possible to define their exact structure and function [46]. Only a few publications focus on structure-function studies of PSs [34,47–49]. Even less discuss PSs properties to treat allergies. Bai et al. described LJP heterogenous PS from *Lonicera japonica* that when administered intragastrically was able to reduce symptoms of OVA-induced allergy in mice by inhibition of serum IgE levels, nasal eosinophils number, allergy-related cytokines, and expression of *Rorγt* genes in the nasal mucosa [49]. Also, in our previous work, we confirmed an inhibitory effect of exopolysaccharide (EPS) of *Lacticaseibacillus rhamnosus* LOCK900 on the development of allergy inflammation in OVA-treated mice [34]. This effect was assessed by i.n. administration of PS, similar to BAP1. However, the structure of the molecule and possible mechanisms involving IgA and TGF-β activation are distinct from the one presented in this publication.

Conclusions

Here, for the first time, we conducted a detailed analysis of BAP1 PS to evaluate its anti-allergic potential in a mouse model of OVA allergy. Primarily, our results confirmed the neutral effect of BAP1 on the naïve immune system of GF mice. Nevertheless, we observed a significant CCL2 downregulation, a decrease in eotaxin production, and an upregulation of *Rorc* gene expression in lung. Further studies performed on OVA-treated mice showed the ability of BAP1 to reduce allergy inflammation. This effect was achieved by inhibition of OVA-specific IgE and Th2-related cytokine production as well as eosinophil and macrophage recruitment. Moreover, treatment with BAP1 inhibited the level of Th2-related and IL-10 cytokines. Finally, we were able to indicate the possible role of increased *Rorc* and decreased *Il10* gene expression in the BAP1 mechanism. In conclusion, BAP1 appears to be a promising candidate for the management of allergic diseases. Nevertheless, modulation of immune response to bacterial antigens is complex, and many factors, such as cells and tissue type, genetic and environment immunity predisposition, microbiome, etc. need to be taken into account. Also, there is little information about possible PSs' molecular pathways. Thus, further studies will be required to fully confirm the mechanism and potential of BAP1.

Funding

This work was supported by the National Science Centre of Poland (UMO2017/26/E/NZ7/01202), and the Czech Science Foundation (23-04050L) and by the Ministry of Education, Youth and Sports of the Czech Republic grant Talking microbes - understanding microbial interactions within One Health framework (CZ.02.01.01/00/22_008/0004597).

CRediT authorship contribution statement

Katarzyna Pacyga-Prus: Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Visualization. **Tereza Hornikova:** Investigation, Formal analysis, Writing - review & editing. **Dagmar Srutkova:** Methodology, Resources, Writing - review & editing, Funding acquisition. **Katarzyna Leszczyńska-Nowak:** Investigation**. Agnieszka Zabłocka**: Writing - review & editing **Martin Schwarzer:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Sabina Górska:** Conceptualization, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We thank Jaroslava Valterova and Sarka Maisnerova for their excellent technical assistance. Graphical abstract and experimental models were prepared using Biorender online software.

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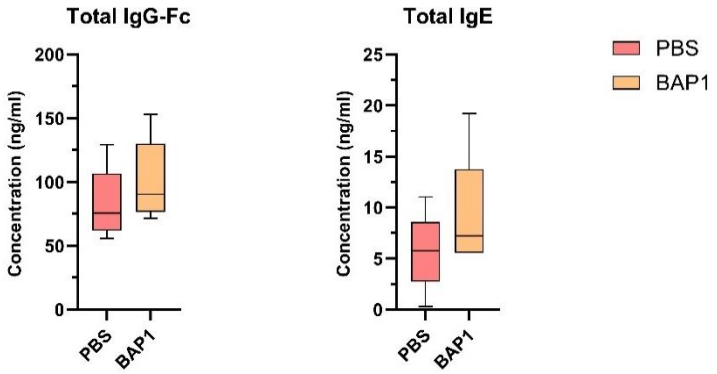
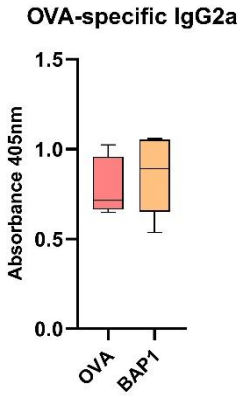
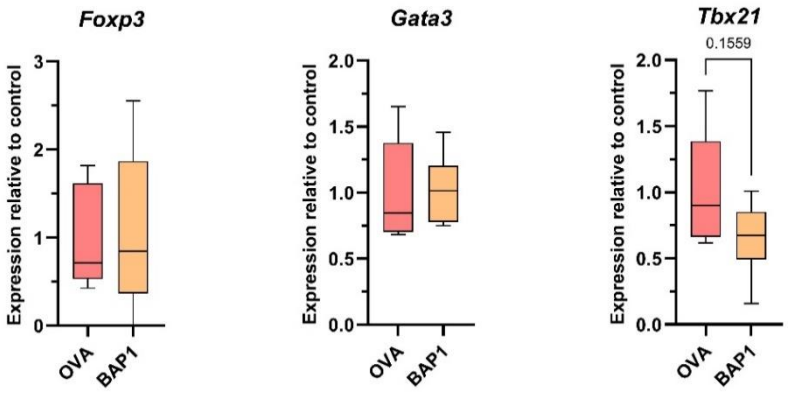
Suplementarny materials



Supplementary Figure 1. Total IgG-Fc and IgE, serum antibodies measured in GF mice and tested by ELISA. An unpaired t-test was performed and significant differences between PBS and BAP-1 treated mice were calculated.



Supplementary Figure 2. OVA-specific IgG2a serum antibodies measured in OVA-induced allergic mice and tested by ELISA. An unpaired t-test was performed and significant differences between PBS and BAP-1 treated mice were calculated (* $p \leq 0.05$).



Supplementary Figure 3. Changes in lung gene expression measured in BAP-1 treated OVA-allergy mice. An unpaired t-test was performed and significant differences between PBS and BAP-1 treated mice were calculated.

Summary of research and prospects

The main idea behind the studies presented in the doctoral dissertation appeared after the evaluation of different *Bifidobacteria* strains' ability to alleviate OVA-sensitization in mice splenocytes. This analysis was performed by Marcelina Pyclik from the Laboratory of Microbiome Immunobiology as a part of her PhD dissertation [22]. Ban218 and Bad368 are among the strains that showed the potential to inhibit Th2-related cytokine response and were subjected to further analysis (1st manuscript – Supplementary Figure S1; Suppl. Fig. S1). First, antigens were isolated from the surface of selected bacteria, thoroughly purified and tested *in vitro* and *ex vivo* to determine their possible anti-allergic properties. The studies started with 10 antigens: 5 belonged to Ban218, including 1 fraction of PG, 1 fraction of LTA, and 3 PSs fractions (B.PAT, PS 2, PS 3) (Suppl. Fig. S2), and 5 belonged to Bad368, including 1 fraction of PG, 1 fraction of LTA, and 3 PSs fractions (BAP1, PS 2, PS 3) (1st manuscript – Supplementary Figure S2).

