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Wpływ pyłu zawieszonego w powietrzu na nabłonek oddechowy w interakcjach pomiędzy nabłonkiem, komórkami dendrytycznymi i makrofagami w obturacyjnych chorobach płuc

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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1. Wykaz stosowanych skrótów

skrót	*pełna nazwa w języku angielskim* pełna nazwa w języku polskim
ACE2	*angiotensin-converting enzyme 2* enzym konwertujący angiotensynę typu 2
ACO	*asthma-COPD overlap* nakładanie astmy i POChP
AHR	*aryl hydrocarbon receptor* receptor węglowodorów aromatycznych
AHRR	*aryl hydrocarbon receptor repressor* represor receptora węglowodorów aromatycznych
ALI	*air-liquid interface* hodowla komórek nabłonka na styku fazy ciekłej i powietrza
BPIFA2	*BPI fold-containing family A member 2* białko wydzielnicze ślinianek przyusznych
CRP	*C-reactive protein* białko C reaktywne
COPD **POChP**	*chronic obstructive pulmonary disease* przewlekła obturacyjna choroba płuc
CYP1B1	*cytochrome P450 1B1* cytochrom P450 1B1
CYP1B1-AS1	*cytochrome P450 B1 antisense RNA 1* cytochrom P450 1B1 antysensowny RNA1
DEP	*diesel exhaust particles* cząstki spalin z silników diesla
EGF	*epithelial growth factor* naskórkowy (nabłonkowy) czynnik wzrostu
EGFR	*epithelial growth factor receptor* receptor dla naskórkowego (nabłonkowego) czynnika wzrostu
ENPEP	*glutamyl aminopeptidase* aminopeptydaza glutamylowa
GATA-3	*GATA binding protein 3* białko 3 wiążące GATA
GBD	*Global Burden of Disease* ogólnoświatowe obciążenie chorobami
GINA	*Global Initiative for Asthma*
GM-CSF	*granulocyte-macrophage colony-stimulating factor* czynnik stymulujący tworzenie kolonii granulocytów i makrofagów
GOLD	*Global Initiative for Chronic Obstructive Lung Disease*
GST	*glutathione S-transferase* S-transferazy glutationu
HLA	*human leukocyte antigen* ludzki antygen leukocytarny
ICAM-1	*intercellular adhesion molecule 1* międzykomórkowa molekuła adhezyjna 1
ILC-2	*innate lymphoid cells 2* nieswoiste komórki limfoidalne 2
INF-γ	*interferon gamma* interferon gamma

LPS	*lipopolisacharides* lipopolisacharydy
MIP-1β	*macrophage inflammatory protein 1 beta* białko zapalne makrofagów 1 beta
MMP	*matrix metallopeptidase* metaloproteinaza
moDC	*monocyte derived dendritic cell* komórka dendrytyczna wyspecjalizowana z monocytu krwi obwodowej
moMφ	*monocyte derived macrophage* makrofag wyspecjalizowany z monocytu krwi obwodowej
MUC5AC	*mucin 5AC* mucyna 5AC
NF-κB	*nuclear factor kappa-light-chain-enhancer of activated B cells* jądrowy czynnik transkrypcyjny NF kappa B
PAMP	*pathogen-associated molecular patterns* wzorce molekularne związane z patogenami
PD-L	*programmed cell death ligand* ligand programowanej śmierci komórki
PLUNC	*palate, lung and nasal epithelium clone proteins* białka klonu podniebienia, płuc i nabłonka
PM	*particulate matter* pył zawieszony
PRR	*pathogen recognition receptors* receptory rozpoznające patogeny
RAGE	*receptor for advanced glycation endproducts* receptor końcowych produktów zaawansowanej glikacji
RANTES	*regulated on activation, normal T-cell expressed and secreted*
RORα	*RAR-related orphan receptor alpha* RAR-powiązany sierocy receptor alfa
ROS	*reactive oxygen species* reaktywne formy tlenu
TEER	*transepithelial electrical resistance* przeznabłonkowy opór elektryczny
TGF-β	*transforming growth factor beta* transformujący czynnik wzrostu beta
Th2	*T helper cell 2* limfocyt pomocniczy typu 2
TIPARP	*TCDD Inducible Poly (ADP-Ribose) Polymerase* polimeraza poli [ADP-ryboza] indukowana przez TCDD
TLR	*toll-like receptor* receptor toll-podobny
TNF-α	*tumor necrosis factor alpha* czynnik martwicy nowotworów alfa
TSLP	*thymic stromal lymphopoietin* limfopoetyna zrębu grasicy
UFP	*ultra fine particles* pył ultradrobny
UPM	*urban particulate matter* pył zawieszony w obszarze miejskim
WHO	*World Health Organisation,* Światowa Organizacja Zdrowia

2. Streszczenie w j. polskim

Zanieczyszczenie środowiska, takie jak podwyższone stężenie pyłu zawieszonego w powietrzu, jest ważnym elementem wpływającym na zdrowie publiczne. Fizjologiczna organizacja nabłonka odgrywa kluczową rolę w tworzeniu bariery dróg oddechowych. Interakcje komórek strukturalnych z komórkami immunologicznymi rezydującymi w drogach oddechowych (makrofagami i komórkami dendrytycznymi), inicjują miejscową odpowiedź immunologiczną. Astma i przewlekła obturacyjna choroba płuc (POChP) są jednymi z najczęściej występujących, przewlekłych chorób układu oddechowego, które charakteryzują się zwiększonym napływem komórek układu immunologicznego do płuc w toku przebiegającej reakcji zapalnej. W konsekwencji, proces ten przyczynia się do podwyższonego uwalniania mediatorów w miejscu zapalenia i może prowadzić do uruchomienia lokalnych mechanizmów naprawczych, przebudowy nabłonka, osłabienia jego funkcji, a także zwiększonej produkcji śluzowej wydzieliny. Przewlekły stan zapalny w drogach oddechowych wpływa na powstanie objawów choroby m.in. duszność (spoczynkową lub wysiłkową), kaszel czy obniżoną czynność płuc.

Pył o średnicy aerodynamicznej cząstek do 10 µm, (PM_{10}) stanowi mieszaninę cząstek frakcji $PM_{2,5}$, o średnicy aerodynamicznej do 2,5 µm, oraz dodatkowych, większych i bardziej zróżnicowanych składników. W związku z tym charakteryzuje się odmiennym od wyizolowanej frakcji $PM_{2,5}$ składem chemicznym i działaniem toksycznym. Do najważniejszych mechanizmów negatywnego oddziaływania pyłu PM_{10} na nabłonek dróg oddechowych zaliczane są: modulacja odporności wrodzonej, odpowiedzi zapalnej oraz indukcja stresu oksydacyjnego, których przebieg i natężenie zależne są od składu chemicznego pyłu z zanieczyszczenia powietrza atmosferycznego. Ekspozycja na zanieczyszczenia powietrza, w tym PM_{10}, wykazuje negatywny miejscowy wpływ na nabłonek oddechowy, moduluje odziaływania międzykomórkowe i powoduje osłabienie zdolności obronnych organizmu, a to z kolei może prowadzić do zaostrzeń i progresji obturacyjnych chorób układu oddechowego.

Badania przeprowadzone w ramach niniejszej pracy polegały na określeniu zmian zachodzących w nabłonku z jamy nosa w odpowiedzi na stymulację pyłem PM_{10} z obszarów miejskich. Analizę przeprowadzono na poziomie molekularnym, biochemicznym i strukturalnym, a wykorzystane komórki nabłonka zostały pobrane od chorych na astmę, POChP oraz osób zdrowych. Wyróżniono następujące cele pracy

(1) przegląd światowej literatury charakteryzującej wpływ pyłu PM_{10} na fizjologię nabłonka dróg oddechowych w astmie i POChP, (2) analiza i porównanie wpływu ekspozycji na pył PM_{10} na odpowiedź zapalną nabłonka z jamy nosa od chorych na astmę lub POChP i osób zdrowych z wykorzystaniem mono- i wielowarstwego modelu *in vitro*, (3) określenie integralności nabłonka dróg oddechowych oraz ekspresji markerów remodelingu w odpowiedzi na stymulację PM_{10} w nabłonku osób zdrowych, chorych na astmę lub POChP, (4) analiza transkryptomiczna odpowiedzi nabłonka z jamy nosowej od chorych na obturacyjne choroby płuc i osób zdrowych, współhodowanego z makrofagami i komórkami dendrytycznymi po ekspozycji na PM_{10}.

Metodyka badania obejmowała rekrutację chorych na astmę, POChP oraz zdrowych ochotników, izolację komórek nabłonka jamy nosa pozyskanego z wymazu szczoteczkowego błony śluzowej nosa oraz izolację i specjalizację makrofagów oraz komórek dendrytycznych z monocytów krwi obwodowej. W badaniach wykorzystano hodowle wielokomórkowe w których komórki od dawcy (makrofagi, nabłonek, komórki dendrytyczne) stanowiły spójny, indywidualny model doświadczalny. Hodowle poddawano 24-godzinnej ekspozycji na PM_{10} o stężeniu 100 µg/ml. Komórki nabłonka hodowano na granicy faz powietrze-ciecz (*air-liquid interface*, ALI) w wyniku czego uzyskano hodowlę składającą się z wielu typów wyspecjalizowanych komórek nabłonkowych, w tym komórek podstawnych, urzęsionych i wydzielniczych. Komórki dendrytyczne oraz makrofagi uzyskane z monocytów krwi obwodowej (*monocyte derived dendritic cells,* moDC; *monocyte derived macrophages*, moMφ) stanowiły odpowiednik komórek immunologicznych napływających do dróg oddechowych. Zastosowany model badawczy pozwolił na określenie wpływu frakcji PM_{10} na nabłonek dróg oddechowych, z uwzględnieniem wpływu prostych interakcji międzykomórkowych, które modulują tkankową odpowiedź immunologiczną. Dodatkowo, wykorzystanie wielkoskalowej analizy, tj. badania transkryptomu, pozwoliło na kompleksową ocenę różnorodności mechanizmów odpowiedzialnych za procesy patofizjologiczne po ekspozycji na zanieczyszczenia powietrza w obturacyjnych chorobach płuc. Wyniki przeprowadzonych doświadczeń oraz analiz zostały przedstawione w formie opublikowanych artykułów stanowiących poszczególne rozdziały pracy doktorskiej.

Przegląd literatury światowej pozwolił dokonać szerokiej analizy opublikowanych wyników badań dotyczących negatywnego działania frakcji PM_{10} i jej istotnego wpływu na powstawanie choroby oraz zaostrzeń objawów u chorych na astmę i POChP. Najistotniejsze wyniki badań własnych wskazały, że nabłonek dróg oddechowych osób

z obturacyjnymi chorobami płuc, a w szczególności chorych na POChP, charakteryzuje się najsilniejszą odpowiedzią prozapalną po ekspozycji na PM_{10}. Stymulacja PM_{10} zwiększała ekspresję IL-6 i IL-8 w nabłonku współhodowanym z komórkami dendrytycznymi i makrofagami w porównaniu do hodowli mononabłonkowych, co wskazuje na istotną rolę komórek napływowych w miejscowej odpowiedzi zapalnej dróg oddechowych. 24-godzinna ekspozycja na pył PM_{10} nie spowodowała zmian integralności połączeń międzykomórkowych w nabłonku we wszystkich analizowanych grupach, jednak wpłynęła na ekspresję EGFR i ST2 na nabłonku astmatycznym. Wyniki analizy transkryptomicznej nabłonka dróg oddechowych wskazują na odmienny niż u osób zdrowych charakter miejscowej odpowiedzi biologicznej w chorobach obturacyjnych po ekspozycji na miejski pył zawieszony. W zastosowanym przez nas modelu *in vitro* stymulacja pyłem PM_{10} u osób z chorobami obturacyjnymi wzmagała ekspresję mRNA genów odpowiedzialnych za odpowiedź immunologiczną, w szczególności napływ leukocytów, dodatkowo w astmie zwiększała ekspresję mRNA genów związanych z procesami przebudowy komórek strukturalnych, natomiast w POChP modulowała odpowiedź na stres oksydacyjny. U osób zdrowych, ekspozycja na PM_{10} aktywowała ścieżki związane z procesami regeneracyjnymi w komórkach nabłonkowych.

3. Streszczenie w j. angielskim

The impact of airborne particulate matter on respiratory epithelium in the interactions between epithelium, dendritic cells and macrophages in obstructive lung diseases

Environmental pollution, such as elevated concentrations of airborne particulate matter (PM), is an important element affecting public health. The physiological organisation of the epithelium plays a key role in the formation of the airway barrier. Interactions of structural cells with immune cells residing in the airways (macrophages and dendritic cells), initiate a local immune response. Asthma and chronic obstructive pulmonary disease (COPD) are among the most common chronic respiratory diseases, which are characterised by an increased influx of immune cells into the lungs during inflammatory response. In consequence, this process leads to an increased release of mediators at the site of inflammation, activation of local repair mechanisms, epithelial remodelling, epithelial function impairment and increased mucous secretion. Chronic inflammation in the airways contributes to the symptoms of the disease, including dyspnoea (at rest or during exercise), coughing or reduced lung function.

Particulate matter with an aerodynamic diameter up to 10 µm, (PM_{10}) is a mixture of particles of the $PM_{2.5}$ fraction, with an aerodynamic diameter up to 2.5 µm, and additional, larger, and more diverse components. Therefore, it is characterised by a different chemical composition and toxic effects from the isolated $PM_{2.5}$ fraction. The most important mechanisms of PM_{10} negative action on the airway epithelium include modulation of innate immunity, the inflammatory response and induction of oxidative stress, which course and intensity depends on the chemical composition of the ambient air pollution. Exposure to air pollutants, including PM_{10}, shows negative local effects on the respiratory epithelium, modulates intercellular interactions and impairs the organism's defence capabilities, which in turn can lead to exacerbations and progression of obstructive lung diseases.

The research conducted in the present study was aimed to determine the changes occurring in the nasal epithelium in response to stimulation by PM_{10} collected from urban areas. The analysis was carried out at the molecular, biochemical and structural level, the epithelial cells used in the study were collected from asthma patients, COPD patients and healthy individuals. The following objectives of the study were formed (1) a review of the world literature characterizing the effects of PM_{10} on airway epithelial physiology

in asthma and COPD, (2) analysis and comparison of the effects of PM_{10} exposure on the inflammatory response of nasal epithelium from patients with asthma or COPD and healthy subjects using a mono- and co-culture *in vitro* model, (3) determination of airway epithelial integrity and expression of remodelling markers in response to PM_{10} stimulation in the epithelium of healthy subjects, asthma or COPD patients, (4) transcriptomic analysis of the response of nasal epithelium from patients with obstructive lung diseases and healthy subjects co-cultured with macrophages and dendritic cells after exposure to PM_{10}.

The methodology of the study included the recruitment of asthma, COPD patients and healthy volunteers, isolation of nasal epithelial cells obtained from nasal mucosa brushing, isolation and specialisation of macrophages and dendritic cells from peripheral blood monocytes. The study used co-cultures in which cells from the donor (macrophages, epithelium, dendritic cells) consisted as an individual experimental model. Cultures were exposed for 24 hours to PM_{10} at a concentration of 100 µg/ml. Epithelial cells cultured at the air-liquid interface (ALI) consisted of multiple types of specialised epithelial cells, including basal, ciliated, and secretory cells. Dendritic cells (moDC) and macrophages (moMφ) derived from peripheral blood monocytes were used as a model of immune cells recruited into the airways. The research model used in experiments assessed the effects of PM_{10} on the airway epithelium, taking into account the influence of simple intercellular interactions that modulate the tissue immune response. In addition, the use of large-scale methods of analysis, i.e., transcriptome analysis, estimated a comprehensive assessment of the diverse mechanisms responsible for pathophysiological processes following exposure to air pollutants in obstructive lung diseases. The results of the experiments and analyses are presented in the form of published articles that constitute the individual chapters of the dissertation.

A review of the world literature allowed for a broad analysis of published research findings on the negative effects of PM_{10} fraction and its significant impact on disease onset and symptom exacerbations among patients with asthma and COPD. The most relevant results of our own research indicated that the airway epithelium of people with obstructive lung disease, COPD patients in particular, has the strongest pro-inflammatory response after exposure to PM_{10}. Stimulation with PM_{10} increased IL-6 and IL-8 expression in epithelium co-cultured with dendritic cells and macrophages compared to epithelial monocultures, indicating an important role for recruited cells in the local airway inflammatory response. 24h exposure to PM_{10} did not alter

the integrity of intercellular junctions in the epithelium in all analysed groups but affected EGFR and ST2 expression on the asthmatic epithelium. The results of transcriptomic analysis of airway epithelium showed the distinct nature of the local biological response in obstructive lung diseases after exposure to urban particulate matter compared to healthy subjects. In the applied *in vitro* model, PM_{10} stimulation among subjects with obstructive lung diseases increased mRNA expression of genes responsible for the immune response, particularly leukocyte influx, additionally, in asthma, it increased mRNA expression of genes related to structural cells remodelling processes, while in COPD it modulated the oxidative stress response. In healthy subjects, exposure to PM_{10} activated pathways associated with regenerative processes in epithelial cells.

4. Wstęp

4.1 Charakterystyka obturacyjnych chorób płuc

Astma i przewlekła obturacyjna choroba płuc (POChP) zaliczane są do najczęściej występujących przewlekłych chorób układu oddechowego. Światowa Organizacja Zdrowia (*World Health Organisation,* WHO) szacuje, że na całym świecie w 2019 roku na astmę chorowały 262 miliony ludzi a zmarło na nią 461 tysięcy osób[1]. Według ogólnoświatowego badania obciążenia chorobami (*Global Burden of Disease*, GBD) w tym samym roku na całym świecie odnotowano 212,3 miliona przypadków POChP[2]. Szacunkowe dane z 2021 roku wskazują, że POChP była czwartą najczęściej występującą przyczyną śmierci na całym świecie i przyczyniła się do ponad 3,6 miliona zgonów[3]. W związku z wydłużeniem się średniej długości życia, wzrastającą urbanizacją oraz zanieczyszczeniem powietrza prognozuje się, że do 2050 roku zapadalność na POChP w Europie wzrośnie o 39,6%[4]. Diagnostyka i rozpoznanie astmy i POChP opiera się na wytycznych publikowanych i aktualizowanych odpowiednio w raportach GINA (*Global Initiative for Asthma*)[5] i GOLD *(Global Initiative for Chronic Obstructive Lung Disease*)[6]. Cechą wspólną astmy i POChP jest zwężenie światła oskrzeli (obturacja), prowadzące do ograniczenia przepływu powietrza przez drogi oddechowe. W przebiegu obydwu chorób obserwuje się jednak odmienny przewlekły proces zapalny dróg oddechowych, a kliniczna manifestacja obejmuje m.in. świszczący oddech, duszność (spoczynkową lub wysiłkową) oraz kaszel. Wyniki licznych badań naukowych wskazały zespół czynników genetycznych predysponujących do zachorowania na astmę lub POChP. Szczególnie dobrze zostało poznane zróżnicowane podłoże genetyczne astmy[7]. Badania asocjacyjne genomu z zastosowaniem próbek pochodzących od osób z różnych ras i grup etnicznych wykazały, że wariant HLA-DQB1 (*human leukocyte antygen,* HLA) może być czynnikiem predykcyjnym podwyższonego poziomu całkowitego IgE w surowicy[8]. Z kolei w badaniach nad genetycznymi czynnikami wpływającymi na powstawanie łagodnej lub umiarkowanej postaci astmy wskazano udział kilku innych genów kodujących m.in nabłonkowe alarminy (IL-33 i *thymic stromal lymphopoietin,* TSLP), cytokiny produkowane w czasie zapalenia typu Th2 (IL-5 i IL-13) oraz czynniki transkrypcyjne (GATA-3 i RORα) oddziałujące na różnicowanie limfocytów pomocniczych typu 2 (*type 2 T helper cells*, Th2) oraz rozwój nieswoistych komórek limfoidalnych 2 (*innate lymphoid cells 2,* ILC2) zaangażowanych w odpowiedź immunologiczną typu 2[9]. W przypadku bardziej

zaawansowanych stadiów choroby oprócz wymienionych wyżej genów wykazano związek ekspresji genu *MUC5AC*, kodującego mucyny, z rozwojem ciężkiej postaci astmy[10]. W przypadku POChP najlepiej udokumentowanym czynnikiem genetycznym predysponującym do rozwoju tej choroby jest ciężki dziedziczny niedobór α-1-antytrypsyny warunkowany mutacją genu *SERPINA1*[11]. Z ryzykiem rozwoju POChP powiązano także geny kodujące metaloproteinazę 12 (*matrix metallopeptidase,* MMP12) oraz S-transferazy glutationu (*glutathione S-transferase,* GST)[12]. Należy jednak pamiętać, że równie ważną rolę w patogenezie tych chorób odgrywa ekspozycja na czynniki środowiskowe takie jak alergeny, dym papierosowy oraz inne szkodliwe cząstki zawieszone w powietrzu.

Ogólna definicja astmy podaje, że jest to choroba heterogenna, która zazwyczaj charakteryzuje się przewlekłym stanem zapalnym w drogach oddechowych. Diagnozowana jest na podstawie objawów ze strony tego układu, które mogą zmieniać się w czasie i nasileniu. Występujące ograniczenie wydechowego przepływu powietrza jest zmienne i często odwracalne[5]. Spośród fenotypów astmy najczęściej diagnozowana jest astma alergiczna. Pierwsze jej objawy pojawiają się często w wieku dziecięcym w powiązaniu z wywiadem chorób alergicznych u danego pacjenta lub w jego najbliższej rodzinie. Kluczowym elementem reakcji alergicznej stanowiącej przyczynę objawów chorobowych u astmatyków jest odpowiedź limfocytów Th2 na alergen wyrażona m.in. produkcją chemokin i cytokin takich jak IL-4, IL-13 and IL-5, co w konsekwencji prowadzi do napływu komórek zapalnych (głównie eozynofilów, komórek tucznych i bazofilów) do dróg oddechowych[13]. Wcześniejsze opracowania wskazywały na istotną rolę atopii, czyli genetycznie uwarunkowanego IgE-zależnego zespołu zaburzeń układu odpornościowego wynikającego z reakcji organizmu na dany alergen, w powstawaniu tego typu astmy. Nowsze wytyczne traktują jednak atopię jako wskaźnik pomocniczy, a decydującą rolę pełni wywiad z osobą chorą, potwierdzający pojawienie się objawów astmy po kontakcie z konkretnym alergenem[5]. Oprócz astmy alergicznej wyróżnia się także a) astmę niealergiczną, b) astmę o późnym początku – zaliczaną często do astmy niealergicznej, pojawiającą się szczególnie często wśród dorosłych kobiet, c) astmę z utrwaloną obturacją, w której na skutek długotrwałego procesu zapalnego nastąpiła przebudowa dróg oddechowych, oraz d) astmę współistniejącą z otyłością, charakteryzującą się silnymi objawami przy niewielkim stanie zapalnym dróg oddechowych. Profil komórek zapalnych napływających do dróg oddechowych w przebiegu w/w fenotypów astmy może być zróżnicowany[5], a wraz z progresją choroby

oraz po ekspozycji na szkodliwe czynniki m.in pyły zawieszone w powietrzu, typ reakcji immunologicznej w astmie może ulegać dalszym zmianom[14].

W przebiegu POChP obturacja oskrzeli jest postępująca i tylko w niektórych przypadkach częściowo odwracalna. U pacjentów występować może nadprodukcja śluzu w drogach oddechowych związana ze zwiększonym odsetkiem komórek wydzielniczych oraz z powiększeniem gruczołów śluzowych jako konsekwencją ekspozycji na szkodliwe cząstki stałe oraz przewlekłe zapalenie[6]. Toczący się proces zapalny może przyczyniać się do zwężenia średnicy oskrzelików i uszkodzenia miąższu płucnego w postaci powiększenia przestrzeni powietrznych przez zapadanie się i niszczenie pęcherzyków płucnych[15]. Najczęściej spotykanymi komórkami efektorowymi reakcji zapalnej są makrofagi, neutrofile, oraz subpopulacje limfocytów pomocniczych Th1 i Th17[16]. W niektórych przypadkach POChP obserwuje się także zwiększony napływ eozynofilów do dróg oddechowych[17]. W zależności od przyjętych kryteriów podziału u 13-61% pacjentów z astmą oraz u 12-55% pacjentów z POChP, obrazy kliniczne obu jednostek chorobowych mogą współistnieć ze sobą[18].

Wzmożony napływ komórek układu immunologicznego do płuc związany z toczącą się reakcją zapalną w obturacyjnych chorobach układu oddechowego, przyczynia się do podwyższonego, miejscowego uwalniania mediatorów zapalenia. Substancje te działają szkodliwie na komórki nabłonka dróg oddechowych, co w konsekwencji prowadzi do uruchomienia komórkowych mechanizmów naprawczych, ich morfologicznej przebudowy (remodelingu), osłabienia ich funkcji, często także nadprodukcji śluzowej wydzieliny.

4.2 Charakterystyka cząstek pyłu zawieszonego w powietrzu atmosferycznym

Zanieczyszczenia w powietrzu atmosferycznym mogą pochodzić ze źródeł naturalnych, jednak dominująca ich część pochodzi ze źródeł antropogenicznych (uwalnianie związane z ruchem ulicznym, budownictwem, przemysłem, rolnictwem, indywidualnym systemem ogrzewania domów). Rozdrobnione pod wpływem działania warunków atmosferycznych lub działań fizyczno-chemicznych cząstki stałe przechodzą do atmosfery (*resuspended dust*), gdzie mogą być transportowane z wiatrem na znaczne odległości i tworzą tzw. pył zawieszony w obszarze miejskim (*urban particulate matter*, UPM). Dodatkowym czynnikiem wpływającym na zmienność cząstek zawieszonych w powietrzu są chwilowe oraz sezonowe zmiany warunków atmosferycznych. Skala niekorzystnych skutków zdrowotnych związanych z ekspozycją na pył zawieszony

w powietrzu zależy od charakteru wchodzących w jego skład cząstek i jest bezpośrednio związana z ich średnicą. Wiele cząstek zawieszonych w powietrzu nie jest idealnie kulistych, dlatego też przy ich charakterystyce stosuje się określenie średnicy aerodynamicznej. Najczęściej spotykaną klasyfikację cząstek stanowi podział na pył PM_{10} i $PM_{2,5}$. W skład pyłu PM_{10} wchodzą cząstki o średnicy aerodynamicznej co najwyżej 10 μm, natomiast pył drobnocząsteczkowy, tzw. $PM_{2,5}$ zawiera cząstki o średnicy aerodynamicznej do 2,5 μm[19]. Bardziej szczegółowa klasyfikacja obejmuje dodatkowo tzw. pył gruby składający się z cząstek o średnicach aerodynamicznych pomiędzy 2,5 a 10 μm (*coarse particulate matter,* $PM_{2,5-10}$) będący częścią frakcji PM_{10}, pył drobny złożony z cząstek o średnicach aerodynamicznych pomiędzy 0,1 a 2,5 μm (*fine particulate matter,* $PM_{0,1-2,5}$) oraz pył ultradrobny o średnicach aerodynamicznych cząstek poniżej 0,1 μm ($PM_{0,1}$, *ultrafine particulate matter,* UFP) wchodzące w skład $PM_{2,5}$. Pył PM_{10} może penetrować do dolnych części dróg oddechowych, jednak w większości jest zatrzymywany w warstwie śluzu i usuwany z dróg oddechowych dzięki kliresowi rzęskowo-śluzowemu. Pył $PM_{2,5}$ jest transportowany w dół dróg oddechowych aż do miąższu płucnego i jest pochłaniany przez komórki nabłonka jak również przez rezydujące tam makrofagi[20]. Pył $PM_{0,1}$ może przechodzić do krążenia ustrojowego i umiejscawiać się również w innych niż płuca narządach.

4.3 Wpływ zanieczyszczenia powietrza na występowanie oraz przebieg obturacyjnych chorób płuc

Zanieczyszczenia powietrza atmosferycznego stanowią jedno z głównych zagrożeń środowiskowych dla zdrowia ludzi na całym świecie i przyczyniają się do 4,2 miliona zgonów rocznie[21]. Jakość powietrza w Polsce na tle innych europejskich państw jest niska i nie spełnia rekomendowanego przez WHO poziomu, który od 2021 roku w ujęciu rocznym wynosi 5 μg/m^3 dla $PM_{2,5}$ oraz 15 μg/m^3 dla PM_{10}[22]. Ekspozycja na zanieczyszczenia powietrza może przyczyniać się do rozwoju oraz zaostrzeń wielu chorób przewlekłych układu oddechowego, krążenia jak i nowotworów, co stanowi nie tylko rozległy problem medyczny, ale także wyzwanie ekonomiczne. Pomimo zauważalnego, korzystnego trendu ostatnich trzech dekad związanego z obniżeniem (o minimum 25%) stężenia $PM_{2,5}$ w powietrzu atmosferycznym w większości krajów w Unii Europejskiej szacuje się, że związane z nimi koszty zdrowotne (*health related costs*) sięgną w 2025 roku 224-749 miliadrów euro[23,24].

Wykazano istotny związek pomiędzy podwyższonym poziomem zanieczyszczenia powietrza, a wzrostem ryzyka zachorowalności i umieralności zwłaszcza wśród osób z obturacyjnymi chorobami płuc[25]. Przewlekłe narażenie na pył z zanieczyszczenia powietrza atmosferycznego wpływa na nadprodukcję gęstego śluzu i skurcze mięśni gładkich oskrzeli, co prowadzi do wystąpienia objawów astmy[26]. W przypadku chorych na astmę, ekspozycja na $PM_{2,5}$, przyczynia się do istotnego wzrostu liczby zaostrzeń tej choroby wymagających większego wykorzystania leków wziewnych oraz hospitalizacji[27]. W POChP, gdzie patogeneza choroby związana jest z przewlekłym stresem oksydacyjnym i zaburzonymi mechanizmami obrony antyoksydacyjnej[28], ekspozycja na pyły zawieszone w powietrzu zwiększa poziom stresu oksydacyjnego prowadzącego do uszkodzenia DNA komórek oraz stymuluje wydzielanie MMP-9 i IL-8 w związku z toczącym się zapaleniem o charakterze neutrofilowym[29]. Co ważne, wykazano, że palacze tytoniu są w większym stopniu narażeni na szkodliwe działanie pyłu zawieszonego w powietrzu w porównaniu z osobami niepalącymi[30]. Długotrwałe narażenie na szkodliwe cząstki, w tym $PM_{2,5}$, prowadzi u tych chorych do wzmożonego osłabienia czynności płuc i zmian rozedmowych znacznie obniżających jakość życia[31]. Wyniki metaanalizy dotyczącej oceny związku pomiędzy stężeniem PM_{10} w powietrzu atmosferycznym a częstością hospitalizacji i umieralnością wśród pacjentów z POChP wykazały 2,7% wzrost częstości hospitalizacji oraz 1,1% zwiększenie śmiertelności na każde 10 $\mu g/m^3$ PM_{10}. Jako szczególnie narażone na wpływ PM_{10} grupy osób wskazano mieszkańców krajów rozwijających się oraz osoby starsze[32].

4.4 Wpływ ekspozycji na zanieczyszczenia powietrza na nabłonek dróg oddechowych

Pierwszym miejscem kontaktu organizmu z wdychanym wraz z powietrzem materiałem, w tym cząstkami stałymi, alergenami czy patogenami, jest nabłonek dróg oddechowych. Wykazuje on szereg mechanizmów funkcjonalnych przyczyniających się do ochrony dróg oddechowych przed toksycznym działaniem tych cząstek m.in. zatrzymuje je w warstwie śluzu i wyprowadza na zewnątrz dzięki transportowi śluzowo-rzęskowemu, utrudnia ich penetrację w głąb tkanek dzięki obecności ścisłych i zwierających połączeń między komórkami (*tight junctions* i *adherens junctions*) a także inicjuje lokalną odpowiedź zapalną. Ponadto, do bariery dróg oddechowych oddzielającej środowisko zewnętrzne od wnętrza organizmu zaliczane są także odpowiedzialne za fagocytozę makrofagi obecne po stronie luminalnej nabłonka oraz komórki

dendrytyczne zaangażowane w prezentację antygenów w obrębie i u podstawy nabłonka oraz blaszki podstawnej[33,34]. Pomimo zmiennej morfologii nabłonka wynikającej z różnic w proporcjach poszczególnych typów komórek (podstawnych, urzęsionych i wydzielniczych) jego fizjologiczna i zapalna odpowiedź na stymulację zarówno w strefie przewodzącej jak i wymiany gazowej jest w dużej mierze podobna. Wykazano jednak, że odpowiedź zapalna komórek nabłonka dolnych dróg oddechowych tj. oskrzeli, jest silniejsza niż komórek nabłonka nosa, które produkują większe stężenia cytokin w odpowiedzi na stymulacje prozapalne[35].

Jednym z mechanizmów oddziaływania pyłu zawieszonego w powietrzu z komórkami nabłonka dróg oddechowych jest ich wpływ na spójność połączeń pomiędzy komórkami[36]. Powszechnie stosowaną w hodowlach *in vitro* metodą pomiaru integralności nabłonka jednowarstwowego jest pomiar przeznabłonkowego oporu elektrycznego (*transepithelial electrical resistance*, TEER)[37]. Jednowarstwowe hodowle nabłonka pobranego od pacjentów z chorobami obturacyjnymi charakteryzują się większa plastycznością i słabszą siłą połączeń międzykomórkowych, co można zaobserwować w postaci obniżonych wartości TEER w porównaniu do komórek osób zdrowych[38]. Ekspozycja na cząstki pyłu zawieszonego w powietrzu obniża napięcie połączeń międzynabłonkowych (co w rezultacie obserwowane jest jako spadek wartości TEER). Co ciekawe, mechanizm ten nie zawsze jest związany z zahamowaniem ekspresji białek m.in. z rodziny klaudyn, okludyny i e-kadheryny a ze zmianami organizacji połączeń międzykomórkowych[39]. Wyniki badań doświadczalnych wykorzystujących zaawansowane techniki *in vitro* np. hodowle wielokomórkowe, pozwalają na bardziej kompleksową analizę problemu. Wykazano, że w hodowlach wielokomórkowych, obejmujących oprócz warstwy nabłonka, komórki układu odpornościowego tj. makrofagi i komórki dendrytyczne, zachodzą przeznabłonkowe interakcje międzykomórkowe, przez wypustki cytoplazmatyczne typu pseudopodia, które dodatkowo stabilizują połączenia między komórkami nabłonka[40]. Z tego powodu ekspozycja na wysokie dawki pyłu pochodzącego ze spalania paliwa w silnikach diesla (*diesel exhaust particles*, DEP) powoduje istotne obniżenia TEER w monokulturach nabłonka oskrzelowego w porównaniu do hodowli wielokomórkowych[41].

Komórki nabłonka oddechowego stanowią nie tylko fizyczną barierę dla szkodliwych cząstek, lecz także inicjują nieswoistą odpowiedź immunologiczną poprzez ekspresję różnorodnych receptorów rozpoznających patogeny (*pattern recognition receptors,* PRR) zaangażowanych w identyfikację wzorców molekularnych związanych

z patogenami (*pathogen-associated molecular patterns,* PAMPs). Pyły o średnicy cząstek do 10 μm (PM_{10}) zawieszone w powietrzu atmosferycznym mogą na swojej powierzchni kumulować osady pochodzenia mikrobiologicznego w tym endotoksyny, które są rozpoznawane przez receptory *toll*-podobne (*toll-like receptor,* TLR). Odpowiedź ta prowadzi do zwiększonej syntezy czynników prozapalnych przez nabłonek w tym IL-8[42]. IL-8 jest uważana także za czynnik promujący ekspresję genu kodującego MUC5AC, co bezpośrednio przyczynia się do nadprodukcji mucyny będącej składnikiem śluzu wyścielającego drogi oddechowe[43]. Wykazano, że nagromadzenie PM_{10} w drogach oddechowych zwiększa ekspresję receptora końcowych produktów zaawansowanej glikacji (*receptor for advanced glycation endproducts,* RAGE) oraz aktywuje czynniki transkrypcyjne z rodziny NF-κB (*nuclear factor kappa-light-chain-enhancer of activated B cells*) odpowiedzialne za dalszą nadprodukcję cytokin prozapalnych[44].

Ze względu na złożoną organizację i różnorodność interakcji zachodzących w nabłonku dróg oddechowych, narażenie na pył zawieszony może nieść za sobą wiele szkodliwych skutków. Duża zmienność poszczególnych składowych pyłu zawieszonego utrudnia szczegółową charakterystykę możliwych działań biologicznych powodowanych przez te substancje w drogach oddechowych. Szczególne zagrożenie stanowią cząstki posiadające w swoim składzie związki metali (przede wszystkim żelaza) pochodzące np. ze źródeł związanych z ruchem ulicznym i transportem szynowym. Pył ten może penetrować w głąb warstwy śluzu, być pochłaniany przez komórki nabłonka i swobodnie przemieszczać się w cytoplazmie komórek[45] lub wykazywać zależne od dawki prozapalne działanie bez przechodzenia do wnętrza komórek nabłonka[46].

Nabłonek dróg oddechowych jest zaangażowany w lokalne procesy naprawcze w odpowiedzi na szkodliwe czynniki. Zmiany chorobowe związane z długotrwałym zapaleniem toczącym się w obrębie tej tkanki prowadzą do jej zmian strukturalnych m.in. przerostu komórek wydzielniczych oraz wzrostem grubości ścian dróg oddechowych poprzez zwiększenie udziału masy mięśni gładkich i fibroblastów[47]. Transformujący czynnik wzrostu beta (*transforming growth factor β*, TGF-β) wydzielany z uszkodzonych komórek nabłonkowych w odpowiedzi na stymulację Th2-zależną m.in. IL-13 odgrywa istotną rolę w modulowaniu przebudowy nabłonka u chorych na astmę[48]. Innym ważnym czynnikiem uczestniczącym w procesach naprawczych nabłonka dróg oddechowych jest nabłonkowy czynnik wzrostu (*epidermal growth factor,* EGF). Zmieniony chorobowo nabłonek charakteryzuje się także zmniejszoną liczbą rzęsek i ich zaburzoną funkcją, co w połączeniu z nadprodukcją gęstego śluzu prowadzi do

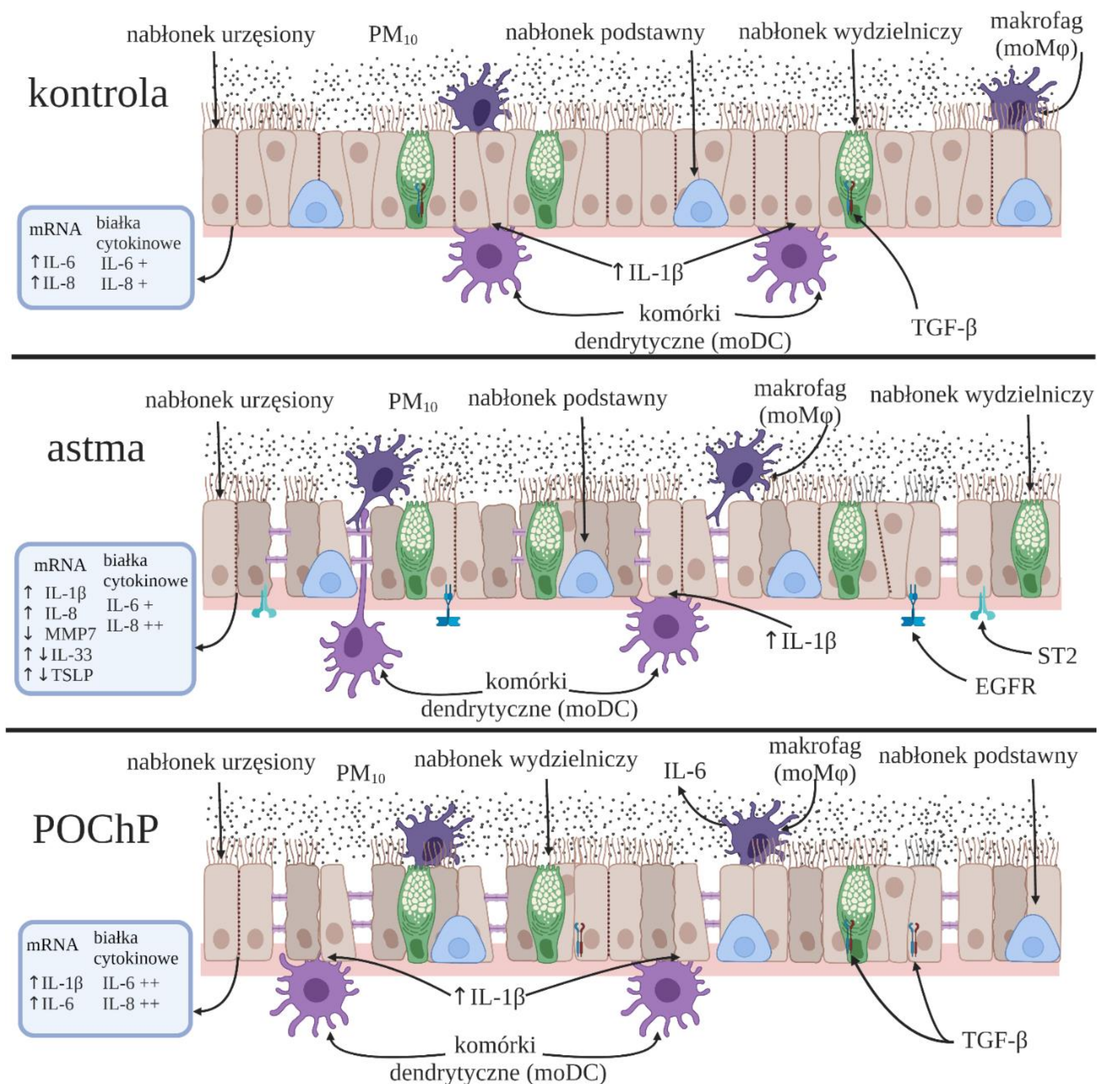
nieefektywnego usuwania zalegającej wydzieliny z dróg oddechowych oraz kumulacji cząstek pochodzących z zanieczyszczenia powietrza[49].

Zapalenie oraz stres oksydacyjny są najważniejszymi elementami odpowiedzi nabłonka na toksyczne działanie zanieczyszczeń powietrza w układzie oddechowym. Po kontakcie z pyłem zawieszonym nabłonek zwiększa ekspresję mediatorów takich jak TSLP, IL-33 czy czynnika stymulującego tworzenie kolonii granulocytów i makrofagów (*granulocyte-macrophage colony-stimulating factor,* GM-CSF), stanowiących element odpowiedzi immunologicznej Th2-zależnej[50,51]. Dodatkowo nabłonek po ekspozycji na PM wydziela związki o charakterze prozapalnym, np. IL-1β, IL-6, IL-8 czy czynnik martwicy nowotworów alfa (*tumor necrosis factor alpha*, TNF-α), które w konsekwencji mogą przyczynić się do rozwoju zapalenia o charakterze neutrofilowym[52]. Co więcej, cytokiny produkowane przez nabłonek indukują syntezę białka C-reaktywnego (*C-reactive protein,* CRP) i białka zapalnego makrofagów (*macrophage inflammatory protein 1 beta,* MIP-1β), co prowadzi do akumulacji makrofagów w drogach oddechowych. Aktywacja makrofagów oraz akumulacja pyłu posiadającego właściwości prooksydacyjne indukuje i nasila stres oksydacyjny w drogach oddechowych. Makrofagi produkują i uwalniają enzymy proteolityczne z grupy metaloproteinaz (MMP), które degradują składniki macierzy zewnątrzkomórkowej, oraz mogą modulować odpowiedź immunologiczną poprzez zmianę aktywności cytokin i chemokin[53]. Ekspresja MMP może być dodatkowo aktywowana poprzez reaktywne formy tlenu (*reactive oxygen species,* ROS)[54]. Powstawanie ROS związane jest przede wszystkim z obecnością na powierzchni cząstek stałych wielonienasyconych węglowodorów aromatycznych (WWA) oraz jonów metali przejściowych tj. miedzi, chromu, niklu i cynku[55]. Wykazano, że komórki nabłonka oskrzelowego po ekspozycji na $PM_{2,5}$ charakteryzowały się znacznymi zmianami morfologicznymi, uszkodzeniami struktury i funkcji mitochondriów, biosyntezy DNA oraz akumulowały ROS[56].

4.5 Interakcje nabłonka z komórkami układu odpornościowego

Wzajemne oddziaływanie komórek nabłonkowych z komórkami układu odpornościowego w drogach oddechowych związane jest z ich bezpośrednim kontaktem oraz lokalnym wydzielaniem mediatorów. Istotną rolę z punktu widzenia tych interakcji odgrywają TSLP, IL-33 i IL-25 należące do cytokin produkowanych przez nabłonek. Odpowiedzialne są one za inicjację reakcji zapalnej aktywując odpowiedź immunologiczną Th2-zależną związaną ze wzmożoną produkcją IL-5, IL-4 i IL-13.

Ponadto, kontrolują rekrutację, przeżywalność i degranulację eozynofilów oraz bazofilów w drogach oddechowych. TSLP pobudza występowanie wyspecjalizowanych populacji komórek dendrytycznych, które stymulują dziewicze limfocyty T CD4+ do różnicowania się w kierunku komórek Th2[57]. Komórki dendrytyczne wraz z alternatywnie aktywowanymi makrofagami (M2), których różnicowanie zależy od cytokin Th2, należą do kluczowych komórek regulatorowych zaangażowanych w inicjację odpowiedzi immunologicznej jak również są istotnymi modulatorami stanu zapalnego. Toczące się procesy zapalne w przebiegu chorób obturacyjnych oraz ekspozycja na czynniki uszkadzające nabłonek takie jak patogeny lub cząstki stałe zawieszone w powietrzu może znacznie zmieniać przebieg tych interakcji (Ryc. 1).