Prepared antigens were tested in cells isolated from OVA-sensitized mice. The 1st manuscript described the selection of BAP1 of the Bad368 strain as a potential candidate for allergy treatment. Thorough analysis based on GLC-MS spectrometry and NMR spectroscopy enabled, for the first time, the determination of a unique structure of BAP1 PS with high molecular mass. Polymer consists of rhamnose, galactose, and glucose residues creating a hexasaccharide repeating unit.

Subsequently, the intranasal administration of BAP1 was tested in GF mice and mice with airway allergy to OVA, which was described in detail in the 3rd manuscript. The obtained results showed the unquestionable potential of BAP1 to alleviate allergy responses by inhibiting Th2-related and IL-10 cytokines both in splenocytes and lung cells of OVA-allergy mice. It reduced lung cell infiltration (with a significant decrease in eosinophils) and OVA-specific IgE levels in sera. The investigation of transcription factors in the lungs of GF and OVA-allergy mice indicated the possible role of the *Rorc* gene activation over BAP1 stimulation. Also, it confirmed the BAP1's influence on the IL-10 inhibition. Moreover, it was shown that the beneficial effect of this PS may be related to its recognition by the TLR2 receptor. It was indicated by BAP1's ability to activate TLR2 receptors in transfected HEK-Blue™ cells (1st manuscript – Supplementary Figure S3) and loss of PS's modulatory properties in BMDCs isolated from *Myd88* knockout C57BL/6 mice (Suppl. Fig. S3).

The comprehensive research on BAP1 showed its strong anti-allergic potential that can be further exploited. However, a thorough analysis of signaling pathways is necessary to understand the BAP1's mechanism of action. It would increase the safety of its use and allow the recognition of BAP1's possible side properties. Next, clinical trials are needed to confirm the results obtained in

mice models. All of the above may contribute to the creation of a novel, intranasal-delivered antigen-based drug that would alleviate airway allergy symptoms by targeting both local and systemic responses. Moreover, it may overcome the side effects demonstrated by currently available treatments, however, further investigation is needed to confirm that assumption.

At the beginning of the studies, a hypothesis was established claiming that the surface antigens of *Bifidobacterium animalis* ssp. *animalis* CCDM 218 and *Bifidobacterium adolescentis* CCDM 368 are responsible for the anti-allergic potential of the whole bacteria. Our results regarding the Bad368 strain proved it right by describing BAP1 polysaccharide exhibiting anti-allergic potential similar to its parental bacteria. However, studies on Ban218 showed no promising molecules among tested antigens (Suppl. Fig. S4) since none of the compounds was able to decrease Th2-related cytokine responses in splenocytes. Given that specific modifications can enhance the function of certain molecules, we turned our attention to B.PAT PS (Suppl. Fig. S2), which was found to be phosphorylated. Further examination identified B.PAT as a PS with a molecular mass of approximately 1.96×10^4, whose structure has not yet been defined. This polysaccharide features a branched, nonasaccharide repeating unit that includes galactose, glucose, and rhamnose residues, predominantly in the pyranose form, with one exception being galactofuranose. Importantly, B.PAT is substituted by glycerol phosphate at the 6th position of the glucose residue. Consequently, the idea emerged to dephosphorylate this PS and assess whether this modification would enhance B.PAT's immunoregulatory properties and alter its structure (2nd manuscript). Indeed, the loss of glycerol phosphate substitution enhanced the functionality of B.PAT, leading to significantly stronger immunomodulatory effects in mouse BMDCs and improved anti-inflammatory properties in the IL-1β model of human cell line inflammation.

Bacterial PSs are widely applied molecules with desirable properties. They can be found among others in cosmetics (e.g. hyaluronic acid as a moisturizing agent) or in the food industry (e.g. Xanthan gum as a stabilizer and thickening agent) [45,46]. Health-promoting properties of certain molecules have been documented in the literature [47–50]. However, not all microbial PSs exhibit beneficial properties. The key determinant of a PS's functionality lies in its structure. Modifications such as acetylation, sulfation, or phosphorylation can affect its charge, molecular weight, and spatial configuration, thereby, significantly influencing its biological activities [51,52]. Additionally, research highlights the importance of sugar conformation, backbone linkages, and the size of the PS in determining its chemical properties and functionalities [53,54]. These findings underscore the necessity of structural analysis of PSs prior to conducting immunological studies.

Supplementary data

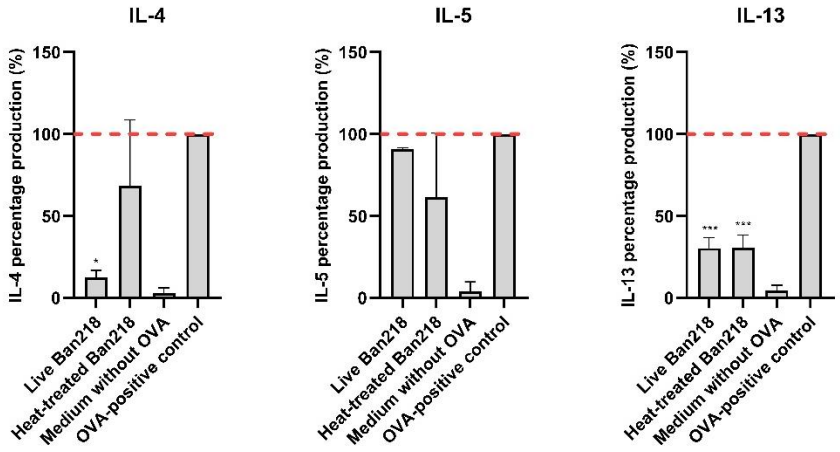
1. Stimulation of splenocytes derived from OVA-sensitized mice with live and heat-treated Ban218

1.1. Materials and methods

The experiment was performed as described previously (1st manuscript, Supplementary Materials 1.2. Splenocytes isolation and stimulation).

1.2. Results

Determination of the Th2-related cytokines production in OVA-treated splenocytes isolated from OVA-sensitized mice showed the ability to significantly reduce IL-13 (live and heat-treated) and IL-4 (live) levels (Suppl. Fig. S1).



Suppl. Fig. S1. Th2-related cytokine production by splenocytes isolated from OVA-sensitized mice, pretreated with OVA, and stimulated with live and heat-treated Ban218 (1 cell : 10 bacteria ratio). Data on the graph are shown with mean ± SD and one-way ANOVA with Dunnett's multiple comparisons was performed to compare obtained results with the OVA-positive control (100%, red dashed line) (*** $p \leq 0.001$, * $p \leq 0.05$).

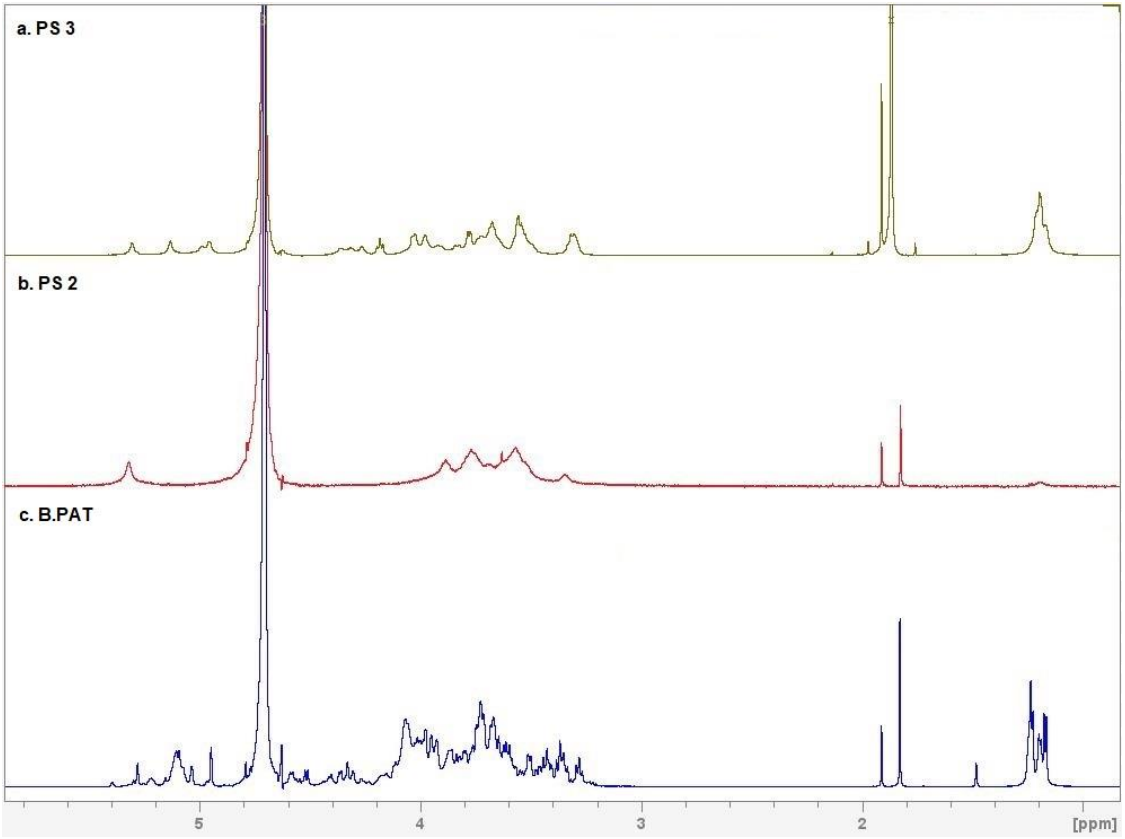
2. Isolation of the PSs' fractions from the Ban218

2.1. Materials and methods

The experiment was performed as described previously (1st manuscript, 2.6.1. NMR spectroscopy and size determination).

2.2. Results

Ban218 strain consists of 3 different PS structures on its surface. One of them (B.PAT) is phosphorylated (Suppl. Fig. S2).



Suppl. Fig. S2. 1H-NMR spectra of PSs produced by Ban218. Spectra (a – c) represent fractions after chromatography separations. The chemical shift range of 4.2 – 5.8 ppm corresponds to the anomeric signals of PS, while the range of 3.2 – 4.5 ppm is associated with the ring signals. Alkyl groups are observed at shifts below 2 ppm.

3. Investigation of importance of Myd88 pathway activation for BAP1 function

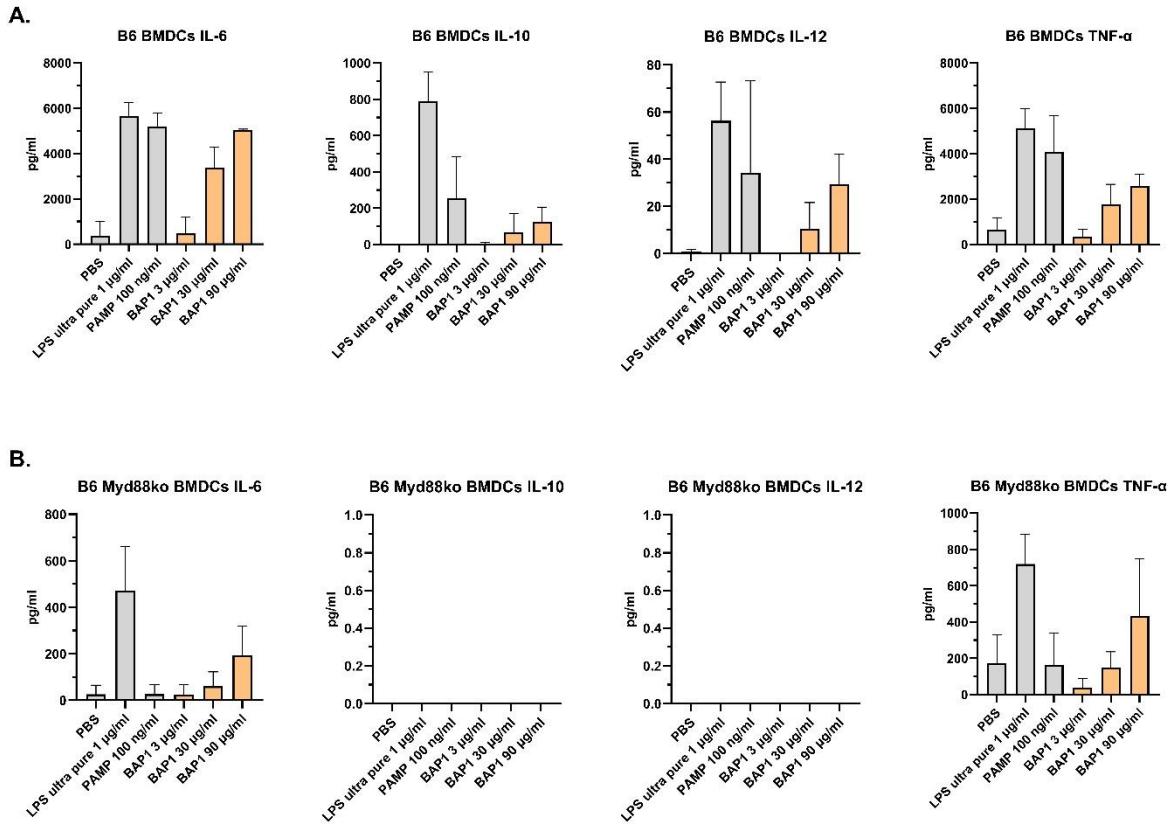
3.1. Materials and methods

BMDCs were isolated from female C57BL/6 wild-type (B6) and *Myd88* knockout (B6 *Myd88* ko) mice (8–12 weeks of age) according to the previously described method (1st manuscript, Material and Methods 2.4. Mouse cell isolation and stimulation). Before, the mice were anesthetized with 3 % isoflurane and euthanized by cervical dislocation. All experiments were performed following the EU Directive 2010/63/EU for animal experiments. Animal experiments were approved by the Committee for the Protection and Use of Experimental Animals of the Institute of Microbiology, The Czech Academy of Sciences (No. 91/2019).