Rycina 1. Schemat odpowiedzi nabłonka współhodowanego z makrofagami oraz komórkami dendrytycznymi na stymulację PM_{10} u osób zdrowych i chorych na astmę lub POChP. moMφ- makrofag wyspecjalizowany z monocytu krwi obwodowej, moDC-komórka dendrytyczna wyspecjalizowana z monocytu krwi obwodowej. [Zaadaptowano z publikacji Paplińska-Goryca et al. "*Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD"*]

Komunikacja pomiędzy nabłonkiem a komórkami immunologicznymi jest dynamicznym i wielokierunkowym procesem. Wykazano, że makrofagi komunikują się z nabłonkiem za pomocą jonów wapnia Ca^{2+} poprzez połączenia szczelinowe (*gap junctions*)[58]. W czasie infekcji o podłożu bakteryjnym makrofagi wydzielają egzosomy, które pobudzają sąsiadujące makrofagi do wydzielania TNF-α. Nabłonek oskrzeli moduluje ten proces wydzielając IL-25, która uruchamia kaskadę reakcji prowadzącą do obniżenia produkcji interferonu gamma (*interferon gamma,* INF-γ) w drogach oddechowych[59]. Komunikacja pomiędzy nabłonkiem a makrofagami typu M2 poprzez szlak IL-33/ST2 oddziałuje na prawidłowe procesy naprawcze uszkodzeń nabłonka[60]. IL-33 wpływa także na aktywację komórek dendrytycznych, które nie tylko reagują na tę stymulację, ale mogą także wytwarzać TSLP, IL-33 i IL-25[61,62]. Integralność warstwy nabłonka jest niezbędna do prawidłowej migracji cząstek obcych sfagocytowanych przez makrofagi przez nabłonek dróg oddechowych do komórek dendrytycznych[63]. U chorych z ciężką astmą sygnalizacja międzykomórkowa jest odmienna od tej obserwowanej u osób zdrowych. Zdrowy nabłonek wykazuje nadekspresję markerów programowanej śmierci komórkowej m.in. ligandu 2 programowanej śmierci komórki (*programmed cell death ligand 2,* PD-L2), inicjujących odpowiedź zapalną typu Th1 z podwyższoną ekspresją IL-10 i CCL2[64]. U chorych na astmę obserwuje się odmienną reakcję immunologiczną manifestującą się zwiększoną ekspresję zarówno PD-L1 jak i PD-L2, a także cytokin m.in. TSLP i IL33, co w rezultacie skutkuje nadmiernie wyrażoną odpowiedzią zapalną[65]. Interakcje komórek dendrytycznych z nabłonkiem osób zdrowych prowadzą do ekspresji wielu cząstek powierzchniowych (HLA-DR, CD40, CD80 i CD86), które warunkują ich prawidłowe funkcjonowanie[65]. Z kolei nabłonek chorych na astmę, produkując znaczne ilości IL-8, przyczynia się do obniżenia ekspresji cząsteczek kostymulujących na powierzchni komórek dendrytycznych, z wyjątkiem CD86, co może wpływać na ich funkcję m.in. aktywację limfocytów T[66]. Wyniki naszych wcześniejszych badań z zastosowaniem współhodowli nabłonka z komórkami dendrytycznymi i makrofagami, wykazały, że nabłonek jamy nosa od chorych na astmę wykazał wyższą ekspresję TSLP i IL-33 w porównaniu do nabłonka osób zdrowych i chorych na POChP[67].

Makrofagi obecne w świetle oskrzeli współpracują z komórkami nabłonka w całym układzie oddechowym i stanowią kluczowy składnik nieswoistej odpowiedzi immunologicznej poprzez fagocytozę patogenów i ich pozostałości oraz uwalnianie mediatorów regulujących zapalenie[68]. Ponadto, makrofagi posiadają szeroki profil

enzymów biorących udział w metabolizmie ksenobiotyków, przez co chronią drogi oddechowe przed szkodliwym działaniem pyłu z zanieczyszczenia powietrza[69]. Makrofagi poddane wcześniejszej ekspozycji na cząstki stałe pochodzące z różnych źródeł wykazywały zwiększone zatrzymywanie, a przez to obniżone wydzielanie prozapalnych cytokin tj. TNF-α i IL-8 w odpowiedzi na bakteryjne lipopolisacharydy (*lipopolisacharides,* LPS)[70]. Reakcja ta może świadczyć o zmniejszonej reaktywności makrofagów i prowadzi do większej zachorowalności na choroby infekcyjne układu oddechowego w okresach podwyższonych stężeń pyłu w powietrzu, np. w sezonie grzewczym.

Bliska lokalizacja komórek nabłonka oddechowego i makrofagów sugeruje, że mogą one jednocześnie pochłaniać pył zawieszony oraz współdziałać w regulacji reakcji immunologicznej[71]. Wyniki badań doświadczalnych wykazały, że współhodowle nabłonka oddechowego i makrofagów cechowała silniejsza odpowiedź immunologiczna po ekspozycji na PM_{10}. Komórki nabłonka oskrzelowego stymulowane pożywką z hodowli makrofagów eksponowanych na cząstki pyłu PM_{10} wytwarzały zwiększone ilości IL-1β oraz cytokin z grupy IL-6 i IL-8 w porównaniu do stymulacji medium z hodowli bez pyłu[72]. Negatywny wpływ zanieczyszczeń powietrza na odziaływania międzykomórkowe powoduje osłabienie zdolności obronnych organizmu przy bakteryjnych infekcjach układu oddechowego, a to z kolei może prowadzić do zaostrzeń i progresji chorób obturacyjnych układu oddechowego.

5. Założenia i cele pracy

Badania stanowiące moją rozprawę doktorską dotyczą zagadnień związanych ze zmianami zachodzącymi w nabłonku dróg oddechowych od chorych na astmę, POChP oraz osób zdrowych w odpowiedzi na ekspozycję pyłem PM_{10} na poziomie molekularnym, biochemicznym i strukturalnym. W części eksperymentalnej wykorzystałam model zaawansowanej, wielokomórkowej hodowli nabłonka z makrofagami i komórkami dendrytycznymi, odzwierciedlający *in vitro* zależności międzykomórkowe występujące w drogach oddechowych. Ocena oddziaływania cząstek stałych na nabłonek jamy nosa uwzględniająca wpływ innych komórek immunologicznych oraz wykorzystanie wielkoskalowych analiz może przyczynić się do opracowania bardziej spersonalizowanych metod terapii, m.in. leczenia zaostrzeń obturacyjnych chorób płuc związanych z ekspozycją na zanieczyszczenia powietrza.

Sformułowano następujące cele badawcze:

1. Przegląd światowej literatury dotyczącej wpływu pyłu PM_{10} na fizjologię nabłonka dróg oddechowych.
2. Analiza i porównanie odpowiedzi zapalnej nabłonka nosa od osób zdrowych, chorych na astmę lub POChP po ekspozycji na pył PM_{10} w mono- i wielowarstwowym modelu *in vitro*.
3. Ocena integralności nabłonka dróg oddechowych oraz ekspresji markerów remodelingu po ekspozycji na PM_{10} w astmie, POChP i grupie kontrolnej.
4. Analiza transkryptomiczna odpowiedzi nabłonka jamy nosa od osób zdrowych i chorych na obturacyjne choroby płuc współhodowanego z komórkami dendrytycznymi i makrofagami po stymulacji PM_{10}.

6. Kopie opublikowanych prac

I. Biological effect of PM10 on airway epithelium-focus on obstructive lung diseases

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Review Article

Biological effect of PM_{10} on airway epithelium-focus on obstructive lung diseases

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ABSTRACT

Recently, a continuous increase in environmental pollution has been observed. Despite wide-scale efforts to reduce air pollutant emissions, the problem is still relevant. Exposure to elevated levels of airborne particles increased the incidence of respiratory diseases. PM_{10} constitute the largest fraction of air pollutants, containing particles with a diameter of less than 10 μm, metals, pollens, mineral dust and remnant material from anthropogenic activity. The natural airway defensive mechanisms against inhaled material, such as mucus layer, ciliary clearance and macrophage phagocytic activity, may be insufficient for proper respiratory function. The epithelium layer can be disrupted by ongoing oxidative stress and inflammatory processes induced by exposure to large amounts of inhaled particles as well as promote the development and exacerbation of obstructive lung diseases. This review draws attention to the current state of knowledge about the physical features of PM_{10} and its impact on airway epithelial cells, and obstructive pulmonary diseases.

1. Introduction

Indoor and outdoor air pollution pose a huge threat to public health, regardless of the societies' economic status. In general, airborne pollution is a mixture consisting of several gaseous components and particulate matter (PM) dispersed in ambient air. The magnitude of adverse health effects of particulate air pollutants hinges on factors such as particle characteristics. In this paper, we present a broad description of PM_{10} – a complex fraction of particles composed of $PM_{2.5,}$ which constitutes its most considerable component, and bigger, more varied constituents. Those elements are characterised by a distinct chemical and toxicological layout, which may have a more diverse impact on human health than the $PM_{2.5}$ fraction alone.

2. General information on particulate matter

PM_{10} is the most popular indicator used to determine the exposition to ambient air pollution [1]. This fraction combines the coarse (particles with an aerodynamic diameter between 10 and 2.5 μm), fine ($PM_{2.5}$, particles with an aerodynamic diameter between 2.5 and 0.1 μm) and ultrafine particulate matter (UFP, particles with an aerodynamic diameter equal or less than 0.1 μm). According to the World Health Organisation (WHO), in the majority of European countries, $PM_{2.5}$ constitutes 50-70% of PM_{10} [1]. The diameter of particles forming PM is critically related to its biological properties. PM_{10} constitutes an inhalable fraction, which can easily reach the lower respiratory tract but is mostly retained in the mucus layer and wiped away from airways by respiratory clearance. It is worth noting that some of its soluble constituents, endotoxins or trace elements, such as metals, can remain in the mucus lining of the airways and trigger oxidative stress or tissue inflammation [2]. Smaller-sized particles such as $PM_{2.5}$, known as a respirable component, are able to reach alveolar parenchyma where part of them interact with resident alveolar macrophages and epithelium cells [3]. The smallest particles ($PM_{1<0.1}$) are found in large numbers in ambient air. Even though their total mass is irrelevant in relation to the less numerous larger particles, they can penetrate within the respiratory tract down to the alveoli. They can migrate further into capillaries and cause hazardous effects in different tissues [4].

2.1. Sources and chemical composition of PM

The diverse chemical and physical nature of PM causes difficulties in assessing its impact. The final composition of PM is influenced not only by emission sources and precursors, but also by weather conditions.

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Pollutants emitted directly from both natural and anthropogenic sources are called primary sources of airborne PM. Under specific meteorological factors, these compounds can transform into secondary sources of air pollution, which may exhibit combined properties and subsequently different toxicity to humans [5].

2.2. Natural sources

According to the European Environmental Agency (EEA), the term 'natural source' can refer only to air pollution that does not involve any direct or indirect anthropogenic intervention [6]. Therefore, in this section, we consider sources that clearly meet this principle.

In marine areas, air contains water vapour, which is mainly included in coarse PM. Airborne sodium is supposed to originate entirely from seawater. The concentration of its water-soluble fraction (Na^{+}) is commonly used as a reference to determine the sea-salt component in airborne particles. Also, airborne Cl^{-} is considered to originate from marine aerosol [7].

Volcanoes, seismic activity and wildfires are the main sources of fine particles as well as associated airborne sulphates and nitrates [8]. The composition of volcanic fog (vog) changes depending on the distance from the emission source. At short distances from the volcano, it consists of both fine particle aerosols and unreacted sulphur oxide gases. Over time and distance, the vog contains sub-micron sized sulphate particulates, as well as further secondary sulphate aerosols, which may combine ammonium sulphate, bisulphate and sulphuric acid [9]. The exposition to a high level of volcano dusts may be toxic for humans. An animal study has shown that the ash of the Pichincha volcano induces an inflammatory reaction in mouse lungs. Cytotoxicity and elevated oxidative stress were also demonstrated in human airway epithelial cells and macrophages after volcano ash cell treatment [10].

Mineral dust is also an important source of natural airborne particles. According to the EEA, the major origin of the European wind-blown desert dust are arid regions in North Africa [6]. Dust from Sahara consists mainly of silicate minerals abundant in magnesium and iron, as well as elements like oxygen, potassium, sodium and aluminium [11]. Erosion of Atlantic coastal sedimentary rocks is a source of airborne carbonates of calcium, magnesium and strontium. Other important components in this fraction are iron oxides, magnesium and iron-rich clays [6]. The same mechanism is involved in originating airborne potassium, which mainly comes from dry humus, thick topsoil from fertile soils, as well as igneous and metamorphic rocks formed by wind erosion [12]. The *in vivo* and *in vitro* study demonstrated that persistent exposition to desert dusts is not neutral for living organisms. Dust from northeastern Arizona upregulated proinflammatory (activated p38 and ERK1/2 pathways and pro-inflammatory mediators release) as well as oxidative stress (elevated expression of mRNA for superoxide dismutase-1, heme oxygenase-1, and cyclooxygenase-2) response in respiratory epithelial cells. Moreover, the desert dust provoked an injury in the lower respiratory tract in the animal model [13].

Primary biological aerosols include particles that represent various size distributions. Grains of pollen are typically the largest constituents, fungal spores are included in PM_{10} while bacteria are in the $PM_{2.5}$ fraction, and viruses are generally included in PM_{1} [14]. In ambient air, especially the smallest particles tend to agglomerate [15] or mix with other urban air pollutants, which may boost their proinflammatory properties [16]. PM can attach to pollen grains; the biochemical and microscopy analysis of pollens showed Si-rich, organic-rich, SO-rich, metals, oxides and Cl-rich particles adsorbed to *Pinus*, *Platanus* and *Acer* airborne pollen. The chemical properties of adsorbed particles changed dynamically and were associated with weather parameters and air pollution [17]. The epidemiological study showed that airborne grass, birch and hornbeam pollen act synergically with PM_{10} in seasonal allergic asthma exacerbations. An association between these aeroallergens and asthma hospitalisations in individuals younger (<60 years) on days with high air pollution levels was observed in the Brussels-Capital Region [18].

2.3. Anthropogenic sources

Air pollutants that come from anthropogenic sources constitute the majority of airborne PM. These pollutants are released during various everyday activities, as well as outdoor motions from urban and rural areas, such as fossil fuel combustion. In comparison to natural dust material, those contaminants are more concentrated in the ultrafine and fine fractions, rather than in the coarse fraction of PM [19].

Great interest is directed towards atmospheric pollution, but the air inside our workplaces and houses can also be a significant source of harmful contaminants. The most important sources of indoor UFP are associated with combustion as well as other activities. Cooking, especially frying on a gas stove, emits the highest amount of UFP [20]. Also, indoor cigarette smoking and candle burning contribute to emitting huge amounts of harmful particles [21]. The indoor environment is also a reservoir of PM from house dust mite (HDM), pets dander, as well as insect proteins and mould spores, which can be resuspended by vacuuming [22,23]. Another important indoor source of airborne coarse particles is walking, with an emission rate of up to 10 milligrams per minute [24]. The majority of people spend a considerable amount of time indoors. The composition of indoor air pollution is also influenced by particles of outdoor origin, which penetrate into buildings with shifting efficiencies so that human exposure to outdoor particles often take place indoors. Chen and Zhao [25] determined the infiltration factor as the fraction of ambient particles, which move indoors by cracks in building envelopes without any mechanical ventilation and remain suspended in the air. Despite the existence of significant variations between homes included in the study, it has been calculated that the average infiltration factor for $PM_{2.5}$ was about 0.55, while factors for UFP and PM_{10} were approximately 0.3 [25].

In highly populated regions, urban particulate matter (UPM) differs in chemical composition, resulting in a complex mixture indicating location-specific variability. Some of the contaminant particles may be transported over long distances by ambient air after their emission from the source [26]. The transcriptomic profiling of genes impacted by UPM (from Rome) in human monocytes showed upregulation of genes involved in DNA repair, apoptosis and oxidative stress, but interestingly not in a dose-dependent, but rather a time-dependant manner [27]. Urban ambient particulate matter in Central and Eastern European cities mainly comes from domestic fuel burning [28]. Emissions from household stoves and local boiler plants involving dust and gases from the combustion of solid fuels, such as coal and its derivatives, are called communal pollutants. In areas where houses are heated by timber, a high content of levoglucosan, mannose and galactosan can be found in the air. These substances are used as tracers that appear in ambient air as a result of burning all types of wood [29]. Nishikawa et al. [30] noted that the concentration of coarse and fine PM in ambient air during the heating period was elevated in comparison to the non-heating period. The main component of these fractions was total carbon, which combines the amount of organic and inorganic carbon in the air. Metal particles, which are part of ambient air pollution, can originate from different sources. The aerosol of fine or ultrafine particles is characterised by a greater amount of organic matter and surface area per volume unit as well as lower density. These features make them the main source of hazardous metals [31]. The International Agency for Research on Cancer (IARC) allocated the most harmful elements into three sections. Group 1, which comprises carcinogenic agents to humans, consists of arsenic (As), chromium (Cr) (VI) compounds, cadmium (Cd) and cadmium compounds as well as nickel (Ni) compounds. Lead (Pb) compounds are classified as a probably carcinogenic agent in group 2a, while cobalt (Co) (metalic and cobalt compounds) is assigned to group 2b with other possibly carcinogenic substances. Mercury (Hg) belongs to group 3 characterised as toxic elements [32]. It is worth noting that during cold seasons, when solid fuels are extensively burnt for

residential heating, the air concentrations of metals such as zinc (Zn), lead (Pb), arsenic (As) and copper (Cu) are 2-10 fold higher in comparison to the warm period [30]. Firework displays are one of the anthropogenic sources of airborne particles, especially metals. Sulphur (S) is used as a propellant, while several metals are sparkle emitters and colour-providing components. The concentrations of lead (Pb) and barium (Ba) in the air were significantly elevated during pyrotechnic events and both can be considered as firework tracers [31]. Calm weather conditions favour metals to remain in ambient air, which could be associated with local sources of emissions [33]. A similar relationship was found for ambient PM. Particles with a smaller diameter tend to be suspended in the air for longer periods and are more vulnerable to weather conditions. Cold front activity, which causes increased gusts of wind, is able to transfer smaller particles over long distances [34]. Li et al. observed a negative correlation between meteorological conditions such as temperature, relative humidity, precipitation and wind speed as well as levels of air pollutants [35]. Other researchers also determined the specific influence of metrological factors on the special distribution of PM_{10} and $PM_{2.5}$ [36]. The temperature and precipitation revealed the contribution for allocation of both fractions. PM_{10} distribution was also affected by relative humidity, while atmospheric pressure had a great influence on $PM_{2.5}$ relocation. The authors proposed the numerical values of the most favourable conditions for the removal of both fractions of PM in the air [36], which represent a consistent pattern with the one from the previous study (Table 1). It should be noted that, due to fuel availability and economic reasons, about 40% of the world's population still cooks with solid fuel, which significantly contributes to the development of childhood pneumonia, asthma, COPD, cardiovascular diseases and cancer in these societies [37]. A study from England demonstrated that the annual mean concentration of PM_{10} from wood burning in London was 1.1 mg /m^3 and was comparable to PM emission from traffic sources in this area [38]. The study of 75 female homemakers from rural areas of China showed that IL-6 levels in the urine of solid fuel users was increased compared to controls during the heating season [39]. The use of solid fuels in the winter contributes to systemic inflammation and poses an adverse effect on human health.

The growing number of vehicles and increased traffic volume in urban areas also significantly contribute to airborne PM pollution, especially fine PM [40]. The main source of these particles is diesel-powered vehicles with faulty particulate filters or catalytic converters [19,41]. Since 2012, experts from IARC have classified exhaust from diesel engines as a carcinogenic agent to humans (group 1) [42]. Diesel exhaust is a complex aggregate of carbonic nucleus and adsorbed substances including metals like aluminium (Al), transition metals such as zinc (Zn), iron (Fe), chromium (Cr), vanadium (V), nickel (Ni) and/or different high-molecular-weight organic compounds such as polycyclic aromatic hydrocarbons (PAHs), halogenated aromatic hydrocarbons and redox-active quinones [43–45]. Cu, Fe and Zn from the PM_{10} fraction correlated with an increased risk of cardiovascular and respiratory mortality but not lung cancer incidence in Greater London. The studies showed that these metals originate from non-tailpipe road traffic emissions (brake and tyre wear) [46,47], and suggest that not only exhaust from diesel engines but also non-tailpipe emissions are tread to human health and life.

PAHs are mostly associated with particulate air pollution connected with anthropogenic activity, including vehicle exhaust and fossil fuel combustion [48]. Natural sources of PAHs, such as volcanic activity, petroleum seeps, forest fires and decomposition of organic matter, have a minor impact on total emissions of these compounds [49]. PAHs are persistent organic pollutants that can easily bioaccumulate, have a long half-life and high mobility in the environment. They consist of two or more benzoic rings taking different mutual positions in the molecule. PAHs containing more than three fused aromatic rings are mainly associated with ambient fine particles ($PM_{2.5}$), while low molecular weight compounds are found mostly in the vapour phase [50]. The aromatic structure of these condensed compounds is basically composed of carbon and hydrogen atoms but it can also contain sulphur, nitrogen or oxygen, as well as different substituent moieties [51]. The individual compounds belonging to PAHs have different lipophilicity, which affects their absorption rate in the human body. Studies confirm that the identification of one PAH compound in the environmental sample is equivalent to the presence of other PAHs. In this case, benzo[a]pyrene is adopted as a marker of the PAH mixture. It is a hazardous compound that acts without a threshold. Longhin et al. [43] showed that the total content of PAHs in diesel samples was more than ten times higher than in biomass combustion samples. Exposure to any concentration of this PAH can cause harmful effects, and even lead to cancer development [52].

The biological effect of PM_{10} from anthropogenic sources is well described in the context of its potential, harmful effect on human health. The studies suggest that both PM fractions (PM_{10} and $PM_{2.5}$) are able to elicit the adverse effect on human health and some of the effects are induced by the larger particle fraction exclusively [53,54]. In 2016, one in nine deaths globally was caused by air pollution [55]. There is no clear evidence on the mechanism by which coarse PM disrupts the molecular paths of cells. Ljubimowa et al. [2] determined that exposure of rats to coarse PM for one month caused a significant upregulation of genes associated with inflammatory responses and tumour formation in the brain. Moreover, a strong correlation during the same exposure period was found between these genes and cerebral nickel accumulation. Zhou et al. [56] demonstrated that in the Chinese city of Nanchang, PM_{10} constitute the most critical air pollutant leading to increased lung cancer incidence. In this study, the highest impact of that factor on lung cancer development was seen in urban residents, women and smokers. Facing the problem, the WHO Regional Office for Europe created a software tool for air pollution health risk assessment, named AirQ+[57], for more detailed knowledge of this issue.

Despite the harmful effect of anthropogenic-derived PM_{10} on the respiratory tract, which is described in detail below, the other important harmful actions of pollutants from PM_{10} are well documented in the context of its impact on pregnancy [58], fetal growth [59] and child health [60], diabetes [61], cardiovascular diseases [53] and a retinal microvascular response [62]. A high number of publications demonstrated the important association of PM_{10} with type-2 diabetes mellitus development and course due to pathophysiology of this disease related to chronic systemic inflammation. Long-term exposure to PM_{10} increases the risk of diabetes – the homozygotes with polymorphism of *IL6* are more susceptible to the diabetogenic effects of particulate matter, through an increased level of systemic inflammation caused by PM [63]. Exposure to PM_{10} caused prothrombotic changes in diabetic patients; however, these were not associated with systemic inflammation [64]. The authors suggest that people predisposed or suffering from diabetes should be in special concern of health control in areas with high emissions of air pollutants.

Table 1
Weather conditions for the removal of PM_{10} and $PM_{2.5}$ in the air.

Factor	Level	Source
Temperature	<10°C or >21°C	[35,36]
Precipitation	>1500 mm	
Atmospheric pressure	<900 hPa	
Wind speed	>3 m/s	
Relative humidity	>65%	

3. The impact of PM_{10} on respiratory epithelium biology

The respiratory epithelium is formed of pseudostratified differentiated mucociliary epithelial cells, anchored to the basement membrane by a basal epithelial cell layer [65]. The basal epithelial cells also act as cell progenitors, which can differentiate and replace mucociliary epithelial cells after injury [66]. The morphology of the epithelium

alters throughout the respiratory tract; secretory and ciliated epithelial cells predominate the upper airways and are absent in the bronchioles. Goblet cells synthesise, store and secrete mucin granules, which absorb water to form the mucus layer covering the upper respiratory tract. This mucus is cleared by the beating action of cilia from ciliated cells [67]. Submucosal glands are also present in the upper airways and contain serous and mucus-secreting cells. The serous cells secrete antimicrobial proteins. Simple cuboidal epithelial cells and secretory Clara cells are present in the bronchioles. Clara cells are also secretory cells and produce surfactant (a fluid layer comprising phospholipids and proteins covering the epithelium of bronchioles and alveoli) [68]. The morphology of the airway epithelium changes again as the bronchioles descend into the alveoli, where Type 1 pneumocytes are abundant with few Type 2 pneumocytes.

The airway epithelium, together with macrophages, build the first barrier for contact for inhaled material, including infectious pathogens and PM. Tight junctions between cells, the surfactant film and the presence of ciliated cells are important components of this hermetic barrier. The airway epithelium is not only a passive anatomical structure, but it is also an immunologically active interface between inhaled air and the respiratory system. Because of the complex organisation and multitude of interactions taking place within the airway epithelium, the tissue could be affected by exposure to coarse PM in many deleterious ways. There is evidence that air pollution impairs mucociliary clearance (MCC) by affecting the cilia structure and function. Exposure to several pollutants, such as nitric dioxide, cigarette smoke and other oxidants, resulted in a decrease in the number of cilia, their shortening or reduced beating frequency even in healthy subjects [69,70]. Furthermore, bronchial epithelial cell cultures of asthmatics and healthy subjects did not differ in terms of cilia beating frequency and both cultures responded to diesel exhaust particle exposure by a reduction of the cilia beating frequency [71]. Cilia function can also be disrupted by bacterial toxins, viral infection, allergic rhinitis or steroid treatment, and contributes to the induction and exacerbation of obstructive lung diseases [72]. Some substances presented the ability to increase ciliary beating; these included osmotic agents modulating the mucus layer or acting directly on cilia, prostaglandins and NO, while among patients with stable COPD, salmeterol and salbutamol can be used to restore the proper frequency [72,73].

Some studies suggest PM_{10} can easily reach the lower respiratory tract but are usually accumulated usually in the mucus layer and removed from the airways by respiratory clearance. Loxham et. al. [74] determined that Fe-rich PM_{10} obtained from an underground railway station can penetrate the mucus layer and enter bronchial epithelial cells cultured in the air-liquid interface (ALI). Internalised coarse PM was found free in the cytosol and all PM-containing cells appeared intact. It is possible that the intrusion of PM_{10} into epithelial cells is not necessary to provide its harmful effect on the cells.

3.1. The impact of PM_{10} on innate immune response associated with epithelial cells

Airway epithelial cells express heterogeneous pattern recognition receptors (PRRs), which are involved in the identification of infectious components known as pathogen-associated molecular patterns (PAMPs) and initiate innate immune responses. This group consists of Toll-like receptors (TLRs) and the nucleotide-binding domain as well as leucine-rich repeat-containing receptors (NLRs) [75].

Toll-like receptors (TLRs), which are expressed on the cell surface, are involved in the identification of infectious substances of viruses, bacteria and fungi [76]. The impact of coarse PM exposition on the activation of the TLR pathway in normal human bronchial epithelial cells (NHBE) seems unclear. Airborne PM_{10} significantly increased the level of TLR-4 mRNA expression, while the TLR-2 mRNA expression level remained stable [77]. Coarse PM among other fractions of PM showed the greatest positive influence on heat shock protein Hsp70 mRNA expression, which is an endogenous ligand of both mentioned receptors [77,78]. Activation of human airway epithelial cell membrane TLRs stimulated increased production of pro-inflammatory molecules, such as IL-8, mediated through an NF-κ B - dependent signalling pathway [79].

The presence of biological components, such as bacterial endotoxins - lipopolysaccharides (LPS) in the PM fraction, has been proved as a modulator of the *in vitro* and *in vivo* inflammatory reaction, which may result in lung injury. Due to meteorological, spatial and seasonal variations of the PM chemical composition, the pattern of endotoxin content in ambient air is difficult to determine [80]. Human bronchial epithelial cells (BEAS-2B) revealed significantly elevated production of IL-8 after exposure to LPS from the coarse fraction of PM [81]. Spring and summer PM_{10}, which may contain up to three times more endotoxins than wintertime-collected PM_{10}, induces a significant increase in IL-8 release both by BEAS-2B and A549 cultures [29,82].

It was shown that air pollution downregulates the antimicrobial properties of airway epithelium and impacts innate immunity. Exposure to $PM_{2.5}$ or PM_{10} deregulated the ability of bronchial epithelial cells to express antimicrobial peptides: human β-defensin 2 (HBD-2) and HBD-3 upon infection with *M. tuberculosis* [83]. *Streptococcus pneumoniae*, the main microorganism causing bacterial pneumonia, exploits another mechanism to attach to target cells. By mimicking the typical ligand for the platelet-activating factor, this bacteria enhanced the mobilisation of surface platelet-activating factor receptors (PAFR), which resulted in an increase of its adhesion and intracellular penetration [84]. Mushtaq et al. [85] demonstrated that exposure to airborne urban PM promoted dose-dependent adhesion of *S. pneumoniae* to airway epithelial cells but did not directly trigger the pathogen's proliferation.

The exposure to the influenza A virus upregulated the nucleotide-binding domain and leucine-rich protein 3 (NLRP3) inflammasome in the PM_{10} pre-treated human airway epithelial cell line (HBEC-6KT). The authors observed enhanced IL-1β release in a multiplicity of infection (MOI) manner. These results indicate that exposure to airborne PM augments the innate immune response to the influenza A virus, which can have a harmful effect especially among people with pre-existing respiratory diseases [86]. During pathogenic infection by RNA viruses, particularly influenza virus, pre-exposure of human alveolar type II epithelial cells (A549) to PM_{10} induced the downregulation of some important antiviral genes, such as type-I interferon beta (IFN-β) and interferon-sensitive response element (ISRE), as well as enhanced viral replication [87]. What is more, upregulation of several metabolic pathway-related genes encoding cytochrome P450, family 1, subfamily A, polypeptide 1 (*CYP1A1),* intestinal peptide 1 *(VIPR1)* and protein phosphatase 1 regulatory subunit 14A *(PPP1R14A*) was noted in the study. All the above-mentioned up and downregulated genes collectively support the virus infections, replication and act as suppressors of innate immune response-related genes, which contributes to the severity of viral infections [87].

Recent studies demonstrated that airborne PM can be a driver for the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The impact of PM_{10} concentrations on SARS-CoV-2 transmission seems ambiguous. A 10-$\mu g/m^3$ increase in PM_{10} concentration was significantly associated with a 1.76% enlargement in the daily number of confirmed COVID-19 cases in mid-February of 2020 in China [88]. Another study from this country revealed that, for each 10 $\mu g/m^3$ increase in PM_{10} concentrations, the COVID-19 case fatality rate between January and March 2020 in Wuhan increased by 0.83% [89]. What is more, on average, 3.6 times more patients infected with COVID-19 were observed on 7th April 2020 in North Italy cities located in highly air-polluted areas, with more than 100 days per year with exceeding PM_{10} standard values, compared to zones with lower air pollution (<100 days with exceeded PM_{10} standard values) [90]. The data from UK biobank revealed that a one-unit increase in PM_{10} concentration was associated with an 8% rise in COVID-19 cases in April 2020 [91]. Wannaz et al. [92] suggested a possible vector role of PM_{10} to SARS-

CoV2. The authors revealed a significant correlation between COVID-19 infections and increased PM_{10} concentration 15 days after the air-pollution incidence between May and October 2020. In contrast to these results, Bontempi et al. [93] revealed no association between PM_{10} concentration and COVID-19 cases in Lombardy (Italy) at the beginning of the pandemic (between 10th February and 27th March 2020). Some researchers suggest a low infection capacity of PM embedded viruses [94] while others revealed only detectable remnants of viral RNA on airborne particles [95]. A more probable mechanism by which PM contributes to high COVID-19 morbidity is an elevated expression of the alveolar angiotensin-converting enzyme 2 (ACE-2) receptor, the main cellular gate for SARS-CoV2 [96,97]. Most of the published studies seem to present preliminary observations; therefore, more research should be conducted in this area, which would allow to draw more reliable conclusions.

3.2. *The impact of PM_{10} on inflammation mediated by epithelial cells*

Inflammatory response is a cascade of reactions, which can be initiated by exposure to toxic stimulants including PM. The complex relationship between PM-specific constituents and induction of inflammatory response, as well as the mediating role of airway epithelial cells in this pathway, is still not sufficiently explained. Furthermore, the effect of the coarse fraction of PM on pro-inflammatory cytokine production seems unclear. The results of murine studies showed increased mRNA expression for mediators of inflammation, such as tumour necrosis factor-alpha (TNF-α), IL-5, IL-22, and IL-10 in lungs of PM-exposed mice after inhalation of coarse PM water solution (40 μg PM/ml) [98]. The best described inflammatory pattern after PM_{10} exposure in airway epithelial cells is known for the IL-8 pathway. IL-8 is an important pro-inflammatory cytokine, a strong chemoattractant agent for neutrophilic accumulation in the airways. The persistent presence of a high number of neutrophils in the airways leads to the release of their cytotoxic and profibrotic agents (e.g. matrix metalloproteinases (MMPs), neutrophil elastase, lysozyme), which results in tissue injury and fibrosis. Additionally, by the production of a wild range of cytokines and chemokines (IL-1α, IL-1β, IL-8, vascular endothelial growth factor (VEGF), TNF-α, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)), the neutrophils start the cascade of inflammatory reaction by recruiting macrophages and T cells to the site of inflammatory reaction [99]. IL-8 is considered to regulate the mucin 5AC gene (MUC5AC) expression (which is directly contributed to mucin overproduction) at the posttranscriptional level in a human bronchial epithelial cell culture model [100]. Ambient particulate matter induces IL-8 expression in human airway epithelial cells through a mechanism associated with nuclear factor-kappa B (NF-κB) that occurs via a pathway involving direct phosphorylation of the transcription factor p65 in the absence of IκBα degradation [79]. The coarse PM fraction had the highest pro-inflammatory capacity in IL-8 secretion by NHBE cells when compared to other PM fractions [77]. On the other hand, the expression of IL-8 after stimulation with the same PM dosage was lower in A549 cells in comparison to other PM fractions [101]. The increased amount of crustal elements such as silicon, barium, sodium and iron, in the coarse fraction of PM may induce IL-8 overproduction [102]. The coarse fraction of ambient PM from Sydney contained more iron than traffic-derived PM and caused a stronger injurious effect on airway epithelial cells [103]. The impact of PM_{10} exposure on IL-8 production in airway epithelial cells is illustrated in Fig. 1.

Not only the PM fraction but also various epithelial cell lines used in the experiment may have resulted in some differences in the observed pro-inflammatory response. Results obtained by Gilmour et al. [104] revealed that bronchial epithelial cells (16HBE) showed a more marked pro-inflammatory response to PM_{10} exposure than A549 cells. Differences in response to agricultural dust treatment between human bronchial epithelial cells (NHBE) and human nasal epithelial cells (HNE) were also observed. Exposure to the PM_{10} fraction of airborne dust from

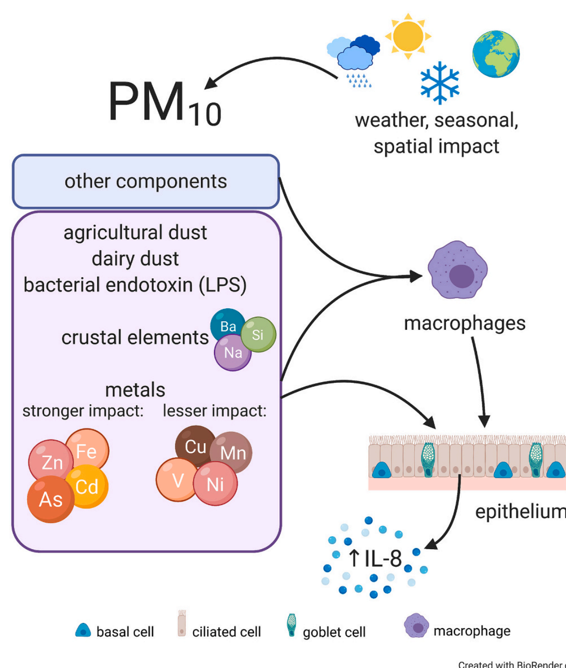

Fig. 1. Impact of PM_{10} exposure on IL-8 expression and production by airway epithelial cells.
PM_{10} characteristics shows significant variability caused by weather, seasonal and spatial changes. Differences among its composition result in various harmful effects on biological processes. One of them is epithelial stimulation to synthesize the increased amounts of proinflammatory cytokines, especially IL-8. PM_{10} exposition impacts IL-8 expression in respiratory epithelium by multiple different pathways. Increased level of bacterial lipopolysacharides, crustal material and metals induce direct IL-8 secretion from epithelial cells. Additionally, interaction between PM_{10}-activated macrophages and epithelium affects IL-8 synthesis. The macrophages have an ability to phagocyte particles and stimulates epithelium for increased alarmin production.

a local dairy parlour resulted in a significant increase of IL-8, IL-6, and TNF-α mRNA expression in NHBE cells only [105].

The pro-inflammatory potential of PM_{10} can vary depending on the fuel source. Expression of intracellular IL-8 was significantly higher in A549 cells treated with PM_{10} from pellet combustion (50 μg/ml), while exposure to charcoal and wood originated PM_{10} did not affect IL-8 levels. The extracellular level of soluble IL-8 and IL-6 was not increased by exposure to all the mentioned biomass PM_{10}. The observed inflammatory response pattern was attributed to the elevated content of metals in PM_{10} obtained during pellet combustion [106]. Also, the content of transition metals in ambient air seems to have a significant impact on airway epithelial cells. Soluble iron, copper and zinc are considered to be inducing compounds in pro-inflammatory cytokine production [107]. Aqueous solutions of air pollution-related Cd^{2+}, Zn^{2+} and As^{3+} had a higher pro-inflammatory capacity in BEAS-2B cells, than Mn^{2+} and Ni^{2+}. Ions such as VO_4^{3-}, Cu^{2+} and Fe^{2+} had the lowest potential to induce synthesis of IL-8 and IL-6. Cd^{2+}, Zn^{2+} and As^{3+} act as mediators of phosphorylation and activators of three major mitogen-activated protein kinase (MAPK) pathways (p38, JNK and ERK), but p38 is considered to be a crucial inductor of IL-8 and IL-6. What is more, exposure to Zn^{2+} and As^{3+} increases the phosphorylation of p65, a subunit of NF-κB transcription complex involved in triggering transcription of both mentioned cytokines [108].

The close proximity of airway epithelial cells and macrophages suggests that these cell populations encounter inhaled stimuli

simultaneously and regulate the inflammatory response. A murine study showed that macrophages phagocyted particles and did not interfere with epithelial cells as long as this was not additionally stimulated with particles. PM_{10} may interact directly both with macrophages and epithelium resulting in IL-8 release. A549 cells exposed to conditioned media from monocyte-derived macrophages stimulated with PM_{10} produced significantly higher levels of IL-8. This enhancement in pro-inflammatory cytokine synthesis is triggered by TNF-α and other soluble mediators released from PM-exposed macrophages activating NF-κB and activator protein 1 (AP-1) signalling pathways [109].

The exposure to secretome from PM_{10} exposed alveolar macrophages (AMs) upregulated IL-1B, leukemia inhibitory factor (LIF) and IL-8 mRNA expression in bronchial epithelial cells. Co-cultivation of AMs with bronchial epithelial cells exposed to PM_{10} increased the production and release of TNF-α, GM-CSF, IL-1β, IL-6, IL-8, LIF and oncostatin M in comparison to control co-culture. The results suggest a collaboration between these cells in the production of these pro-inflammatory mediators [110].

The cross-talk between bronchial, structural and immunological cells is a dynamic process. One of the mechanisms by which macrophages can communicate with airway epithelial cells is Ca^{2+} signalling via gap junction channels [111]. This signal may be induced by dust particles and result in transient receptor potential melastatin 2 (TRPM2) activation and cause increased transcription of genes encoding pro-inflammatory cytokines. The disruption of cell-cell communication by ambient particulate matter impacts the response of airway epithelial cells to external stimuli. This action may accelerate the inflammatory processes in the respiratory tract. NLRP3 is an intracellular danger-sensing complex known to be present in various cell types including human airway epithelial cells [112]. This inflammasome reacts to danger signals such as adenosine triphosphate (ATP) or reactive oxygen species by promoting caspase-1 autocleavage, which leads to the secretion of active IL-1β, CC chemokine ligand-20 (CCL20) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [112]. Hirota et al. [113], using a murine model, confirmed that exposure to PM_{10} activated the NLRP3 inflammasome and cytokine production, which contributed to the migration of immature dendritic cells and their maturation within the lung as well as increased inflammatory cell recruitment.

The production of PM_{10}-induced pro-inflammatory cytokines can be attenuated by several drugs. Statins decreased cytokine production by alveolar macrophages causing downregulation of the TLR4 receptor involved in PM_{10} mediated pro-inflammatory signalling or by suppression of the phagocytic capacity of the cells [114]. Interestingly, bronchial epithelium pre-treated with a combination of clotrimazole (CLZ) and N-acetylcysteine (NAC) resulted in decreased IL-8 mRNA expression. These compounds may act as Ca^{2+} signal blockades, so their potential application in dust particle-mediated airway diseases should be considered [115].

The actin cytoskeleton has a significant impact on the maintenance of proper cellular structure and function in different cell types. Any amendment of this structure, especially increased actin filament stabilisation, could cause changes in physiological processes, including cell migration and adhesion, leading to tissue pathologies such as senescence-like phenotype in lung cells [116]. Exposure of lung epithelial A549 cells to 10 μg/cm^2 of PM_{10} from a high-traffic residential zone caused enlarged expression of cytoplasmic Rho-associated coiled-coil-containing protein kinase (ROCK) constituting a phosphorylating agent of myosin phosphatase-targeting 1 (MYPT-1) and myosin light chain (MLC). An increased amount of phosphorylated MLC (p-MLC) resulted in extended F-actin stress fibre formation but had no detrimental influence on cell viability [117]. Long-lasting inflammation and exposure to reactive oxygen species (ROS) resulted in the activation of the signal transducer and activator of the transcription-3 (STAT3) protein via phosphorylation of several kinases such as tyrosine protein kinase (Src), extracellular signal-regulated kinases 1 and 2 (ERK1/2), and protein kinase C zeta (PKCζ) [118]. Phosphorylation of $p21^{CIP1/WAF1}$ protein induced by the STAT3-dependent pathway resulted in its cytoplasmic stabilisation and suppression of its growth-inhibitory activity, G1-G0 cell cycle arrest, decreased apoptosis and increased actin polymerisation implicating the carcinogenic potential of PM_{10} [119].

3.3. The impact of PM_{10} on oxidative stress in epithelial cells

Oxidative stress (OS), synergistically with inflammation, are the leading pathways of PM-induced toxicity and adverse health effects. PM contains a variety of polyaromatic hydrocarbons and transition metals that induce oxidative stress [112]. ROS are considered the main cause of PM-mediated OS. ROS such as hydrogen peroxide (H_2O_2) and superoxide (O_2•$^-$) can be generated by reducing molecular oxygen in a series of subsequent one-electron reductions, while hydroxyl radicals (OH•) can be generated by the Fenton reaction in the presence of Fe^{2+} [74]. Other redox-reactive metals (Cu, Cr, Ni and Zn), similarly to organic compounds, especially polycyclic PAHs, can be associated with PM-induced ROS production [80]. A concentration-dependent (3.1-12.5 μg PM_{10}/cm^2) increase in ROS-sensitive dye (2,7-dichlorofluorescein (DCF)) fluorescence was observed in the bronchial epithelial cells culture 3 hours after exposure to Fe-rich PM_{10} obtained from an underground railway station [74]. PM_{10} obtained from a commercial zone was able to induce (5-20 μg PM_{10}/cm^2) ROS formation dose-dependently even after 1h of exposure in the A549 cell culture. This effect was sustained for 12 h [120]. ROS can interact with a variety of biological molecules like DNA, proteins, lipids and may also interrupt signalling pathways via nitric oxide (NO) [121]. Vignal et al. showed an induction of a systemic OS expressed by enhanced serum concentration of malondialdehyde (MDA) after exposure to airborne PM in mice [98]. OS affects the production of antioxidant enzymes. mRNA expression of various OS markers such as catalase (CAT), cytochrome c oxidase 2, heme oxygenase 1 (HO-1), nitric oxide synthase 2 (NOS2) and superoxide dismutase 2 (SOD2) was increased in the lungs. These effects were eliminated by applying an aqueous solution of antioxidant N-acetyl-L-cysteine (NAC) [98]. HO-1 is one of the most important antioxidant enzymes, belonging to the heat shock proteins, which present both anti-inflammatory and airway epithelial-protective functions [122]. HO-1 mRNA expression as well as A549 cells viability significantly correlated with transitional metal content in the coarse fraction of ambient PM [101]. On the other hand, Marchetti et al. demonstrated that exposure to indoor PM_{10} significantly increased the HO-1 expression in A549 cells, independently of their chemical compositions [106]. As opposed to HO-1 levels, A549 cells exposed to PM_{10} collected in urban and rural sites for 48h (50 μg/ml) showed a significant decrease in SOD activity (>50%) [80]. A similar inhibitory effect was noted after exposure to PM_{10} from a commercial zone (10 μg/cm^2) for 24h. In the study, reduced activity of SOD, glutathione reductase (GR) and catalase by 65%, 61.5% and 31.2%, respectively, was observed [120]. The authors suggest that PM_{10} particles cause the inhibition of enzyme activity by direct interaction with their molecules or by mediators such as metal ions and PAH. On the other hand, a decrease in enzymatic activity can be caused by PM_{10}-induced cytotoxicity [120]. Microarray analysis of the gene expression profile of BEAS-2B cells after 24-h exposure to PM_{10} also confirmed previous reports on the significant increase among genes participating in the cellular response to OS. Extension of cell exposure time to 96 hours expressed in terms of changes involving cell mobility and lipid metabolism was revealed [123].

It is well established that mitochondria are the main source of ROS but little is known about PM_{10} exposure-induced mechanisms involved in mitochondrial alternations. The level of structural remodelling, including changes in their shape, an increase of intermembrane spaces and disruption in cristae, as well as the decrease in dense granules, were observed in mitochondria isolated from rat lung tissue after PM_{10} exposure in a concentration-dependent manner [124]. Functional disorganisation noted in those organelles after exposure to PM_{10} involved

the dysfunction of the respiratory chain, reduction of oxygen consumption and ATP synthesis [124], as well as a decrease in membrane potential and enlarged activity of apoptotic mediators like caspase-3/7 [125]. The important role of the mitochondria-targeted gene, neuronal pentraxin 1 (*NPTX1*), in cellular protection against PM_{10} exposure was also emphasised in human olfactory mucosa (hOM) cells [125]. *NPTX1* is a neuron-specific gene that targets the mitochondria to trigger the pro-apoptotic signalling cascade including BAX regulation. The activated BAX protein translocates from the cytosol to mitochondria and forms a destructive membrane pore, which leads to cell death [126]. The exact protective mechanism of NPTX1 protein on hOM cells has not been explained yet, but a lack of this protein dysregulates the controlled mitochondrial ROS production mechanism and was strongly associated with the extension of PM-induced adverse effects on cellular metabolism and increased mitochondrial ROS production. What is more, mitochondrial defects caused by PM exposure cannot be restored by transient suppression of *NPTX1* in hOM cells [125].

4. Relationship between epithelial dysfunction caused by PM_{10} and exacerbation as well as morbidity in asthma and COPD

Asthma and chronic obstructive pulmonary disease (COPD) are obstructive lung diseases in which airflow limitation caused by chronic inflammation is a fundamental pathophysiological symptom. Susceptibility to asthma and COPD is genetic but environmental factors such as allergens, which increase the risk of asthma and tobacco exposure - the main cause of COPD, are also of great importance. It is considered that the type of inflammation that occurs in the airways in asthma and COPD is different. In asthma, two principal phenotypes are most common: allergic (in which asthma symptoms are induced by allergen exposure), and non-allergic. In the asthmatic immunological response, T-helper cells infiltrate the airways in abundance and induce inflammation by secretion of chemokines and cytokines (e.g. IL-4, IL-13 and IL-5). The pro-inflammatory molecules promote migration and activation of effector inflammatory cells including eosinophils, mast cells, basophils and leukocytes [127,128]. Inflammation observed in COPD is characterised by airflow limitation that is progressive and non- or only partially reversible. The inflammation in COPD is characterised by the presence of CD8+ T lymphocytes, macrophages and neutrophils [129]. Different cellular profile and prevalence of Th1 response are associated with an airway cytokine panel which differs from asthma. The most characteristic is IL-8, a strong chemoattractant for neutrophils and macrophages, IL-1β, IL-6, MMPs and TNF-α which promote and support local inflammation in the lungs.