For the stimulation, 0.5 x 10^6 cells were seeded on a 48-well plate and stimulated with a growing concentration of BAP1 (3 – 90 µg/ml). The cells were incubated for 20 h at 37 °C with 5 % CO_2. After, the supernatant was collected and cytokine production was determined with the use of ELISA Ready-Set-Go! kits (eBioscience) according to the manufacturer's instructions.

3.2. Results

BAP1 was able to induce dose-dependent production of IL-6, IL-10, IL-12 and TNF-α. Interestingly, stimulation of cells from knockout mice resulted in the inhibition of the BAP1 effect (Suppl. Fig. 3).



Suppl. Fig. S3. Cytokine production after BMDCs treatment with the BAP1. **S3A.** Production of IL-6, IL-10, IL-12, and TNF-α by BMDCs isolated from wild-type C57BL/6 mice. **S3B.** Production of IL-6, IL-10, IL-12, and TNF-α by BMDCs isolated from *Myd88* knockout C57BL/6 mice.

4. Stimulation of splenocytes derived from OVA-sensitized mice with surface antigens isolated from Ban218

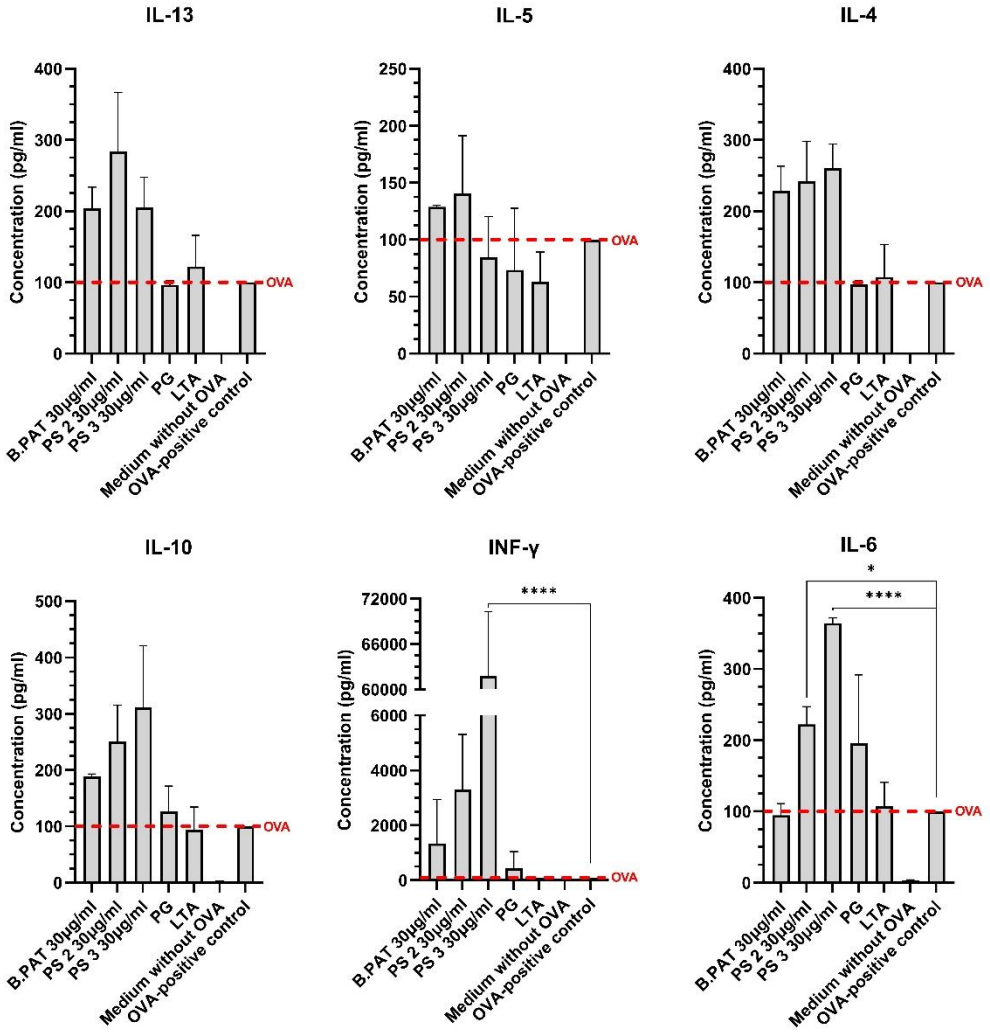
4.1. Materials and methods

The experiment was performed as described previously (1st manuscript, Materials and methods 3.4.1. Stimulation of the splenocytes isolated from OVA-sensitized mice).

4.2. Results

None of the tested surface antigens isolated from Ban218 could inhibit Th2-related cytokine response by reducing IL-13, IL-5, or IL-4 levels. Interestingly, a tendency was observed for all PSs

to increase IL-10 production. Also, PS3 significantly increased the level of INF-γ and IL-6 (Suppl. Fig. S4).



Suppl. Fig. S4. Stimulation of OVA-sensitized mice splenocytes with surface antigens isolated from Ban218. Cells were treated with 500 µg/ml of OVA and bacterial compounds at a concentration of 10 µg/ml (PG, LTA) or 30 µg/ml (B.PAT, PS 2, PS 3). Stimulation with PBS served as an OVA-positive control, while unstimulated splenocytes – as a negative one (medium without OVA). Data on the graph are shown with mean ± SD and one-way ANOVA with Dunnett's multiple comparisons was performed to compare obtained results with the OVA-positive control (100 %, red dashed line) (**** $p \leq 0.0001$, * $p \leq 0.05$).

Conclusions

The findings of this doctoral dissertation allowed us to evaluate the proposed hypothesis, which was confirmed only for the Bad368 strain. In contrast, the tested antigens from Ban218 did not exhibit notable anti-allergic properties, leading to the rejection of the hypothesis for this strain. It is possible that other Ban218 components (not tested in this study), such as surface proteins or metabolites, may contribute to its bacterial effects.