The pathophysiology of both diseases is associated with dysfunction of the respiratory epithelium, its remodelling or damage. Airway epithelial cells take part in the local wound repair. The remodelling process starts when the epithelial injury and repair are abnormal, for example, in asthma [130]. These structural changes are characterised by epithelial goblet cell hyperplasia and metaplasia, an increase in bronchial smooth muscles mass and an increase in airway wall thickness. Transforming growth factor β (TGF-β) (which is released from damaged/repairing epithelium in response to inflammatory mediators, such as IL-13) and epithelial growth factor (EGF) are the main mediators of remodelling in asthma [131]. The airway remodelling, which includes hyperplasia of airway epithelial cells, thickening of the basement membrane, collagen deposition, and bronchial smooth muscle cell proliferation, is a hallmark of asthma. In COPD, two main phenotypes associated with different epithelium injury organisation are observed: emphysema and chronic bronchitis [132]. Long-lasting OS, airway inflammation and epigenetic changes are the leading mechanisms in respiratory epithelium causing the development and exacerbation of respiratory diseases, such as asthma and COPD [133]. ROS, the formation of which is mediated via transition metals from ambient PM, contributes to airway epithelium damage and increased inflammation as well as airway hyperresponsiveness [134]. It can lead to an increased number of exacerbations of obstructive lung diseases.

4.1. Epidemiological and in vivo studies of PM_{10} association with asthma and COPD development and exacerbations

The incidence of asthma and COPD, as well as the increased exacerbation rate of these diseases, are correlated with the elevated concentration of harmful dust in the surrounding atmosphere, which suggests a significant contribution of air pollution to the course of obstructive pulmonary diseases. Air pollutants are significantly linked to increased asthma and COPD exacerbation frequency as well as hospitalisations [135,136]. An association between an increased level of airborne PM_{10} and SO_2 (during solid fuel usage in the heating period in Turkey), as well as a significantly higher number of COPD and asthma hospital admissions in comparison to the non-heating period, was shown [137]. In this study, the level of airborne PM_{10} highly correlated with the number of patients with COPD. In addition, a linear relationship was found between the levels of PM_{10} and SO_2 as well as the number of COPD hospital admissions. Intensive exposure to PM_{10} and endotoxins included in PM are important factors of asthma pathogenesis [138]. Islam et al. [139] examined a cohort of healthy children to assess the long-term influence of exposure to airborne pollutants on respiratory health. The authors associated better lung function at the baseline of the study with a reduced risk of asthma development in the teenage years. It was also determined that exposure to elevated concentrations of fine and coarse PM as well as gaseous pollutants significantly diminish this effect [139]. Endotoxin load in PM_{10} emitted by livestock farm has the potential to impact human health, depending on endotoxin concentration. Lower endotoxin concentrations are considered as securing agents while their increased levels have a possible adverse effect on residents in the context of respiratory symptoms [140].

The association between asthma development and exposure to air pollution was analysed using data from a large study group based on six European cohorts. The most important associations between asthma incidences and airborne pollutants were stated for nitrogen oxides and dioxides, but not for $PM_{2.5}$, $PM_{10,}$ as well as for traffic load and its intensity. These results do not allow to draw unequivocal conclusions on the harmful effect on airways leading to adult-onset asthma [141]. James et al. observed a significant association between 3-day averaged ambient PM_{10} concentrations and emergency admissions as well as hospitalisations for asthma exacerbations in a rural community. Each 15 $\mu g/m^3$ increase in ambient PM_{10} resulted in a 3.1% increase in asthma symptoms demanding urgent hospital visits among all adult patients, and a 5.0% increase in the likelihood of an asthma event requiring hospital admission in children [142]. Another study showed a 7.2% increase in emergency admissions for asthma among young children for each 5.92 $\mu g/m^3$ 3-day mean $PM_{2.5}$ concentration. The authors of that study revealed an association between these emergency visits for respiratory diseases in low-polluted areas and enlarged glutathione-related oxidative potential of airborne $PM_{2.5}$ [143]. Hwang et al. also suggested a higher susceptibility of children to the harmful effects of short-term $PM_{2.5}$ exposition on asthma emergency room visits in comparison to adults. The authors considerably associated asthma emergency admissions and nitrate levels, which constitute a component of $PM_{2.5}$ fraction [144]. An observational study showed that school children living in urban areas were exposed to higher concentrations of indoor fine and coarse PM than their peers residing in rural areas. This exposure was associated with an increased level of IL-6 measured in induced sputum implicating a significant impact on the prevalence of respiratory symptoms, which lead to improper development of childrens' lungs [145]. Exposure to elevated levels of airborne pollutants at a young age impairs respiratory health for life.

The interaction between airborne PM and biological allergens affects allergic diseases. Combined exposure to both of these factors is considered to have a synergetic or even additive harmful effect on respiratory health [146]. Interestingly, a significant association was found between

outdoor activity duration and provocation of allergic symptoms such as nasal obstruction, sneezing, rhinorrhea, itching and sleep disturbance implicating a higher effect of the time-dependent exposure than the PM_{10} concentration itself on increased allergy symptoms [147].

Exposure of susceptible residents to wildfire smoke resulted in adverse health outcomes [148]. During the period of major fires, when the daily mean $PM_{2.5}$ concentration reached 89.1 μg/m^3, researchers noted an immediate 34% increase in emergency room visits for overall respiratory conditions and a 112% increase for asthma exacerbations. Exposition to wildfire PM caused a lagged effect manifested as a 72% increase in acute bronchitis cases in five days following the peak fire period, when the daily mean concentration dropped to 9.33 μg/m^3. In this research, effects after wildfire PM exposure on children's respiratory health was also considered because of its possible long-term consequences to children's lung health. Emergency admissions for asthma among children aged less than 1 had a 243% increase, while among children aged 1-4, a 136% increase was noted [148]. Elevated levels of airborne zinc were also related to a 123% increase in emergency room visits for paediatric asthma exacerbations on the following day. In the same study, synergistic effects of zinc with other co-pollutants such as nitrogen dioxide, carbon monoxide and elemental carbon nitrogen dioxide were also marked [149]. The study was based on a validated Asthma Quality of Life Questionnaire (AQLQ) conducted among patients with asthma and revealed decreased scores associated with increasing exposure to PM_{10} from ambient air. The risk of impaired quality of life related to PM_{10} exposure was the highest in terms of symptoms regardless of the concentration of this air pollutant, while in terms of activity limitation, emotional functioning and environmental stimuli, the risk was significantly higher only among patients exposed to more than 80 μg/m^3 of airborne PM_{10} [150]. Not only PM but also meteorological factors affected respiratory health. A higher number of emergency room visits for respiratory-related conditions such as allergic rhinitis, acute bronchitis and asthma was associated with increased PM levels and decreased relative humidity. An increase in the incidence of these events was noted especially among children and people of senior age [151].

Measurement of fractional exhaled nitric oxide (FeNO) is one of the respiratory tests used in asthma management. An increased level of FeNO is considered as one of the markers of eosinophilic airway inflammation in asthma diagnosis [152]. Choi et al. found a positive correlation between high levels of PM_{10} and FeNO values both in asthma patients and healthy subjects, as well as showed that FeNO values in healthy people elevated to 8.3 ppb if the PM_{10} concentrations increased by 100 μg/m^3. These results indicate that exposure to PM_{10} boosts respiratory inflammation [153].

In COPD, abnormal inflammatory response can also be triggered by airborne particles or gases [154]. Short-term average exposure to PM_{10} was linked with increased non-accidental mortality among patients with COPD and with asthma-COPD overlap [155]. Increased vulnerability of women and never smokers to the hazardous effects of PM_{10} exposition was also revealed [155]. Zhu et al. [156] conducted a systematic study evaluating the relation between concentrations of airborne PM_{10} and hospital admissions as well as mortality related to COPD. The study showed a 2.7% increase in hospital visits and a 1.1% increase in COPD fatality for each 10 μg/m^3 PM_{10} concentration, especially among patients from developing countries and the elderly population. Another research demonstrated that a short-term increase in airborne ozone and PM_{10} levels was associated with pneumonia and COPD hospitalisations. The authors noted a 0.41% and 0.27% increase in COPD hospital admissions for pneumonia and COPD, respectively, as a result of 5-ppb increase in airborne ozone concentration. On the other hand, a 1.47% and 0.84% increase in emergency visits for COPD and pneumonia, respectively, were observed for each 10 μg/m^3 PM_{10} increase in ambient air [157]. Similar results were obtained by Ko et al. [158]. The authors associated the higher number of hospitalisations for acute COPD exacerbations with increased concentrations of air pollutants such as SO_2, NO_2, fine and coarse PM, but the relative risk of O_3 exposure was the highest. It was also shown that each 10 μg/m^3 increase in airborne PM_{10} and $PM_{2.5}$ resulted in a 2.4% and 3.1% increase in COPD emergency admissions, respectively [158]. Exposure to PM_{10} from natural sources such as dust storms also contributes to an increased number of emergency visits for COPD exacerbation. The highest number of admissions was noted two days after the event when the relative risk for PM_{10} exposition was 1.05 for each 10 μg/m^3. It can be beneficial, especially for susceptible subjects, to reduce outdoor activity during dust storms; this would probably contribute to a decrease in acute exacerbations of pulmonary diseases [159]. The association between $PM_{2.5}$ and PM_{10} concentrations of up to 150 μg/m^3 with an elevated risk of respiratory symptoms, such as chronic cough, wheezing, chest tightness and prevalence of chronic bronchitis, allergic rhinitis, asthma and especially COPD, was demonstrated [160]. Liu et al. noted a decline in respiratory function in patients exposed to airborne PM. Each 10 μg/m^3 increase in PM_{10} was related to a 14 ml decrease in forced expiratory volume in the first second (FEV_1) and 16 ml in forced vital capacity (FVC) as well as a 0.024% reduction in FEV_1/FVC [160].

4.2. The impact of PM_{10} on the immune response of airways in asthma

Results of *in vitro* studies indicate that exposure to airborne PM contributes to onsets of asthma via heterogeneous mechanisms. Airway epithelial cells are involved in the regulation of airway response to ambient air pollution [161]. Thymic stromal lymphopoietin (TSLP) synthesised by these cells seems to be a crucial alarmin linking the innate and adaptive immune responses to airborne particles that can mediate an allergic reaction, which may result in asthma development. Exposure of human bronchial epithelial cells to ambient PM upregulated TSLP synthesis, which promotes Th2-related response. One of the proposed mechanisms of increased TSLP expression may be mediated by human mi-RNA (hsa-miR-375) with regulatory influence on the aryl hydrocarbon receptor (AhR) [162]. An elevated risk of adult-onset asthma development was associated with an increased plasma concentration of IL-1 receptor agonist (IL-1RA) [163]. IL-1RA belongs to the IL-1 protein family and acts as a natural inhibitor of IL-1β by binding to IL-1 receptors. It is also considered to adjust the exacerbation of immune response by suppressing the synthesis of mediators like IL-6, IL-8 and TNF-α [164]. Moreover, the authors of the mentioned study observed a positive association between the levels of IL-1RA and long-lasting exposure to all tested air pollutants, PM_{10}, $PM_{2.5}$ and NO_2 [163]. Also, higher concentrations of macrophage inflammatory protein-1 beta (MIP-1β) and C-reactive protein (CRP) were correlated with the increased level of IL-1RA after exposure to UFPs. Further analysis showed that the indirect effect of the immune system accounts for 15% of the total effect of airborne pollution on asthma incidence in adults and could be upregulated in the group of patients with allergic asthma and overweight subjects [163]. In a murine model of mild chronic asthma, the intranasal exposure to PM_{10} resulted in an increased eosinophil and neutrophil influx as well as upregulation of IL-33 expression and protein production characteristic for the disease exacerbation induced by allergens [165]. IL-33 is a member of the IL-1 cytokine family and has a dual function in cells. It may act both as a classic cytokine, as well as an intracellular nuclear factor, functioning as a transcription regulator associated with the induction of Th2-type immune response and IL-4, IL-5 and IL-13 synthesis [166]. As IL-33 mediates the release of relevant inflammatory mediators involved in the exacerbation of chronic allergic asthma, it could be considered as a potential therapeutic target to downregulate airway inflammation [165]. The hazardous mechanisms of PM_{10} in asthmatic epithelium are shown in Fig. 2.

Bayram et al. [71] noted a significant increase in IL-8, GM-CSF and soluble intercellular adhesion molecule-1 (sICAM-1) after diesel exhaust particle (DEP) exposure in asthmatic epithelium cultures compared to non-asthmatic ones. What is more, only in asthma group the release of chemokines like regulated on activation, normal T-cell expressed and

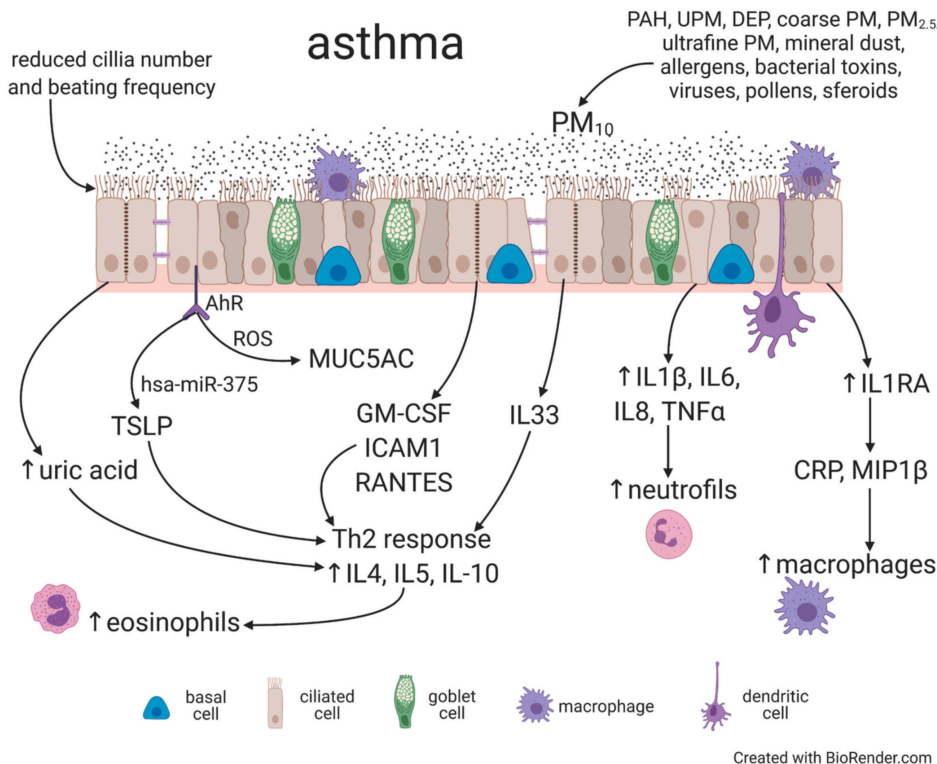

Fig. 2. Hazardous mechanisms of PM_{10} in asthmatic epithelium.
Cilia number reduction and their improper function are the features of asthma which lead to inefficient particle clearance. The accumulated particles in the airways interact with epithelium by several mechanisms. Oxidative stress induced by PM_{10} exposure results in mucus overproduction. After contact with airborne particles epithelium releases alarmins such as TSLP, IL-33, GM-CSF, ICAM-1, RANTES and uric acid which are involved in Th2 response and leads to eosinophilic inflammation in the lungs. Asthmatics epithelium after PM_{10} stimulation produces chemical signals such as IL-1β, IL-6, IL-8 and TNF-α which attract neutrophils. Additionally, epithelial derived cytokines induce CRP and MIP1β synthesis which lead to macrophage accumulation their overstimulation and dysfunction.

secreted (RANTES) was observed. Overexpression of these mediators caused by higher susceptibility of the asthmatic epithelium to DEP exposure is considered to be a key mechanism leading to the exacerbation of asthma symptoms [71]. Stimulation by high doses of PM_{10} in a murine model of asthma (250 μg per mouse) not only contributed to enhanced neutrophil influx and induction of Th1 cell-related cytokine production (TNF-α and IFN-γ) but also resulted in allergic-like immune responses, including elevated eosinophil recruitment and increased production of Th2-related cytokines (IL-5 and IL-13) [167]. IL-13 is an important mediator in asthma pathophysiology – this cytokine causes airway remodelling through smooth muscle cell hypertrophy, fibroblast proliferation and hypersecretion by airway epithelial cells [168]. Increased number of eosinophils affects the respiratory epithelial integrity and vascular permeability. Furthermore, ROS and other mediators released by these white blood cells resulted in mucus overproduction and airway smooth muscle constriction [169], which constitutes the symptoms of asthma.

In humans, purine metabolism by xanthine dehydrogenase (XDH) leads to uric acid generation, which cannot be catabolised because of a non-functional enzyme - uricase and can be found in the lower and upper airways even in healthy subjects [170]. The soluble fraction of uric acid released by airway epithelial cells is considered an antioxidant, while its overproduction may lead to its precipitation and trigger innate immune response [171]. Interestingly, researchers found a link between pulmonary uric acid production and Th2-related allergic sensitisation to house dust mite (HDM) [172]. The airborne PM_{10} exposure in mice, similarly to HDM, selectively induces uric acid production in the airway mucous membrane [173]. This study revealed that airway epithelial cells can produce an active form of uric acid and are able to release it by active transport via MRP4 (multidrug resistance-associated protein 4), an ATP-dependent urate export transporter. The authors hypothesised that uric acid production in the mucosa constitutes the supporting mechanism of PM_{10}-associated allergic sensitisation, as well as coordinates the proliferation of antigen-specific T-cells [173]. On the other hand, the secretome of airway epithelial cells pre-exposed to PM_{10} impacts the maturation of dendritic cells independently of the soluble uric acid pathway [174].

4.3. The impact of PM_{10} on airways in COPD

The impact of air pollution on the pathophysiology of COPD has been widely reported [135]. Prolonged chronic exposure to $PM_{2.5}$ resulted in decreased lung function, emphysematous destruction by OS, proteolysis and apoptosis of lung parenchyma as well as airway inflammation in COPD patients [175]. UFPs, especially particles with an aerodynamic diameter between 0.1 and 0.001 μm, tend to concentrate in emphysematous lesions and initiate the inflammation constituting the mechanism of COPD development or accumulate in the lung regions with pre-existing inflammation, causing further harmful effects [176]. This intensified OS can cause upregulated transcription of pro-inflammatory genes via the NF-kB pathway and histone acetylation [177]. Histone modification resulted in DNA unfolding and enlarged transcription of pro-inflammatory genes [178]. Long-lasting exposure to high amounts of airborne PM induced small airway remodelling involving tissue

proliferation and wall thickening, which, in some cases, resulted in COPD-like airflow limitation [179]. Elderly people with an age-related reduction of antioxidant defence are especially inclined to more pronounced inflammatory responses after ambient PM exposure. Another possible mechanism of COPD progression associated with airway pollution is acute adenoviral infection co-existing with PM_{10} exposure or presence of latent adenoviral infection [180,181], both of which enhanced the pro-inflammatory effects of inhaled particles. Elevated transcription of pro-inflammatory genes was also triggered by free radical-mediated OS and increased the intracellular calcium level in macrophages after exposure to ultrafine carbon black particles [182]. The relationship between ambient air pollution and lung function along with COPD susceptibility factors has been widely investigated. The relation between impaired lung function and elevated exposure to all tested airborne pollutants was revealed in COPD patients, while COPD incidence was linked only with elevated concentrations of $PM_{2.5}$, PM_{10} and NO_2, but not with the coarse fraction of PM [183]. This study revealed a stronger impact of air pollution on subjects with excess body fat, those from a lower income group, individuals with occupational exposure to fumes or dust, and males. Schikowski et al. [184] analysed the results of participants from four cohorts who met the criteria for the European Study of Cohorts for Air Pollution Effects (ESCAPE). These researchers observed some differences in the prevalence and incidence of COPD in the tested groups. A weak association was revealed between long-lasting exposure of residents to PM_{10}, NO_2 and traffic rate as well as COPD prevalence. The only significant relationship was established between the traffic rate and the prevalence of COPD in women, as well as COPD incidence among women and never smokers [184]. The role of ambient air pollution, as well as gender-dependent factors, in the development and incidence of COPD, seems to be ambiguous and uncertain. Exposure to airborne PM disrupts proper lung function, which may result in acute respiratory symptoms. Accumulation of damages caused by PM in the respiratory tract seems to be a credible explanation linking ambient air pollution and COPD development [185], but there is no sufficient evidence for this hypothesis. COPD patients were more vulnerable to PM_{10}–induced genotoxicity in comparison to healthy subjects [186]. In this study, the researchers observed an increased risk of buccal cells with micronuclei and binucleated cell formation in the group of patients with COPD during periods when the airborne PM_{10} level exceeded 50 $\mu g/m^3$. The above mentioned cell types are considered to appear due to DNA damage caused by OS and failure of cytokinesis, respectively [186]. The cells from patients with moderate to very severe stages of COPD exposed to high levels of airborne PM_{10} responded with a significantly higher fraction of cells with condensed chromatin, constituting a well-established feature of ongoing apoptosis associated with COPD progression [186,187].

Exposure to high airborne PM_{10} concentrations during a short period of seasonal smog resulted in a significant reduction of pulmonary function parameters, such as FEV_1 and FVC, exacerbation of symptoms, including sleep disturbance and chest tightness, leading to a reduction in the quality of life in COPD patients, regardless of the severity of the disease [188]. The study conducted among patients from Korean COPD in Dusty Areas (CODA) cohort revealed that long-lasting exposure to PM_{10} was significantly associated with decreased lung function manifested as reduced FVC and exacerbated emphysema, as well as increased airway wall thickness, which is considered as an indicator of disease severity [189]. Moreover, Chen et al. [190] associated a long-term residential-site exposure of elderly people from Taiwan to selected fractions of ambient PM, fine and coarse PM, with decreased lung function. The authors observed an intensified negative impact of exposure to $PM_{2.5\text{-}10}$ on airway function parameters associated with a decrease of FEV_1 as well as reduced maximal mid-expiratory flow (MMEF) [190]. The impact of air pollution on airway inflammation in patients with COPD may also be evaluated in induced sputum. The baseline levels of sputum MMP-9, MMP-3 and pulmonary surfactant protein (SP-D) were higher in patients with stable COPD compared with the control group [191]. Further analysis showed that short-term exposure of COPD patients to $PM_{2.5}$, PM_{10}, NO_2, and CO can cause neutrophil-mediated airway inflammation and resulted in increased levels of MMP-9 and IL-8, which contributed to acute exacerbation of symptoms related with COPD [191]. The possible harmful effects of PM_{10} in COPD are presented in Fig. 3.

5. Conclusions

In this paper, we characterised PM_{10} based on the physical characteristics of particles included in this fraction, their origin, biological effects on living organisms and contribution to innate immunity, oxidative stress and inflammatory processes. A large number of studies indicates the toxic effects of PM_{10} and its important impact on asthma and COPD pathophysiology. Concentrations of airborne pollution and PM_{10} should be considered as a potential contributor exacerbating the symptoms of asthma and COPD. Further research to fully understand this issue, as well as an investigation focused on the targeted treatment of PM_{10}-mediated processes leading to asthma and COPD exacerbations, is also needed.

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Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

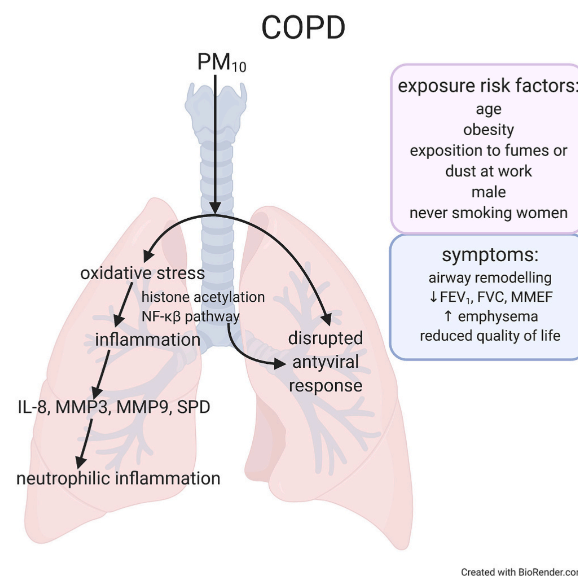

Fig. 3. PM_{10} harmful effects in COPD.
Airborne particles inhaled by patients with COPD contribute to several harmful processes. They induce oxidative stress and inflammation which lead to enhanced production of alarmins and neutrophil accumulation in the lungs. Additionally, air derived pollutants disturb antiviral response. Negative impact of PM_{10} exposure is related to age, gender, BMI, working conditions and smoking status. What is more, chronic stimulation by PM_{10} results in airway remodelling, increased emphysema, decreased lung function parameters and worsening of overall life quality among COPD patients.

Consent to participate

Not applicable

Data availability

Authors' contributions MPG brought the concept and overall manuscript design. PMS wrote the first manuscript. MPG, PMS edited, proofread and finalised the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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II. Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD

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Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD

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Urban particulate matter (UPM) is an important trigger of airway inflammation. The cross-talk between the external and internal matrix in the respiratory tract occurs due to the transepithelial network of macrophages/dendritic cells. This study characterized the immune processes induced by the epithelium after UPM exposure in special regard to interactions with monocyte-derived dendritic cells (moDCs) and monocyte-derived macrophages (moMϕs) in obstructive lung diseases. A triple-cell co-culture model (8 controls, 10 asthma, and 8 patients with COPD) utilized nasal epithelial cells, along with moMϕs, and moDCs was exposed to UPM for 24 h. The inflammatory response of nasal epithelial cells to UPM stimulation is affected differently by cell–cell interactions in healthy people, asthma or COPD patients of which the interactions with DCs had the strongest impact on the inflammatory reaction of epithelial cells after UPM exposure. The epithelial remodeling and DCs dysfunction might accelerate the inflammation after air pollution exposure in asthma and COPD.

Environmental conditions significantly affect human health. Air pollution may alter the health status on different levels (individual patients and societies) and based on different mechanisms. These include not only a harmful effect but also changes in gene expression as well as metabolic and inflammatory pathways in the respiratory and cardiovascular system. The increased morbidity and mortality caused by air pollution are largely related to exposure to particulate matter (PM) with an aerodynamic diameter from 10 µm (PM_{10}) to less than 2.5 µm ($PM_{2.5}$), especially in people with pre-existing obstructive lung diseases[1,2]. Urban particulate matter (UPM) is a mixture of predominating PM_{10} and less amount of $PM_{2.5}$[3]. When inhaled, large PM are mainly deposited in conducting airways where the smallest particles can even reach small peripheral airways, including terminal bronchioles and alveoli. Several factors were identified in $PM_{2.5}$ from rural and urban areas including secondary sulphate, secondary nitrate, biomass burning, gasoline combustion, diesel combustion, dust, industry and winter salt[4]. The air pollutants are significantly associated with an increased risk of asthma exacerbation and hospitalizations[5]. Associations between chronic obstructive pulmonary disease (COPD) and air pollution have also been widely reported[6]. It was shown that chronic exposure to $PM_{2.5}$ resulted in decreased lung function, development of emphysematous lesions and enhancement of airway inflammation in COPD patients[7].

The mechanisms of the harmful effect of ambient PM on the airways of asthma and COPD patients have not been sufficiently explained. This refers in particular to the interactions between PM and airway epithelial cells that form a layer of direct contact between airway pollution and the human respiratory tract. Due to a variety of inhaled chemical compounds, complex organization and multiple interactions occurring within airway epithelium, the tissue could be affected in many deleterious ways. A Fe-rich PM_{10} fraction from an underground railway station can penetrate the mucus layer and enter primary bronchial epithelial cells (PBEC)[8]. Bronchial epithelial cells exposed to increasing concentrations of urban PM_{10} containing Cu, Ni, Zn and endotoxins show significantly enhanced dose-dependent production of IL-8.

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	Control n = 8	Asthma n = 10	COPD n = 8	Overall p value[a]	Pairwise p value*		
					Asthma versus control	COPD versus control	Asthma versus COPD
Age (years)	38.5 (32.5–48)	55 (38–62)	62 (59.5–72.5)	0.005	0.138	0.0002	0.138
Gender (F/M)	6/2	3/10	5/3	0.046			
BMI (kg/m^2)	22.1 (20.7–24.1)	26.9 (26–27.7)	28 (25.4–30.3)	0.002	0.002	0.0003	0.696
Atopy (n)	3	8	2	0.03			
Smoking exposure (pack-years)	0 (0–0)	0 (0–4)	32.5 (22.5–50)	0.0002	0.277	0.0002	0.0003
FEV_1 (% predicted)	103 (81–111)	81 (75–94)	61.5 (51.5–76.5)	0.006	0.043	0.004	0.034
FEV_1/VC (%)	106 (81.8–112)	82 (75–86)	54 (50–68)	0.0003	0.02	0.0006	0.0004
FeNO (ppb)	11.0 (9.3–12.6)	52.3 (31.3–77.6)	17.4 (12.6–26.1)	0.0047	0.03	0.333	0.003
ACT (points)	N.A	20.5 (17–25)	N.A	N.A	N.A	N.A	N.A
ICS treatment (n)	N.A	6	1	N.A	N.A	N.A	N.A
CAT (points)	N.A	N.A	10.5 (8–15)	N.A	N.A	N.A	N.A
mMRC (points)	N.A	N.A	1.5 (1–3)	N.A	N.A	N.A	N.A

Table 1. The patients' characteristics. Data are presented as median (IQR) or n. *ACT* Asthma control test, *BMI* body mass index, *FeNO* fractional exhaled nitric oxide FEV_1 forced expiratory volume at first second, *ICS* inhaled corticosteroids, *mMRC* modified Medical Research Council, *N.A.* not applicable, *VC* vital capacity. [a]Kruskal Wallis or Chi square test, *Mann–Whitney U test.

PM_{10} and $PM_{2.5}$ exposure induces oxidative stress and inflammation in the airways. Many animal and in vitro studies reported increased mRNA expression and protein secretion of IL-1α, IL-1β, IL-6, IL-8, TNF-α in epithelial cells after treatment with PM[9–11]. Some research indicated the acceleration of Th2 dependent inflammation in the airways after air pollutant exposure as well as upregulation of TSLP and IL-33 expression[12,13]. Moreover, PM_{10} stimulation is related to epithelial cell dysfunction and remodeling by inducing an increased matrix metalloproteinases (MMPs) activity and downregulation of E-cadherin/b-catenin or claudin in these cells[14,15]. Chronic exposure to air pollution contributes to airway remodeling and mucus hypersecretion[16,17]. The expression of epidermal growth factor receptor (EGFR) ligands was upregulated in human airway epithelial cells exposed to urban $PM_{2.5}$[18]. Additionally, $PM_{2.5}$ had been connected to epithelial-mesenchymal transition (EMT) pathogenesis. The components of $PM_{2.5}$ activated EMT related gene expression (e.g. transforming growth factor β (TGF-β), extracellular signal-regulated protein kinase (ERK), phosphatidylinositol 3-kinase (PI3K), high mobility group box B1 (HMGB1), receptor for advanced glycation end-products (RAGE), aryl hydrocarbon receptor (AHR)) in epithelial cells, which finally leads to changes in the morphology and functions of airway epithelia[19]. All these mechanisms contribute to epithelium dysfunction, which is a dominant factor of obstructive lung disease pathophysiology.

An important component of airway defence is a transepithelial cellular network, which includes macrophages and dendritic cells (DCs). These cells play the role of sentinels against foreign environmental antigens. Macrophages simultaneously encounter inhaled stimuli and regulate the local inflammatory response. Exposure to medium from PM_{10} treated alveolar macrophages (AMs) upregulated the mRNA expression of IL-1β, leukemia inhibitory factor (LIF) and IL-8 in human bronchial epithelial cells (HBEC)[20]. Co-cultivation of AMs with HBEC exposed to PM_{10} increased the production and release of tumor necrosis factor alpha (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-6, IL-8, LIF and oncostatin M in comparison to control co-cultures.

Understanding how information about UPM signaling is processed and translated into distinct cellular mechanisms remains elusive. In order to better explore cellular responses and interactions in patients with obstructive airway diseases, we undertook a study aimed at the evaluation of (1) the immune response (IL-1β, IL-6, IL-8, MMP7, MMP9, TSLP, IL-33) of airway epithelial cells co-cultured with monocyte-derived dendritic cells (moDCs) and monocyte-derived macrophages (moMφs) to UPM stimulation, (2) the impact of air pollutants on epithelial integrity and remodeling markers expression (TGF-β, EGFR, ST2) in triple co-cultures.

Material and methods

Patients' characteristics. This was a prospective, cross-sectional study, which involved 8 healthy controls, 10 asthma patients, and 8 patients with COPD. In all patients, the diagnosis of asthma or COPD was previously established according to the current recommendations of the Global Initiative for Asthma (GINA) and the Global Initiative for Chronic Obstructive Lung Disease (GOLD)[21,22]. Details on the patients' examination are available in the supplementary file. The control group consisted of smoking and non-smoking volunteers, with normal spirometry. The clinical characteristics of patients and controls recruited for the study are summarized in Table 1. Nasal brushing and peripheral blood samples were obtained from each participant. The study protocol was approved by the Ethics Committee of the Medical University of Warsaw (KB/37/2020) and informed written consent was obtained from all the participants.

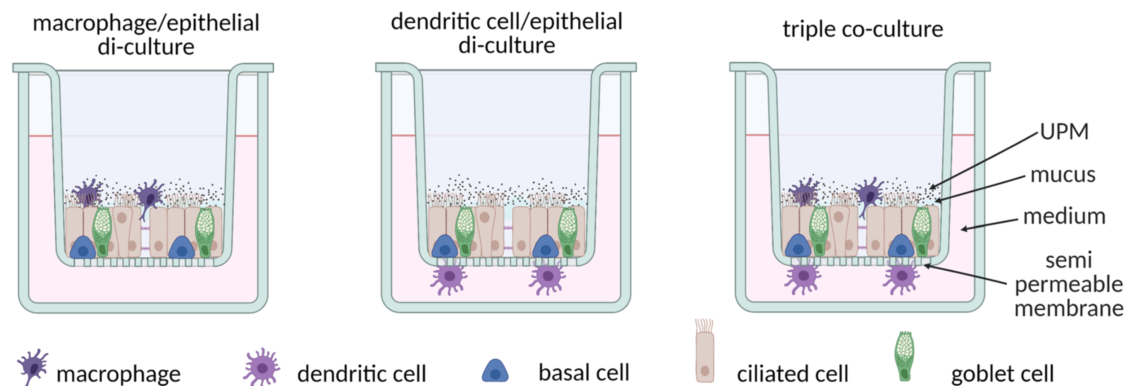


Figure 1. The scheme of di- and triple-co-cultures used in the study.

Cell cultures: epithelial cells in an air–liquid interface (ALI) with monocyte-derived dendritic cells (moDCs) or monocyte-derived macrophages (moMϕs) culture and triple co-culture. The study used a triple-cell co-culture model: the nasal epithelial cells cultured in air–liquid interface (ALI) conditions were grown on a microporous membrane in a two-chamber system (Greiner Bio-One, Austria) with monocyte-derived macrophages (moMφs) placed on the top and monocyte-derived dendritic cells (moDCs) placed underneath the epithelial monolayer (Fig. 1). moMφs added on the apical side of the epithelial monolayer were suspended in 35 µl of macrophage medium DXF (Promocell, Germany). moDCs attached to the basal site of inserts with epithelium monolayer were cultured for 24 h in ALI maintenance medium (Stemcell, Canada). Each experiment was performed on triple-co-cultures containing nasal epithelial cells, moMφs as well as moDCs of the same individual. Nasal epithelial cells were obtained by brushing (Cytobrush Plus GT, CooperSurgical, Germany) the inferior surface of the middle turbinate of both nostrils. Macrophages and DCs were specialized from monocytes obtained from a peripheral blood sample. The cells were isolated, cultivated and specialized as previously described[23] with the protocols of the procedures available in the supplementary file.

The overall scheme of the study. Epithelial cells were cultured with or without stimulation with 100 µg/ml UPM for 24 h as follows:

1. Epithelial cells,
2. +moDCs (co-culture),
3. +moMφs (co-culture),
4. +moMφs + moDCs (triple co-culture).

The scheme of di- and triple-co-culture models used in the study is illustrated on Fig. 1.
Additionally, a combination with previously 24 h UPM treated moMφs was used in co-cultures of:

1. moMφs (24 h UPM) with unstimulated epithelium,
2. moMφs (24 h UPM) + moDCs with UPM stimulated epithelium.

Particles preparation. Urban particulate matter was obtained from the Silesian University of Technology. The samples were collected with a low-volume PM sampler type PNS-15 (Atmoservice) 1.5 m above the ground, at a flow rate of $2.3m^3\ h^{-1}$ in Zabrze, Gliwice or Żory during heating season[24]. PM samples were collected on high-purity quartz (SiO_2) microfiber filters (QM-A Whatman). The filters were weighed, cut into small pieces, suspended in PBS; the particles were detached from filters by sonication, and autoclaved.

UPM trace elements analysis. The QM-A filter was mineralized under high pressure and high temperature in a system for microwave mineralization in 8 ml HNO_3 and 2 ml H_2O_2 (Merck, Germany). Elemental concentrations of nine elements (Zn, Fe, Mn, Pb, Cd, Cu, Cr, Ni and Co) were analysed for each sample by atomic absorption spectrometry (Avanta PM, GBC Scientific Equipment Pty Ltd, Melbourne, Australia)[24,25]. The concentrations of heavy metals in UPM are shown in Table 2.

Cell viability. Cell viability was determined by a fluorescence cell counter (Luna, Logos Biosystems, South Korea) using acridine orange/propidium iodide (PI) staining and a flow cytometric using annexin/PI staining (Alexa Fluor 488 annexin V/PI, Thermo Fisher, MA, USA) according to the manufacturer's protocol.

Measurements of cytokine mRNA expression changes. Quantitative real-time PCR was performed to assess the mRNA expression of IL-1β, IL-6, IL-8, MMP7, MMP9, TSLP, IL-33 and 18 s rRNA in epithelial cells.

Elements	Concentration
Dust mass (g)	3.65 (3.13–4.08)
V (m^3)	55.11 (54.60–55.11)
Zn	
ng/m^3	102.89 (97.19–161.94)
μg/g	1467.48 (1063.76–2075.17)
Fe	
ng/m^3	306.47 (232.74–386.93)
μg/g	4557.63 (3479.91–5727.20)
Mn	
ng/m^3	17.02 (13.11–21.30)
μg/g	222.44 (182.32–271.68)
Pb	
ng/m^3	39.55 (34.77–64.25)
μg/g	562.24 (434.74–737.63)
Cd	
ng/m^3	5.96 (4.63–8.10)
μg/g	93.45 (65.42–106.48)
Cu	
ng/m^3	11.70 (9.66–14.15)
μg/g	143.93 (126.53–199.25)
Cr	
ng/m^3	51.58 (40.52–70.65)
μg/g	662.14 (589.04–832.97)
Ni	
ng/m^3	78.88 (57.98–88.90)
μg/g	1032.39 (653.65–1406.09)
Co	
ng/m^3	22.73 (22.68–23.00)
μg/g	342.0 (306.50–400.0)

Table 2. The characteristic of heavy metals in UPM. The results are presented as median and IQR.

The mean ΔCT of unstimulated epithelial cells from controls was used as a calibrator for all groups. A detailed protocol of total RNA isolation, cDNA synthesis and real-time PCR is described in the supplementary file and Table S1. The fold changes < 1 were converted and presented as negative values.

Protein concentration measurements. The levels of the IL-1β, IL-6, and IL-8 in cell culture supernatants were measured using ELISA kits (Thermo Fisher, MA, USA) according to the manufacturer's procedure. The sensitivity of kits was 2 pg/ml.

Measurements of transepithelial electrical resistance (TEER). TEER measurements were performed using Millicell ERS-2 Voltohmmeter (Merck Millipore, Burlington, MA, USA) according to the manufacturer's protocol. Two hundred microliters of PBS were added to the upper chamber in the cell culture system. The ohmic resistance of a blank (culture insert without cells) was evaluated for each experiment's measurement and was subtracted from the total resistance of the sample.

Flow cytometry analysis. For epithelial FACS measurements, cells from the upper chamber (ALI cultured epithelial cells alone or co-cultured with moMφs or/and moDCs) were evaluated. The cells were stained with antibodies against CD326, CD45, EGFR, mucin 1 (MUC1), TGF-β1, β-tubulin (BD Biosciences, San Jose, CA, USA), IL-33 receptor (ST2) (Biotechne, R&D Systems, MN, USA) and analyzed using the FACSCelesta instrument (BD Biosciences, San Jose, CA, USA). A detailed protocol of FACS analysis is described in the supplementary file, the characterization of fluorochrome-conjugated antibodies used for epithelial cell staining is shown in Table S2.

The epithelial cell subpopulations were defined as follows:

- Epithelial cells: CD45 – CD326 +
- Epithelial cells with secretory phenotype: CD45 – CD326 + MUC1 + β-Tubulin –
- Epithelial cells with ciliary phenotype: CD45 – CD326 + MUC1 – β-Tubulin +.

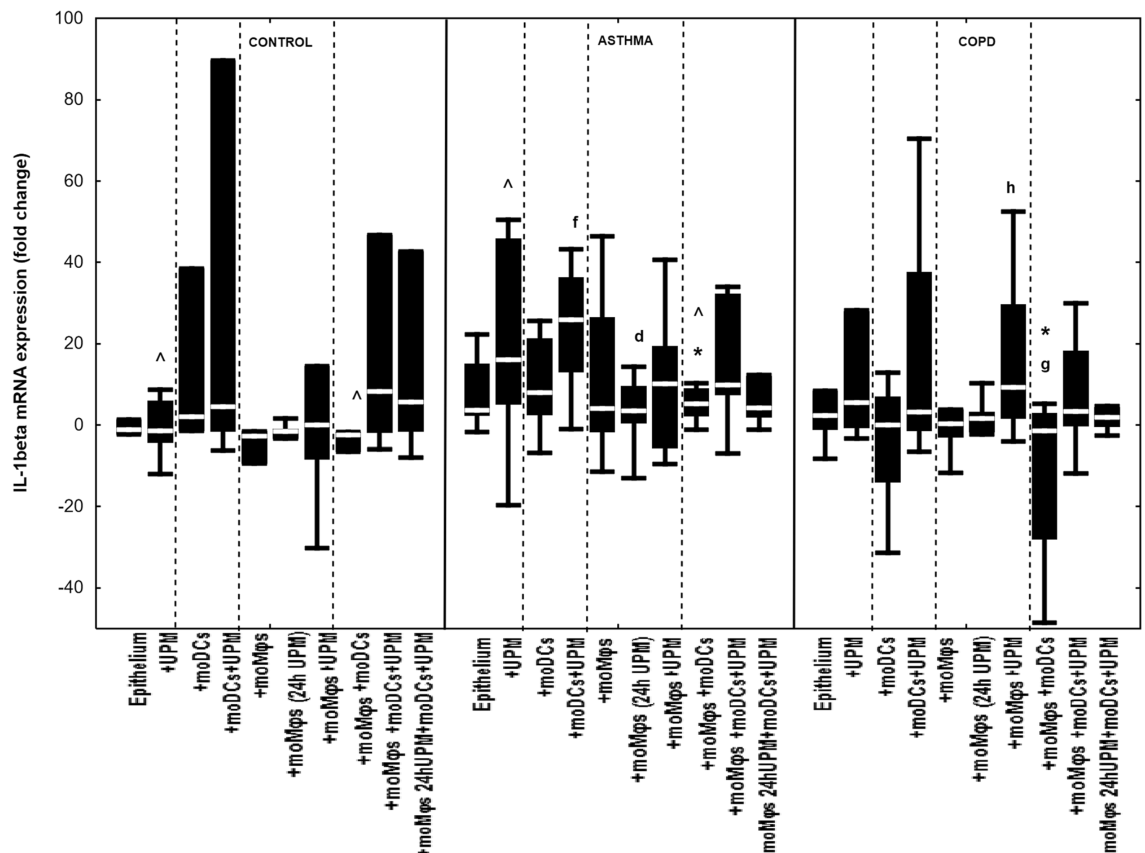


Figure 2. IL-1β mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-cultures in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value < 0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

The proportions of positive cells for EGFR, TGF-β1 and ST2 were presented as a percentage in the epithelial gate or subpopulation of undefined epithelial cells, epithelial cells with secretory phenotype, and epithelial cells with ciliated phenotype gates.

Statistical analysis. Statistical analysis was performed with the use of the Statistica 13.3 software package (StatSoft Inc., OK, USA). The Kruskal–Wallis test was used to assess differences between continuous variables in the three study groups. The Mann–Whitney U test was applied for pairwise comparisons. Pearson Chi-square test was used to compare intergroup differences in the categorical variables. Results are given as a median and interquartile range (IQR). Differences were considered statistically significant at $p < 0.05$.

Statement of ethics. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This work has received approval for research ethics from Medical University of Warsaw Review Board (KB/37/2020) and a proof/certificate of approval is available upon request.

Results

The effect of UPM on cytokines' mRNA expression in epithelial cells. Co-cultivation of epithelial cells with moDCs and/or moMφs without UPM treatment did not change mRNA expression for IL-1β, IL-6, IL-8, MMP7, MMP9, TSLP, and IL-33 in evaluated groups, except for decreased MMP9 and TSLP mRNA expression in asthmatic epithelium co-cultivated with moDCs compared to epithelial cells alone (Figs. 2, 3, 4, 5, 6, 7, 8). The exact medians and IQR values of the fold change of cytokine mRNA expression are shown in Table S3.