Therefore, the doctoral thesis presents the following key findings:

1. **Antigenic Properties**: The Bad368 and Ban218 strains contain antigens with immunomodulatory effects.

2. **Effectiveness of BAP1**: Among the antigens from Bad368, BAP1 demonstrated the most potent ability to restore the balance between Th1 and Th2 responses disrupted in OVA-treated splenocytes isolated from OVA-sensitized mice. Also, it induced TNF-α, IL-10, and IL-6 cytokine responses in BMDCs.

3. **BAP1 Characterization**: BAP1 is a high molecular mass PS recognized by TLR2 and TLR4 innate immune receptors. Its unique structure, previously undetermined, consists of a repeating unit of six monosaccharides, including rhamnose, glucose, and galactose.

4. **Cellular Uptake and Processing**: BAP1 is efficiently processed by cells, with a 99.9 % uptake by epithelial cells and a 71.3 % transfer to DCs.

5. **Impact of Modifications**: Dephosphorylation of B.PAT isolated from Ba218 alters its structure and immunomodulatory properties, leading to increased cytokine production in BMDCs and enhanced anti-inflammatory effects in the IL-1β inflammation model of human cell lines.

6. **Effect in a Naïve Immune System**: BAP1 did not elicit significant local or systemic responses in the naïve immune system of GF mice, indicating its neutral effect despite reduced allergy-associated CCL2 and eotaxin levels, and increased *Rorc* gene expression.

7. **Therapeutic Potential in Airway Allergy**: Intranasal administration of BAP1 to OVA-allergic mice triggered both systemic and local immune responses, resulting in reduced allergic inflammation at both the humoral and cellular levels.

8. **Mechanism of Action**: BAP1 mitigates airway allergy responses by suppressing Th2-mediated immunity in mice. Additionally, the findings highlight the potential role of *Rorc*

gene activation and the inhibition of IL-10 production and gene expression in BAP1 signaling.

9. **Future Applications**: BAP1 holds promise as a potential therapeutic agent for the treatment of airway inflammation and allergic diseases.

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06.08.2024

Declaration

I, Katarzyna Pacyga-Prus, hereby declare that my contribution to the following manuscript:

Pacyga-Prus, K., Jakubczyk, D., Sandström, C., Šrůtková, D., Pyclik, M. J., Leszczyńska, K., Ciekot, J., Razim, A., Schwarzer, M., & Górska, S. (2023). Polysaccharide BAP1 of Bifidobacterium adolescentis CCDM 368 is a biologically active molecule with immunomodulatory properties. *Carbohydrate Polymers*, *315*, 120980. https://doi.org/10.1016/j.carbpol.2023.120980

is correctly characterized in the table below.

Contributor	Description of main tasks
Katarzyna Pacyga-Prus	**Conceptualization:** throughout project implementation. **Methodology and resources:** implementation of the polysaccharide, lipoteichoic acid, and peptidoglycan isolation protocol from *Bifidobacterium* strains; purification of surface antigens. **Investigation:** HEK-Blue™ cells cultivation and stimulation; isolation and stimulation of mouse splenocytes and dendritic cells; Milliplex Cytokine/Chemokine assay for the determination of cytokine production with Luminex 2000 System and ELISA assay; determination of BAP1 structure by NMR and GLC-MS. **Formal analysis:** throughout project implementation; statistical analysis except Figures 3 and 4. **Writing**: original draft. **Visualization:** all tables and figures.
Dominika Jakubczyk	**Methodology**: implementation of the antigen uptake and transfer protocol. **Formal analysis**: throughout project implementation; statistical analysis of Figures 3 and 4. **Investigation:** antigens staining; antigens uptake by epithelial cells; antigens transfer between epithelial and dendritic cells. **Writing:** original draft.
Corine Sandström	**Investigation:** determination of the BAP1 structure by NMR. **Writing:** manuscript review and editing.
Dagmar Šrůtková	**Resources**: preparation of ovalbumin-sensitized mice. **Writing:** manuscript review and editing.
Marcelina Joanna Pyclik	**Methodology:** implementation of the peptidoglycan and polysaccharide isolation protocol from *Bifidobacterium* strains.

	Investigation: isolation and stimulation of mouse splenocytes and dendritic cells; strain selection.
Katarzyna Leszczyńska	**Methodology and resources:** implementation of the peptidoglycan isolation protocol; purification of peptidoglycan.
Jarosław Ciekot	**Investigation:** determination of BAP1 molecular size.
Agnieszka Razim	**Methodology:** implementation of the antigen uptake and transfer protocol. **Writing:** manuscript review and editing.
Martin Schwarzer	**Resources**: preparation of ovalbumin-sensitized mice. **Writing**: manuscript review and editing. **Supervision:** throughout project implementation.
Sabina Górska	**Conceptualization:** initial project proposal and throughout project implementation. **Validation**: throughout project implementation. **Writing:** manuscript review and editing. **Supervision:** throughout project implementation. **Project administration and funding acquisition:** National Science Centre of Poland project SONATA BIS 7, project number UMO 2017/26/E/NZ7/01202.

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06.08.2024

Declaration

I, Katarzyna Pacyga-Prus, hereby declare that my contribution to the following manuscript:

Pacyga-Prus, K., Jakubczyk, D., Sandström, C., Šrůtková, D., Pyclik, M. J., Leszczyńska, K., Ciekot, J., Razim, A., Schwarzer, M., & Górska, S. (2023). Polysaccharide BAP1 of Bifidobacterium adolescentis CCDM 368 is a biologically active molecule with immunomodulatory properties. *Carbohydrate Polymers*, *315*, 120980. https://doi.org/10.1016/j.carbpol.2023.120980

is correctly characterized in the table below.