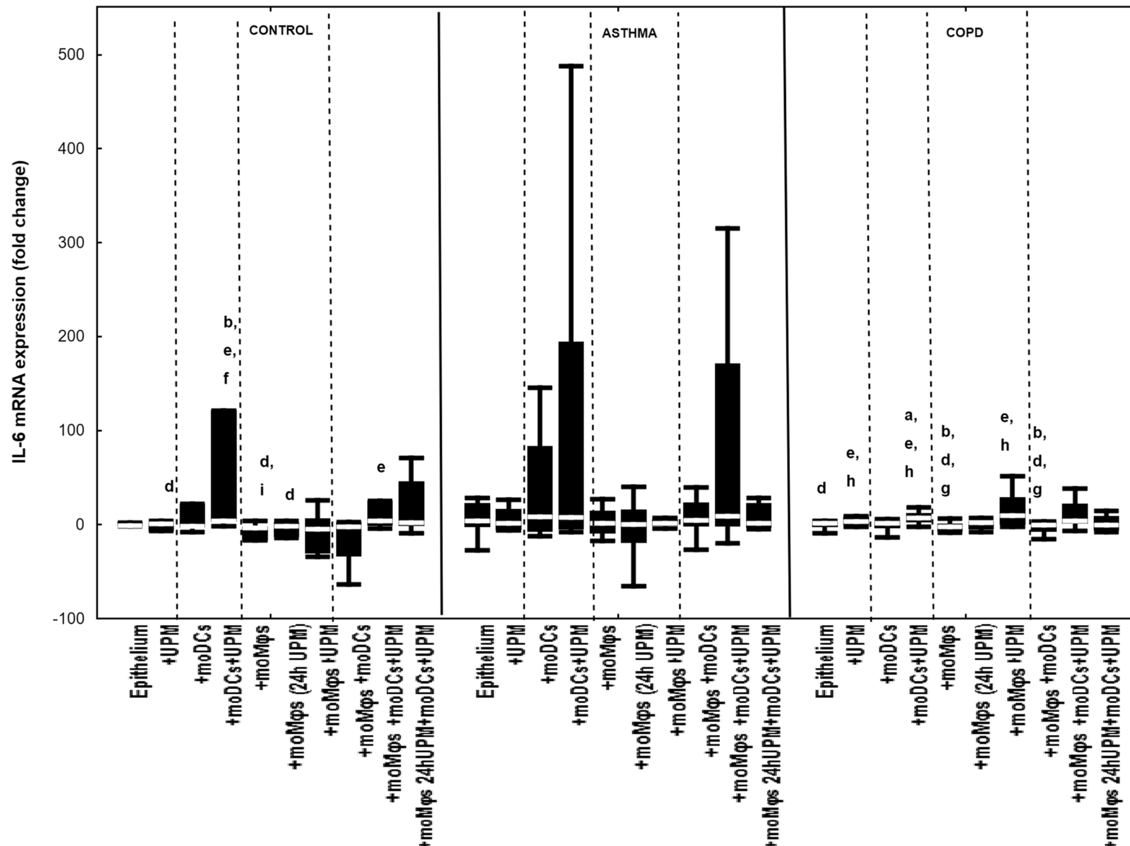


Figure 3. IL-6 mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

24 h cultivation of moDCs in epithelial growth medium did not affect cell viability (84.6% (79.2–89.7%) versus 93.9% (92.6–95.6%), $p = 0.114$ of moDCs cultured in DC and epithelial dedicated medium, respectively) as well as the distribution of viable, apoptotic and necrotic cells within these groups.

Exposure to UPM increased IL-1β mRNA expression in epithelium/moDCs di-cultures (25.9 fold change (13.0–36.2 fold change)) compared to epithelial/moMφs (UPM 24 h) co-cultures (3.5 fold change (0.5–9.5 fold change), $p = 0.015$) in asthma, and in epithelial/moMφs (9.3 fold change (1.6–29.7 fold change) compared to unstimulated triple co-cultures (− 1.5 fold change (− 28.1 to 2.9 fold change), $p = 0.02$) in COPD (Fig. 2).

The comparison between groups showed that the highest changes in IL-1β mRNA expression after UPM exposure were observed in asthmatic epithelial cells alone (16.1 fold change (5.1–45.8 fold change) compared to analogous cell cultures in controls (− 1.4 fold change (− 4.3 to 6.0 fold change), $p = 0.04$) (Fig. 2).

The greatest changes in IL-6 mRNA expression after UPM stimulation were observed in controls in epithelium/moDCs di-cultures (4.0 fold change (3.5–121.1 fold change) compared to UPM treated epithelium alone (1.1 fold change (− 6.5 to 2.6 fold change), $p = 0.03$), unstimulated epithelium/moMφs co-cultures (− 3.7 fold change (− 16.7 to − 2.0 fold change), $p = 0.004$) and epithelium/moMφs (UPM24h) co-cultures (− 0.2 fold change (− 13.0 to 2.3 fold change), $p = 0.01$). UPM exposure upgraded IL-6 mRNA expression in triple co-cultures (3.6 fold change (− 1.2 to 25.1 fold change)) compared to unstimulated epithelium/moMφs, ($p = 0.035$) in controls. A similar pattern of changes after UPM treatment was noted in COPD with additional increased IL-6 mRNA expression in epithelium/moMφs di-cultures (9.8 fold change (0.4 to 20.1 fold change)) compared to unstimulated epithelium/moMφs di-cultures (− 2.4 fold change (− 7.0 to 1.1 fold change)) ($p = 0.02$) (Fig. 3).

The changes in expression of IL-6 mRNA after UPM stimulation did not differ between control, asthma and COPD groups (Fig. 3).

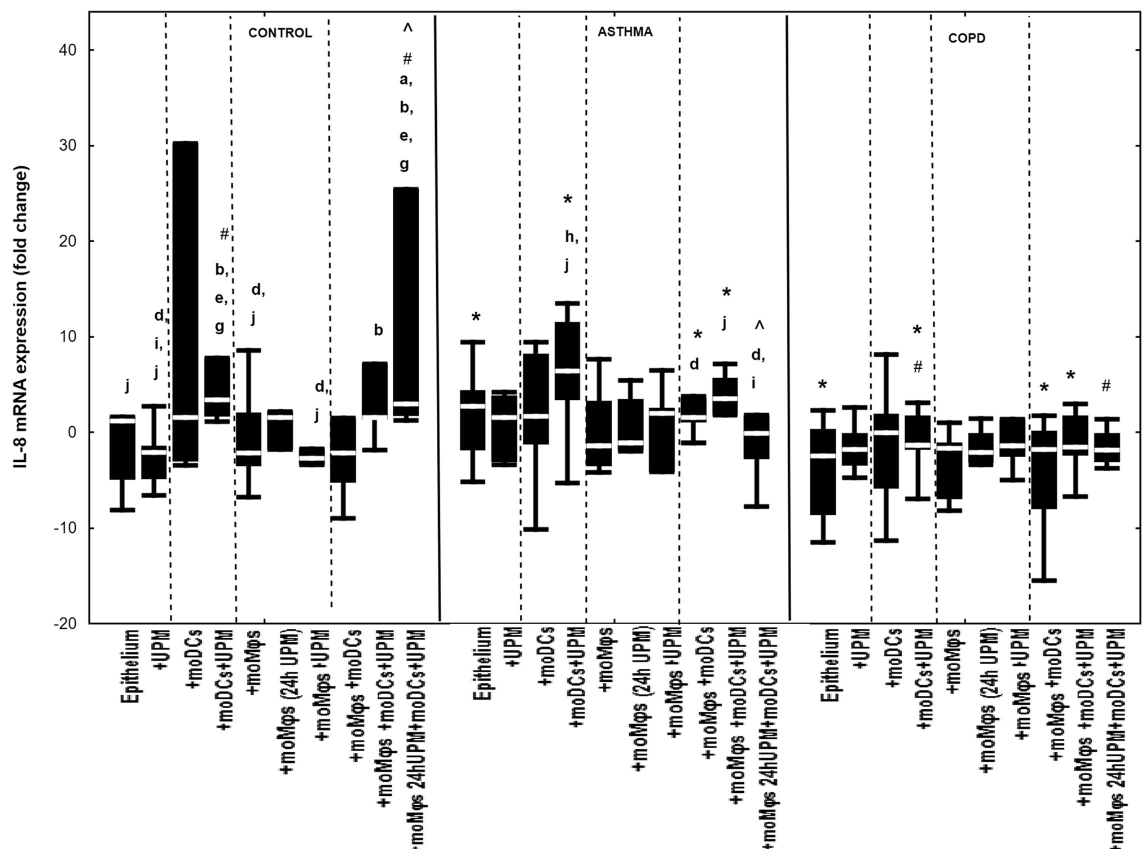


Figure 4. IL-8 mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

In control group, UPM exposure increased IL-8 mRNA expression in epithelium/moDCs di-cultures (3.4 fold change (1.7–7.8 fold change)) compared to UPM treated epithelial cells alone (−2.1 fold change (−4.8 to −1.4 fold change), *p* = 0.002), unstimulated (−2.2 fold change (−3.5 to 2.0 fold change), *p* = 0.04) and UPM treated epithelial/moMφs co-cultures (−2.7 fold change (−3.4 to 1.7 fold change), *p* = 0.04). UPM upgraded IL-8 mRNA expression in triple co-cultures, with the highest changes in triple co-cultures with moMφs (24 h UPM) (3.0 fold change (1.7–25.4 fold change)) in controls. In asthma similarly to control group the biggest changes in IL-8 mRNA expression after UPM exposure was found in epithelial/moDCs co-cultures (6.4 fold change (3.4–11.5 fold change) and in triple co-cultures (3.5 fold change (1.8–5.7 fold change). No significant changes were observed in IL-8 mRNA expression in UPM treated cells in COPD (Fig. 4).

The asthmatic epithelium/moDCs co-cultures (6.4 fold change (3.4 to 11.5 fold change)) as well as triple-co-cultures (3.5 fold change (1.8 to 5.7 fold change)) were characterised by the most substantial changes in IL-8 mRNA expression after UPM exposure compared to COPD (−1.3 fold change (−1.8 to 1.7 fold change), *p* = 0.01; (−1.5 fold change (−2.4 to 1.7 fold change), *p* = 0.04, for epithelial/moDCs and triple co-cultures, respectively) (Fig. 4).

UPM stimulation decreased MMP7 mRNA expression in triple-co-cultures with moMφs (UPM 24 h) (1.0 fold change (−6.3 to 2.6 fold change)) compared to untreated epithelial cells alone (5.6 fold change (3.3 to 11.5 fold change), *p* = 0.049) in the asthma group. UPM treatment did not or slightly impact MMP7 mRNA expression in control and COPD group. (Fig. 5).

UPM exposure did not change MMP9 mRNA expression in control, asthma or COPD group in any of the epithelial co-cultures (Fig. 6).

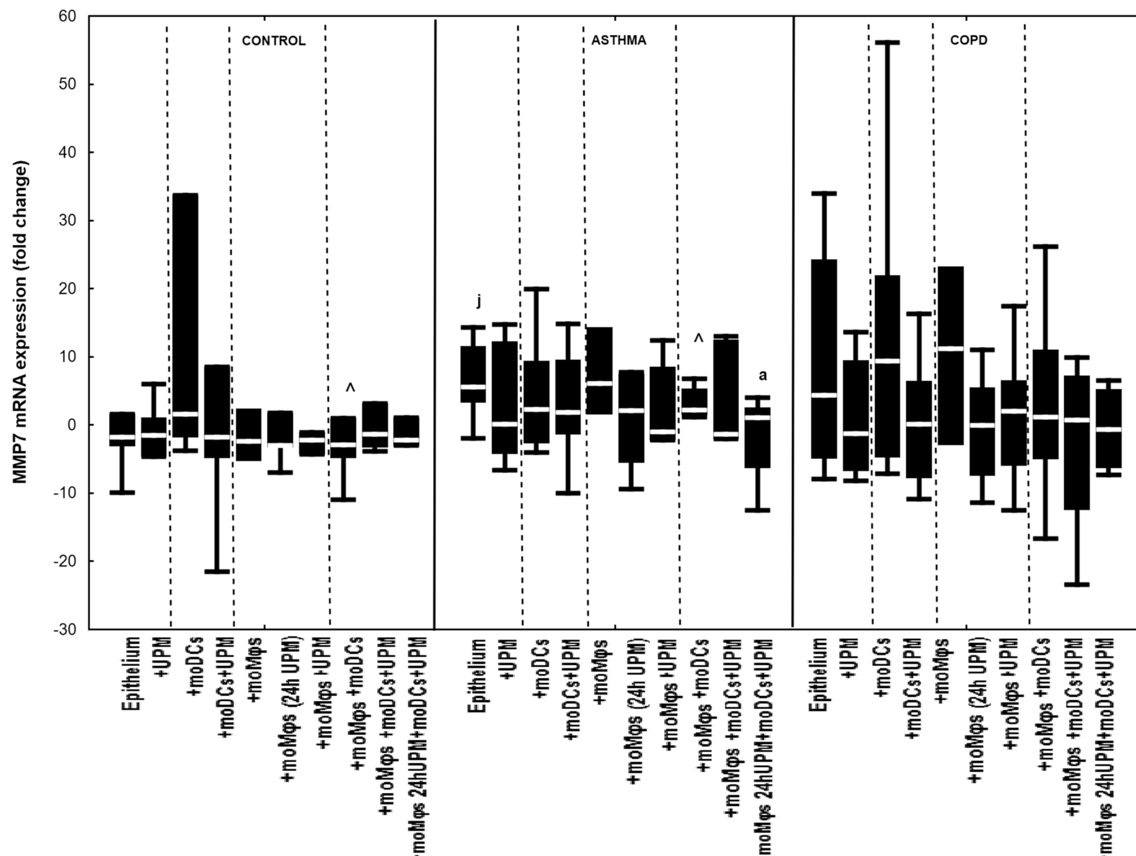


Figure 5. MMP7 mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

The most pronounced effect concerning changes in TSLP mRNA expression after UPM exposure was noted in asthma group. UPM treatment decreased TSLP mRNA expression in epithelial/moDCs co-cultures (1.8 fold change (− 1.3 to 2.4 fold change)), triple co-cultures (− 0.1 fold change (− 2.0 to 2.8 fold change) and triple co-cultures with moMφs (24 UPM) (1.3 fold change (− 2.5 to 2.8 fold change)) compared to unexposed epithelial cells alone (7.1 fold change (2.9 to 45.5 fold change), $p = 0.02$, $p = 0.02$, $p = 0.01$, respectively). No changes for TSLP mRNA expression after UPM exposure were observed in the COPD group (Fig. 7).

MMP7, MMP9 and TSLP presented the same pattern of mRNA expression changes after UPM exposure in all evaluated groups (Fig. 5, 6, 7).

We found no significant alterations of IL-33 mRNA expression in the epithelium after UPM stimulation in the control or COPD group. In asthma, UPM exposure decreased IL-33 mRNA expression in triple co-cultures with moMφs (24 h UPM) (−1.1 fold change (− 2.8 to 1.2 fold change)) compared to unstimulated epithelial cells alone (11.6 fold change (2.9 to 45.5 fold change), $p = 0.01$), unstimulated epithelium/moDCs di-cultures (2.3 fold change (1.3 to 4.7 fold change), $p = 0.04$) and UPM exposed epithelium/moDCs di-cultures (4.2 fold change (1.5 to 6.0 fold change), $p = 0.02$) (Fig. 8).

The asthmatics epithelial cells alone after UPM treatment expressed the highest IL-33 mRNA level compared to controls ($p = 0.02$). However, the most profound changes after UPM stimulation for IL-33 mRNA expression was found for COPD epithelial/moDCs co-cultures (compared to control, $p = 0.02$) epithelial/ moMφs co-cultures ($p = 0.03$) and triple co-cultures ($p = 0.01$) (compared to asthma) (Fig. 8).

The effect of UPM on cytokine proteins' secretion by epithelial cells. Nasal epithelium cultured together with moDCs (both di- and tri-co-cultures) without UPM stimulation produced a significantly higher

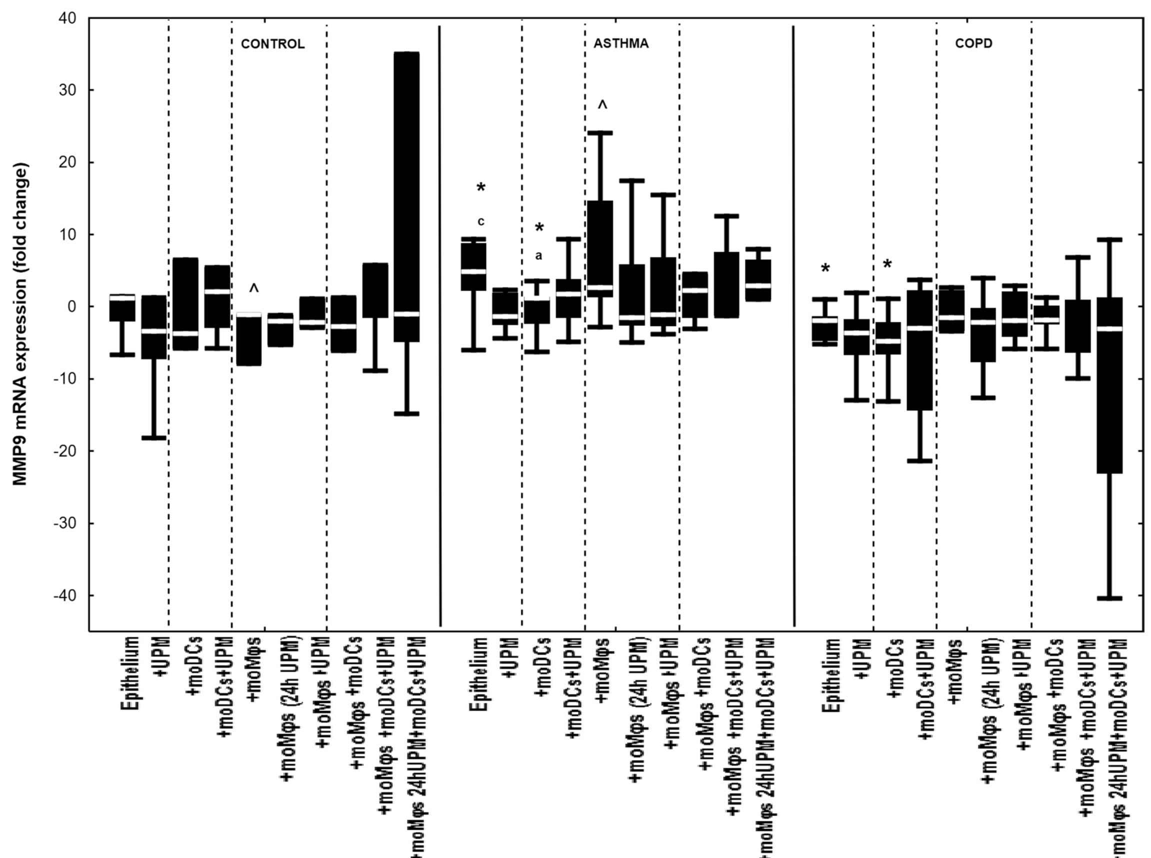


Figure 6. MMP9 mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

amount of IL-1β in all studied groups (Fig. 9). Co-cultivation of epithelial cells with moDCs and/or moMφs without UPM did not change IL-6 or IL-8 protein secretion in the control, asthma, and COPD groups (Figs. 10, 11). The exact medians and IQR values of the fold change of cytokine protein levels are shown in Table S4.

UPM stimulation did not alter the IL-1β level in epithelial cultures compared to corresponding unstimulated control cells, asthma and COPD patients (Fig. 9). Detailed comparisons of the IL-1β protein level in control, asthma and COPD are presented in Tables S5, S6, and S7. UPM exposure impacted IL-1β production in similar pattern in all groups.

We observed a few variations in IL-6 secretion after UPM exposure in the controls, including slightly higher IL-6 levels in epithelial/moDCs (18.0 pg/ml (12.74–21.14 pg/ml)) and triple-co-cultures (with UPM 24 h moMφs) (15.20 pg/ml (12.29–19.09 pg/ml)) compared to epithelial cells alone (3.44 pg/ml (1.13–11.94 pg/ml)) ($p=0.022$ and $p=0.01$, respectively) or epithelium co-cultivated with moMφs (2.16 pg/ml (0.0–10.46 pg/ml)) ($p=0.026$ and $p=0.022$, respectively). In asthma, the highest IL-6 production after UPM treatment was noted in epithelial cells alone (34.33 pg/ml (5.77–57.26 pg/ml)) and in triple-co-cultures with moMφs (24 h UMP) (34.60 pg/ml (6.03–54.27 pg/ml)) compared to unstimulated triple co-cultures (5.63 pg/ml (2.94–11.74 pg/ml), $p=0.038$ and $p=0.008$, respectively). In COPD, an elevated level of IL-6 after UPM exposure was found in epithelial/moMφs di-cultures (55.58 pg/ml (31.92–76.04 pg/ml) compared to unstimulated epithelial/moMφs di-cultures (9.77 pg/ml (3.31–20.57 pg/ml)) ($p=0.021$) and in triple co-cultures (54.50 pg/ml (19.81–117.37 pg/ml)) compared to unexposed epithelial/moMφs di-cultures ($p=0.049$) (Fig. 10).

The highest amount of IL-6 after UPM stimulation was produced by epithelial/moMφs co-cultures ($p=0.005$) and triple co-cultures ($p=0.004$) in COPD group compared to controls (Fig. 10).

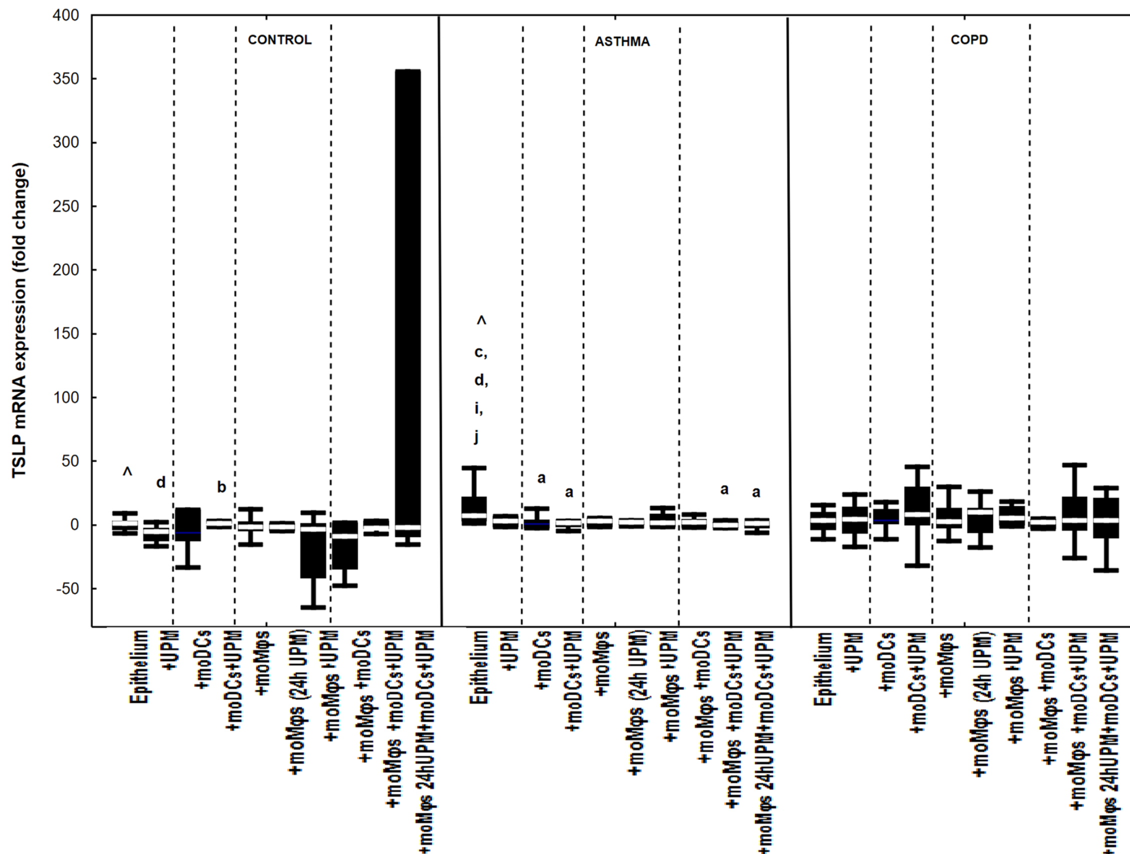


Figure 7. TSLP mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

Upgraded IL-8 production after UPM stimulation was observed in triple co-cultures (238.23 pg/ml (229.59–247.95 pg/ml)) compared to epithelium/moMφs (24 h UPM) di-cultures (105.16 pg/ml (84.86–199.0 pg/ml)) ($p=0.017$) in the control group. UPM exposure enhanced IL-8 secretion in epithelial/moDCs di-cultures (607.66 pg/ml (442.91–804.51 pg/ml)) in asthma and in triple-co-culture with moMφs (24 h UPM) (614.41 pg/ml (425.41–764.81 pg/ml)) compared to unexposed triple co-cultures (315.93 pg/ml (168.30–500.06 pg/ml)) ($p=0.013$ and $p=0.003$, respectively) and epithelial/moMφs co-cultures (288.43 pg/ml (182.33–405.59 pg/ml)) ($p=0.007$ and $p=0.002$, respectively) in asthma. In COPD, UPM exposure increased IL-8 production in triple-co-culture with moMφs (24 h UPM) (708.14 pg/ml (533.57–921.53 pg/ml)) compared to epithelium/moMφs co-cultures without UPM treatment (403.1 pg/ml (237.33–549.72 pg/ml)) ($p=0.040$). However, due to the high production of IL-8 by moDCs in asthma and COPD alone (*p* value >0.05), the evaluation of IL-8 secretion by di-and triple co-cultures with moDCs should be interpreted carefully (Fig. 11).