Contributor	**Description of main tasks**
Katarzyna Pacyga-Prus	**Conceptualization:** throughout project implementation. **Methodology and resources:** implementation of the polysaccharide, lipoteichoic acid, and peptidoglycan isolation protocol from *Bifidobacterium* strains; purification of surface antigens. **Investigation:** HEK-Blue™ cells cultivation and stimulation; isolation and stimulation of mouse splenocytes and dendritic cells; Milliplex Cytokine/Chemokine assay for the determination of cytokine production with Luminex 2000 System and ELISA assay; determination of BAP1 structure by NMR and GLC-MS. **Formal analysis:** throughout project implementation; statistical analysis except Figures 3 and 4. **Writing**: original draft. **Visualization:** all tables and figures.
Dominika Jakubczyk	**Methodology**: implementation of the antigen uptake and transfer protocol. **Formal analysis**: throughout project implementation; statistical analysis of Figures 3 and 4. **Investigation:** antigens staining; antigens uptake by epithelial cells; antigens transfer between epithelial and dendritic cells. **Writing:** original draft.
Corine Sandström	**Investigation:** determination of the BAP1 structure by NMR. **Writing:** manuscript review and editing.
Dagmar Šrůtková	**Resources**: preparation of ovalbumin-sensitized mice. **Writing:** manuscript review and editing.
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	Investigation: isolation and stimulation of mouse splenocytes and dendritic cells; strain selection.
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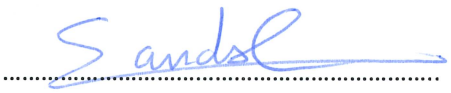
Jarosław Ciekot	**Investigation:** determination of BAP1 molecular size.
Agnieszka Razim	**Methodology:** implementation of the antigen uptake and transfer protocol. **Writing:** manuscript review and editing.
Martin Schwarzer	**Resources**: preparation of ovalbumin-sensitized mice. **Writing**: manuscript review and editing. **Supervision:** throughout project implementation.
Sabina Górska	**Conceptualization:** initial project proposal and throughout project implementation. **Validation**: throughout project implementation. **Writing:** manuscript review and editing. **Supervision:** throughout project implementation. **Project administration and funding acquisition:** National Science Centre of Poland project SONATA BIS 7, project number UMO 2017/26/E/NZ7/01202.



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06.08.2024

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Corine Sandström	**Investigation:** determination of the BAP1 structure by NMR. **Writing:** manuscript review and editing.
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Katarzyna Leszczyńska	**Methodology and resources:** implementation of the peptidoglycan isolation protocol; purification of peptidoglycan.
Jarosław Ciekot	**Investigation:** determination of BAP1 molecular size.
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Pychik M

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Katarzyna Leszczyńska

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J. Ciekot

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Agnieszka Razim .....

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Lubomir Cedula

(co-author signature)

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Contributor	**Description of main tasks**
Katarzyna Pacyga-Prus	**Conceptualization:** throughout project implementation. **Methodology and resources:** implementation of the polysaccharide isolation protocol from *Bifidobacterium* strains; purification of polysaccharides; preparation of dephosphorylated compound. **Investigation:** determination of B.PAT and B.MAT structures by NMR and GLC-MS; isolation and stimulation of mouse dendritic cells; determination of cytokine production by ELISA assay; cytotoxicity assay; UV-vis analysis; investigation of prophylactic and therapeutic effect of the tested polysaccharides on the human cell lines in the IL-1β inflammatory model. **Formal analysis:** throughout project implementation; statistical analysis of the obtained results. **Writing**: original draft. **Visualization:** all tables and figures.
Corine Sandström	**Investigation:** determination of the B.PAT and B.MAT structure by NMR. **Writing:** manuscript review and editing.
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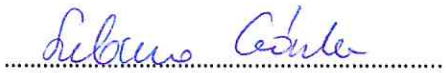

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Tereza Hornikova:	**Resources:** preparation of germ-free mice and mice with airway allergy to ovalbumin. **Investigation:** splenocytes isolation and stimulation from germ-free mice and mice with airway allergy to ovalbumin; BAL total and differential count; histopathological evaluation of lungs. **Writing:** manuscript review and editing.
Dagmar Srutkova:	**Resources:** preparation of germ-free mice and mice with airway allergy to ovalbumin. **Investigation:** cytokine/chemokine determination in germ-free mice

	performed with Luminex 2000 System. **Writing:** manuscript review and editing.
Katarzyna Leszczyńska-Nowak	**Investigation:** isolation and stimulation of lung cells isolated from mice with airway allergy to ovalbumin.
Agnieszka Zabłocka	**Writing:** manuscript review and editing.
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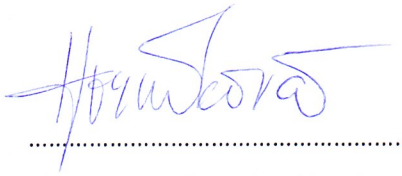

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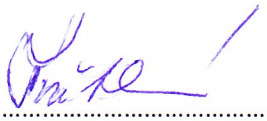
Martin Schwarzer	**Resources:** preparation of germ-free mice and mice with airway allergy to ovalbumin. **Writing:** manuscript review and editing. **Supervision:** throughout project implementation.
Sabina Górska	**Conceptualization:** initial project proposal and throughout project implementation. **Validation:** throughout project implementation. **Writing:** manuscript review and editing. **Supervision:** throughout project implementation. **Project administration and funding acquisition:** National Science Centre of Poland project SONATA BIS 7, project number UMO 2017/26/E/NZ7/01202.



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(co-author signature)

14.09.2024

Declaration

I, Katarzyna Pacyga-Prus, hereby declare that my contribution to the following manuscript (preprint): **Pacyga-Prus, K.**, Hornikova, T., Srutkova, D., Leszczyńska-Nowak, K., Zabłocka, A., Schwarzer, M., Górska, S. (2024). Polysaccharide BAP1 of Bifidobacterium adolescentis CCDM 368 attenuates ovalbumin-induced allergy through inhibition of Th2 immunity in mice. *bioRxiv*, 1 Jan 2024, 2024.09.14.613063. https://doi.org/10.1101/2024.09.14.613063.
is correctly characterized in the table below.

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Katarzyna Pacyga-Prus	**Conceptualization:** throughout project implementation. **Resources:** isolation and purification of BAP1 polysaccharide. **Investigation:** lung cells and splenocytes isolation and stimulation from germ-free mice and mice with airway allergy to ovalbumin; determination of cytokines in mice with airway allergy to ovalbumin performed with Luminex 2000 System; immunological evaluation of sera and BALF; RNA isolation from lungs; cDNA preparation, Real-Time PCR analysis of lungs isolated from germ-free mice and mice with airway allergy to ovalbumin. **Formal analysis:** throughout project implementation; statistical analysis of all the obtained results. **Writing:** original draft. **Visualization:** all tables and figures.
Tereza Hornikova:	**Resources:** preparation of germ-free mice and mice with airway allergy to ovalbumin. **Investigation:** splenocytes isolation and stimulation from germ-free mice and mice with airway allergy to ovalbumin; BAL total and differential count; histopathological evaluation of lungs. **Writing:** manuscript review and editing.
Dagmar Srutkova:	**Resources:** preparation of germ-free mice and mice with airway allergy to ovalbumin. **Investigation:** cytokine/chemokine determination in germ-free mice

	performed with Luminex 2000 System. **Writing:** manuscript review and editing.
Katarzyna Leszczyńska-Nowak	**Investigation:** isolation and stimulation of lung cells isolated from mice with airway allergy to ovalbumin.
Agnieszka Zabłocka	**Writing:** manuscript review and editing.
Martin Schwarzer	**Resources:** preparation of germ-free mice and mice with airway allergy to ovalbumin. **Writing:** manuscript review and editing. **Supervision:** throughout project implementation.
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K. Leszczyńska-Nowak

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Agnieszka Zabłocka

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(co-author signature)

Scientific experience

Education:

10/2019 – now	PhD student, Wrocław Doctoral School of Institutes of Polish Academy of Sciences
10/2017 – 07/2019	Master's degree in Genetics and Experimental Biology, University of Wrocław
10/2014 – 06/2017	Bachelor's degree in Genetics and Experimental Biology, University of Wrocław,

Participations in grants

Project head-leader

06/2022 – now	„Comprehensive research of the Bifidobacterium lipoteichoic acids - structural and functional studies extended by the scavenger receptor processing pathways.” funded by the National Science Centre of Poland (Preludium 20, UMO-2021/41/N/NZ7/03976).

Researcher

10/2023-10/2024	“Epidemiology of Clostridioides difficile infections in long-term-care facilities, analysis of disease mechanisms and immune response with the characteristics of strains and their prevalence using molecular epidemiology surveillance methods.” funded by National Science Centre of Poland (OPUS 21, UMO-2021/41/B/NZ6/00749).
06/2022 – 12/2022	"The structure and biological role of Bifidobacterium components in allergy disease development." funded by the National Science Centre of Poland (SONATA BIS 7, UMO-2017/26/E/NZ7/01202).
12/2020 – 03/2022	"Badania nad stworzeniem innowacyjnej szczepionki przeciwko wirusowi SARS-CoV-2 odpowiedzialnemu za wystąpienie COVID-19." funded by the National Centre for Research and Development (SZPITALE-JEDNOIMIENNE/28/2020).

10/2020 – 11/2021 "Innowacyjny produkt leczniczy przeciwko *Clostridioides difficile* oparty o przeciwciała IgY." funded by the National Centre for Research and Development (TANGOIV-A/0003/2019).

01/2020-01/2022 "The structure and biological role of Bifidobacterium components in allergy disease development." funded by the National Science Centre of Poland (SONATA BIS 7, UMO-2017/26/E/NZ7/01202).

11/2016 – 11/2020 „Epitope mapping of Clostridium difficile surface proteins with characteristics of protective properties of epitope conjugated with carrier protein in combination with novel nanoadjuvant.” funded by the National Science Centre of Poland (OPUS 11, 2016/21/B/NZ6/02286)

Publications:

1. **Pacyga-Prus, K.**, Hornikova, T., Srutkova, D., Leszczyńska-Nowak, K., Zabłocka, A., Schwarzer, M., Górska, S. (2024). Polysaccharide BAP1 of Bifidobacterium adolescentis CCDM 368 attenuates ovalbumin-induced allergy through inhibition of Th2 immunity in mice. *bioRxiv*, 1 Jan 2024, 2024.09.14.613063. https://doi.org/10.1101/2024.09.14.613063.
2. **Pacyga-Prus, K.**, Sandström, C., Šrůtková, D., Schwarzer, M., & Górska, S. (2024). Phosphorylation-dependent immunomodulatory properties of B.PAT polysaccharide isolated from Bifidobacterium animalis ssp. Animalis CCDM 218. Carbohydrate Polymers, 344, 122518. https://doi.org/10.1016/j.carbpol.2024.122518
3. Jakubczyk, D., Leszczyńska, K., **Pacyga-Prus, K.**, Kozakiewicz, D., Kazana-Płuszka, W., Gełej, D., Migdał, P., Kruszakin, R., Zabłocka, A., & Górska, S. (2024). What happens to Bifidobacterium adolescentis and Bifidobacterium longum ssp. Longum in an experimental environment with eukaryotic cells? BMC Microbiology, 24(1), 60. https://doi.org/10.1186/s12866-023-03179-z
4. **Pacyga-Prus, K.**, Jakubczyk, D., Sandström, C., Šrůtková, D., Pyclik, M. J., Leszczyńska, K., Ciekot, J., Razim, A., Schwarzer, M., & Górska, S. (2023). Polysaccharide BAP1 of Bifidobacterium adolescentis CCDM 368 is a biologically active molecule with immunomodulatory properties. Carbohydrate Polymers, 315, 120980. https://doi.org/10.1016/j.carbpol.2023.120980

5. Zabłocka, A., Jakubczyk, D., Leszczyńska, K., **Pacyga-Prus, K.**, Macała, J., & Górska, S. (2023). Studies of the Impact of the Bifidobacterium Species on Inducible Nitric Oxide Synthase Expression and Nitric Oxide Production in Murine Macrophages of the BMDM Cell Line. Probiotics and Antimicrobial Proteins. https://doi.org/10.1007/s12602-023-10093-3
6. Kazana, W., Jakubczyk, D., **Pacyga-Prus, K.**, Leszczyńska, K., Górska, S., Siednienko, J., Macała, J., Piechowiak, G., & Zabłocka, A. (2022). A Novel Mechanism of Macrophage Activation by the Natural Yolkin Polypeptide Complex from Egg Yolk. International Journal of Molecular Sciences, 23(6), 3125. https://doi.org/10.3390/ijms23063125
7. Pyclik, M. J., Srutkova, D., Razim, A., Hermanova, P., Svabova, T., **Pacyga, K.**, Schwarzer, M., & Górska, S. (2021). Viability Status-Dependent Effect of Bifidobacterium longum ssp. Longum CCM 7952 on Prevention of Allergic Inflammation in Mouse Model. Frontiers in Immunology, 12, 707728. https://doi.org/10.3389/fimmu.2021.707728
8. Razim, A., **Pacyga, K.**, Naporowski, P., Martynowski, D., Szuba, A., Gamian, A., & Górska, S. (2021). Identification of linear epitopes on the flagellar proteins of Clostridioides difficile. Scientific Reports, 11(1), 9940. https://doi.org/10.1038/s41598-021-89488-7
9. Razim, A., Pyclik, M., **Pacyga, K.**, Górska, S., Xu, J., Olszewski, M. A., Gamian, A., & Myc, A. (2021). Silicone Oil-Based Nanoadjuvants as Candidates for a New Formulation of Intranasal Vaccines. Vaccines, 9(3), 234. https://doi.org/10.3390/vaccines9030234
10. **Pacyga, K.**, Razim, A., Martirosian, G., Aptekorz, M., Szuba, A., Gamian, A., Myc, A., & Górska, S. (2020). The Bioinformatic and In Vitro Studies of Clostridioides Difficile Aminopeptidase M24 Revealed the Immunoreactive KKGIK Peptide. Cells, 9(5), 1146. https://doi.org/10.3390/cells9051146
11. Razim, A., **Pacyga, K.**, Martirosian, G., Szuba, A., Gamian, A., Myc, A., & Górska, S. (2019). Mapping Epitopes of a Novel Peptidoglycan Cross-Linking Enzyme Cwp22 Recognized by Human Sera Obtained from Patients with Clostridioides difficile Infection and Cord Blood. Microorganisms, 7(11), 565. https://doi.org/10.3390/microorganisms7110565
12. Razim, A., **Pacyga, K.**, Aptekorz, M., Martirosian, G., Szuba, A., Pawlak-Adamska, E., Brzychczy-Włoch, M., Myc, A., Gamian, A., & Górska, S. (2018). Epitopes identified in GAPDH from Clostridium difficile recognized as common antigens with potential autoimmunizing properties. Scientific Reports, 8(1), 13946. https://doi.org/10.1038/s41598-018-32193-9

Conferences

11-13/09/2024	International Conference 3rd Polish-Czech Probiotic Conference Presentation „BAP1 polysaccharide of *Bifidobacterium adolescentis* CCDM 368 alleviates allergy symptoms in the OVA-allergy mice model"; K. Pacyga-Prus, T. Svabova *et al.*
9-11/07/2024	International conferencje ISAPP Annual Meeting 2024 Poster presentation „Complex studies of Bifidobacterium adolescentis CCDM 368 surface polysaccharide BAP1 confirmed its structure and its immunomodulatory properties in preventing allergic reaction"; K. Pacyga-Prus, D. jakubczyk *et al.*
28-29/09/2023	International conference "Paths of Glycobiology. Jerzy Kościelak Memorial Conference" Presentation „Impact of the dephosphorylation on the function of Ba218.3 polysaccharide isolated from *Bifidobacterium animalis* ssp. *animalis* CCDM 218"; K. Pacyga-Prus, C. Sandström *et al.*
22-23/06/2023	International conference "ChemBiotIC" (Chemistry & Biotechnology International Conference) Presentation „The role of the *Bifidobacterium* polysaccharides in the treatment of allergy diseases"; K. Pacyga-Prus, C. Sandström *et al.*
1-3/07/2022	International conference "EAACI Hybrid Congress 2022" 3-minute poster presentation „Potential of the Bifidobacterium adolescentis CCDM 368 antigens to alleviate the OVA sensitization in mice"; K. Pacyga-Prus, D. Jakubczyk *et al.*
6-9/062022	International conference "Food, Microbiota and Immunity (FMI) 2022" Poster presentation "Antigens as key molecules for the immune modulation – influence of the Bifidobacterium components on the Th1/Th2 responses"; K. Pacyga-Prus, M. Pyclik, *et al.*
22-24/01/2021	International conference "EAACI Winter School Digital 2021"

	3-minute poster presentation “Effectiveness Of The *Bifidobacterium* Surface Molecules In The Treatment Of Allergy Diseases”; K. Pacyga-Prus, M. Pyclik, *et al.*
11/06/2019	Conference „7 Forum Młodych Biotechnologów” , IITD PAN
	Presentation „Mapowanie epitopu peptydazy M24 *Clostridium difficile* z wykorzystaniem ludzkich surowic odpornościowych” Pacyga K., Razim A. *et al.*
17/05/2018	Conference „Posiedzenie Wrocławskiego Forum Biologii Eksperymentalnej” (WFBE), UWr
	Presentation „Mapowanie epitopów białek powierzchniowych *Clostridium difficile* z wykorzystaniem ludzkich surowic odpornościowych” Pacyga K., Razim A. *et al.*
26-27/04/2018	International conference „COMBINAT”
	Presentation „Mapowanie epitopów białek powierzchniowych *Clostridium difficile* z wykorzystaniem ludzkich surowic odpornościowych” Pacyga K., Razim A. *et al.*

Courses

04/2024	Bioinformatic training METAVIROMICS WORKSHOP by PhD Ryan Cook, IITD PAN
02/2020	Planning, performing experiments, and killing experimental animals, PolLASA

Awards

06/2024	Travel grant for ISAPP 2024 Annual Meeting
11/2023	Awarded in a Ludwik Hirszfeld student scholarship program organized by Wrocławskie Centrum Akademickie
11/2022	Distinction in a Ludwik Hirszfeld student scholarship program organized by Wrocławskie Centrum Akademickie

June 2022	Awarded at Food, Microbiota and Immunity (FMI) 2022 conference for the best abstract (financed by EFIS-EJI)
10/2018 – 06/2019	Scholarship from the Rector of the University of Wrocław for the best students due to scientific achievements
10/2016 – 06/2017	Scholarship from the Rector of the University of Wrocław for the best students due to high academic results
10/2017 – 06/2018	Scholarship from the Rector of the University of Wrocław for the best students due to high academic results

Internships

05/2023 and 03/2020	Swedish University of Agriculture Sciences, Department of Molecular Sciences
11/2021 and 11/2019	Institute of Microbiology, Czech Academy of Sciences, Laboratory of Gnotobiology
10/2016 – 06/2019	Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Laboratory of Medical Microbiology
12/2015 – 01/2016	Institute of Genetics and Microbiology, Faculty of Biological Sciences, University of Wrocław

Scientific collaborations

1. Medical Joanna Chorbińska (Klinika Urologii i Onkologii Urologicznej, Wrocław, Poland)
   Determining the microbiome composition in patients with kidney stones and bladder cancer.
2. Laboratory of Gnotobiology (Czech Academy of Sciences, Novy Hradek, Czech Republic)
   Determination of the structure and biological role of *Bifidobacterium* components in allergy disease development.
3. Prof. Corine Sandstrom (Department of Molecular Sciences, Uppsala, Sweden)
   Structure determination of the *Bifidobacterium* polysaccharides
4. The Kennedy Institute of Rheumatology (Oxford University, Oxford, England)
   Purification and structure determination of *Helicobacter hepaticus* polysaccharides

5. Prof. Jiri Hrdy (Charles University, Prague, Czech Republic)
   Determination of the anti-allergic properties of antigens isolated from the *Bifidobacterium adolescentis* CCDM 368 in patients allergic to birch pollen.
6. Department of Experimental Biology (Masaryk University, Brno, Czech Republic) Isolation of lipoteichoic acids from vesicles produced by *Enterococcus* and *Lactobacillus* strains.
7. 4th Military Clinical Hospital in Wroclaw (Wrocław, Poland)
   Determination of the impact of pathogenic and probiotic bacteria on patients with sinusitis.

Science Promoting Events

09/2024	Co-organizer of 3rd Polish-Czech probiotic Conference
10/2023	Workshop preparation and conducting as part of the Stacja IITD project
09/2022	Lecture co-host at XXV Lower Silesian Science Festival „Co w brzuchu burczy?" – czyli skąd się biorą alergie pokarmowe i choroby jelit.
09/2019 and 09/2020	Lecture co-host at XXII and XXI Lower Silesian Science Festival „Kto chce zjeść ciastko? – niezwykły świat naszych mikrobów"
01/2017	Workshops co-leader on DNA isolation from plant material during the Lower Silesian edition of the Biologist's Night in Wrocław