Asthmatic and COPD epithelium produced more IL-8 protein after UPM treatment than epithelium from control group, especially in asthma epithelial/moDCs co-cultures ($p=0.003$) and COPD triple co-cultures ($p=0.0007$) compared to controls (Fig. 11).

The effect of UPM on cytokine mRNA expression and proteins' secretion by moMϕs. UPM exposure did not change moMφs viability (76.8% (76.1–83.7%) versus 80.2% (75.1–84.7%), $p=0.886$ for untreated and UPM treated moMφs, respectively), but it shifted necrotic and apoptotic cell distribution within the group from 65.2% (54.3–80.4%) to 34% (31.6–37.1%), $p=0.03$ for necrotic cells, and from 5.04% (4.1–5.1%) to 36.3% (32.2–38.8%) for apoptotic cells in untreated and UPM exposed moMφs, respectively.

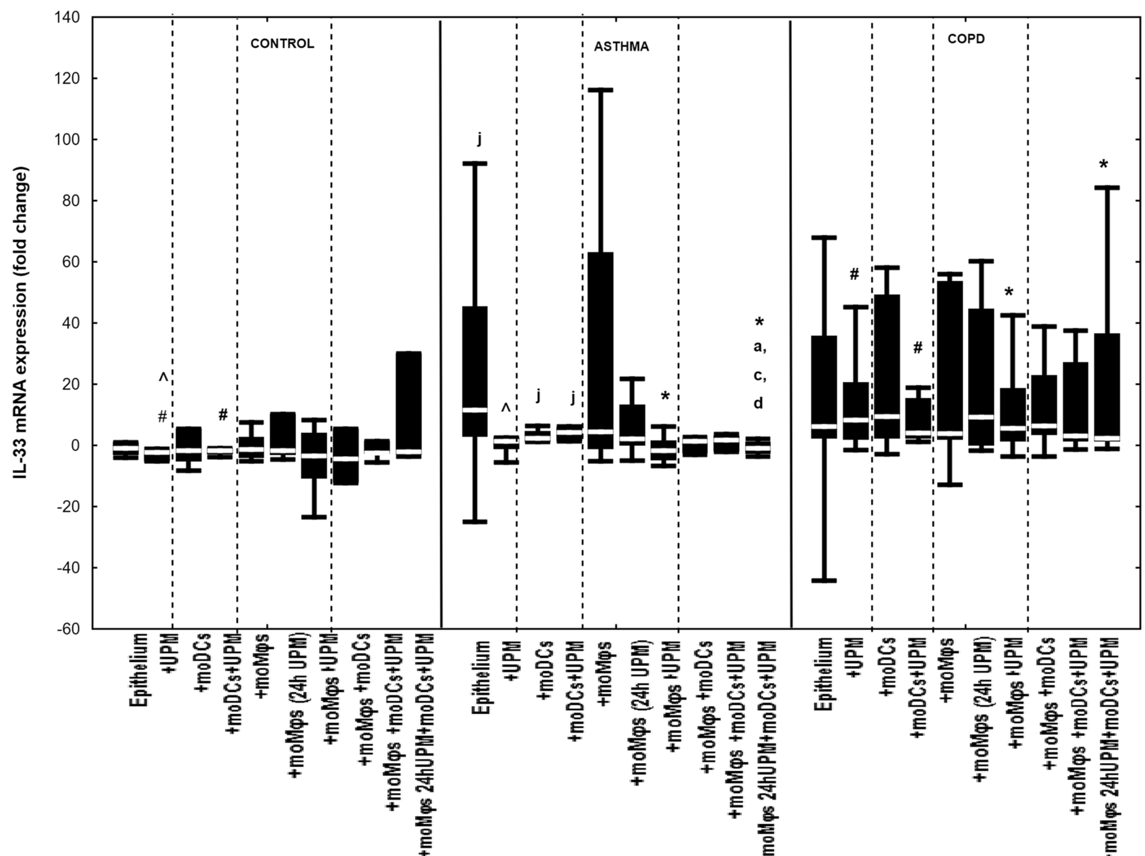
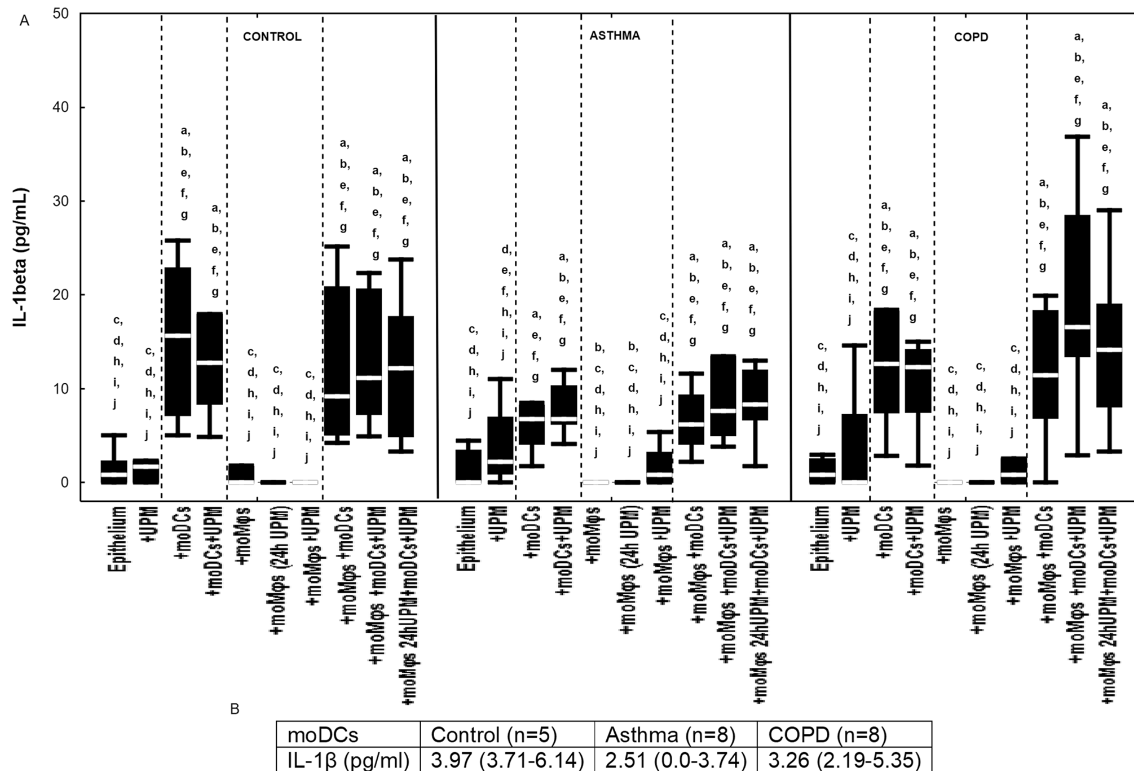


Figure 8. IL-33 mRNA expression in air–liquid interface (ALI) after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g—epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

The mRNA expression of inflammatory mediators, as well as the levels of IL-1β, IL-6 and IL-8 after UPM exposure in control, asthma, and COPD moMφs are presented in Tables 3 and 4. We observed decreased mRNA expression of TSLP in asthma and decreased expression of IL-6 mRNA COPD in moMφs after the exposure to UPM (Table 3). The moMφs in control group secreted increased level of IL-1β protein after the UPM treatment (Table 4).

The comparison between groups revealed that moMφs after UPM stimulation produced higher amount of IL-1β protein in asthma group compared to controls ($p = 0.004$). The untreated moMφs from controls produced more IL-6 protein than moMφs from COPD patients ($p = 0.03$). On the other hand, the unstimulated moMφs in CODP group secreted the highest IL-8 protein level compared to both controls ($p = 0.01$) and asthma ($p = 0.002$) moMφs (Table 4).

The effect of UPM on transepithelial electrical resistance (TEER) changes. Asthma and COPD epithelial cells were characterized by lower TEER values compared to controls (Fig. 12A). A different pattern of TEER changes between mono, di- and triple co-cultures in controls and obstructive lung disease groups was observed: co-cultivation with moDCs and/or moMφs decreased TEER in controls but did not change TEER values in asthma and COPD (Figure S1, S2, S3). Asthma and control epithelial cells differed in terms of changes in TEER values after 24 h in most cultivation combinations, whereas such dependency was not observed in COPD, with the exception of unstimulated epithelial cells alone. Importantly, different TEER changes in epithelial co-cultures after 24 h were demonstrated: decreased in controls (ΔTEER > 0), increased or without changes in asthma (ΔTEER < 0) and without changes in COPD. Only in asthma ΔTEER of epithelial/moMφs (16.88 ΔTEER Ω/0.33 cm^2 (−123.13 to 109.88 ΔTEER Ω/0.33 cm^2), epithelial/moMφs (24 h UPM) (−3.13 ΔTEER



moDCs	Control (n=5)	Asthma (n=8)	COPD (n=8)
IL-1β (pg/ml)	3.97 (3.71-6.14)	2.51 (0.0-3.74)	3.26 (2.19-5.35)

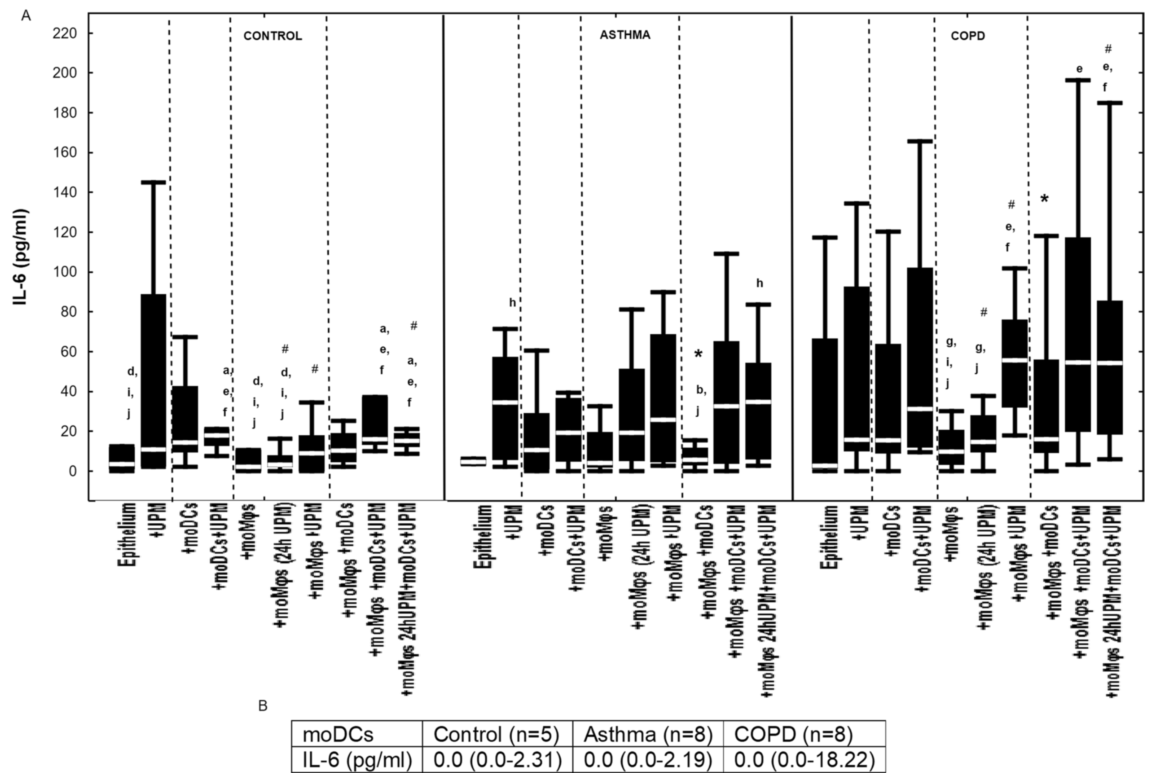
Figure 9. (**A**) IL-1β secretion by air–liquid interface (ALI) cultured nasal epithelium after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. (**B**) IL-1β levels produced by moDCs cultured alone are shown in table. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line). The exact *p* value calculated after Mann–Whitney U test are shown in supplementary file (Table S5, Table S6 and Table S7). The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g- epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

Ω/0.33 cm^2 (− 135.5 to 187.25 ΔTEER Ω/0.33 cm^2), and epithelial/moMφs treated with UPM (23.0 ΔTEER Ω/0.33 cm^2 (− 164.63 to 56.75 ΔTEER Ω/0.33 cm^2) was increased compared to unexposed epithelial cells alone (− 213.78 ΔTEER Ω/0.33 cm^2 (− 294.75 to (− 85.88) ΔTEER Ω/0.33 cm^2) ($p=0.02$, $p=0.04$, $p=0.049$, respectively) (Fig. 12B).

The effect of UPM on the expression of EGFR, TGFβ and ST2 in epithelial cells. The highest proportion of EGFR + epithelial cells was noted in asthma patients. EGFR was expressed mainly in epithelial cells with ciliated phenotype. We did not observe EGFR expression in epithelial cells from controls in unstimulated and UPM stimulated triple co-cultures. UPM exposure decreased the proportion of EGFR + epithelial cells in all evaluated groups with significant changes found in asthma epithelial cells with ciliated phenotype (50.3% (27.7–60.7%) versus 25.2% (18.3–37.9%), $p=0.049$) (Fig. 13).

TGFβ was expressed in COPD triple co-cultures stimulated with UPM, with its predominance in epithelial cells with ciliated phenotype. The largest proportion of TGFβ + epithelial cells with secretory phenotype was found in unstimulated and UPM stimulated triple co-culture of COPD patients. UPM treatment decreased the proportion of TGFβ + epithelial cells with secretory phenotype in control subjects (6.2% (5.5–9.0%) versus 0.9% (0.5–1.5%), $p=0.02$) (Fig. 13).

A small proportion of ST2 + epithelial cells was found in all investigated groups. ST2 was expressed most frequently in epithelial cells with ciliated phenotype and was insignificantly higher in patient groups compared to controls. A higher amount of ST2 + epithelial cells with secretory phenotype was observed in triple co-cultures after UPM exposure in COPD compared to the control group. A decreased number of ST2 + epithelial cells with ciliated phenotype was found in asthma after UPM treatment (6.8% (4.4–8.5%) versus 2.8% (1.9–3.3%), $p=0.002$) (Fig. 13).



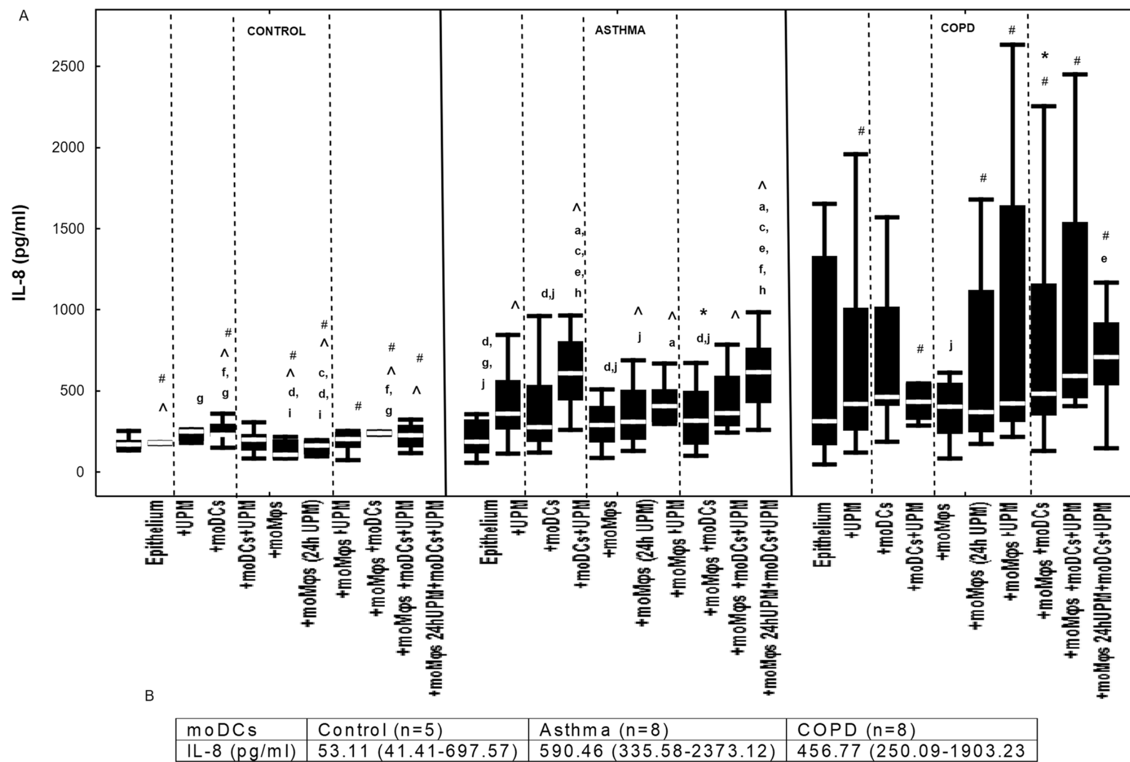
moDCs	Control (n=5)	Asthma (n=8)	COPD (n=8)
IL-6 (pg/ml)	0.0 (0.0-2.31)	0.0 (0.0-2.19)	0.0 (0.0-18.22)

Figure 10. (**A**) IL-6 secretion by air–liquid interface (ALI) cultured nasal epithelium after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. (**B**) IL-6 levels produced by moDCs cultured alone are shown in table. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g- epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

Discussion

Morbidity from asthma and COPD, including acute exacerbations of these diseases, is correlated with an elevated concentration of harmful PM in the atmosphere, suggesting a contribution of air pollution to the pathobiology of obstructive lung diseases. Our study showed a different pattern of epithelial response after UPM exposure in asthma, COPD, and healthy people. Our results revealed that the inflammatory reaction of nasal epithelial cells after UPM treatment was impacted by interactions with moDCs in all studied groups and to a lesser effect with moMφs in COPD. We found different dynamics of changes in the integrity of tight junctions of nasal epithelial cells after 24 h UPM stimulation between asthma, COPD, and control group, and showed, unexpectedly, an increase of the integrity of the cellular barrier of asthmatic epithelium co-cultivated with moMφs only, not related to UPM treatment. The results of our study presented that the inflammatory alternations after UPM exposure are more intense in patients with obstructive lung diseases than in healthy people. Here, for the first time, we characterized the immunological processes induced by airway epithelium after UPM exposure in special regard to interactions with moDCs and moMφs different in asthma, COPD, and healthy people.

The impact of various airborne PM on airway epithelial cells has been well described. Byun et al. analyzed the whole transcriptome of nasal epithelial cells and showed that expression of genes related to inflammation (e.g. IL-8, IL1RL1, PTGS2, ICAM, ADAM8) and adhesion (IL-8, AKAP12, ICAM1, ADAM8, IL-1β) were affected after 24 h UPM exposure[26]. The in vitro studies using ALI cultured airway epithelial cells showed that exposure to $PM_{2.5}$, for example, impacted many processes associated with oxidative stress and pro-inflammatory response in particular: reduced cell viabilities, induced ROS generation, enhanced arginase II levels, heme oxygenase-1 (HO-1), and IL-8 levels[8,27,28]. Our study showed that inflammatory reaction after UPM exposure in the respiratory epithelium is related to cell-to-cell interactions. The inflammation helps the epithelial cells with tolerance and neutralization of noxious stimuli in the airways after air pollution exposure. The crosstalk between immune and epithelial cells regulates Th1- (observed at IL-1β, IL-6 and IL-8 level) inflammatory reaction as it was shown for the control group. IL-6 and IL-8 accelerate the local inflammatory reaction, recruit neutrophils and macrophages for efficient clearance and degradation of inhaled pollutants and apoptotic cells. The role of IL-1β in



moDCs	Control (n=5)	Asthma (n=8)	COPD (n=8)
IL-8 (pg/ml)	53.11 (41.41-697.57)	590.46 (335.58-2373.12)	456.77 (250.09-1903.23

Figure 11. (**A**) IL-8 secretion by air–liquid interface (ALI) cultured nasal epithelium after 24 h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients. (**B**) IL-8 levels produced by moDCs cultured alone are shown in table. The data are shown as non-outlier range (whiskers), interquartile range (box) and median (line); *p* value calculated using Mann–Whitney U test. The *p* value <0.05 in comparison to: a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g- epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM; ^ asthma versus control, # COPD versus control, *asthma versus COPD.

response to PM seems to be complex. The analysis of whole transcriptome expression in ALI cultured epithelial cells showed the highest changes in expression and function of *IL1A* and *IL1B* among the genes upregulated by PM exposure[29]. It is suggested that IL-1α and IL-1β are key mediators of mucus cell metaplasia in the airways after PM exposure[29,30]. Moreover, increased release of IL-1-β from airway epithelial cells may contribute to abnormal collagen remodeling by airway fibroblasts and be associated with EMT[31]. Taken together, exposure to UPM initiates complex inflammatory processes that are efficient in epithelial cells, which interact with airway immune cells. Prolonged PM exposure may lead to uncontrolled inflammation, acute tissue injury and airway remodeling in healthy people. The results of our study found that the immune responses of epithelial cells in asthma and COPD after PM exposure differ in some aspects of inflammatory pattern from healthy individuals. Moreover, the intensity of inflammation is higher in patients with obstructive lung diseases in our study, especially observed for IL-6 in COPD and IL-8 in both asthma and COPD, and include other mediators e.g., TSLP and IL-33 in asthma. This observation suggests that patients with obstructive lung diseases are more prone to negative effects of air pollution because of epithelial dysfunction and impairment of its protective mechanisms, and more pronounced inflammatory response in the airways.

The epithelial/DCs communication is essential for an immunologic response during allergen or environmental triggers in the lungs. Epithelial cells signal and impact DCs to initiate the allergic immune response by producing cytokines (TSLP, IL-33, IL-25, IL-1α, and GM-CSF)[32]. DCs are activated by components of the epithelial layer: after exposure to epithelium-derived mucin, DCs promoted IL-8-dependent neutrophil migration[33]. Only a few authors evaluated the effect of interactions between epithelial cells and DCs on the inflammatory response after PM stimulation. It has been reported that normal bronchial epithelial (submerged)/DCs co-cultivation enhanced pro-inflammatory responses after PM exposure compared to monocultures. Stimulation of immature DCs to conditioned media from airway epithelial cells exposed to PM_{10} caused DCs maturation, not related to the uric acid pathway[34]. In contrast to DCs, the mucosal PM_{10}-facilitated allergic airway response is mediated by uric acid[35]. The results of our work showed that co-cultivation of airway epithelial cells with moDCs upregulated the expression and production of many mediators. In this study, we showed that this is a two-way interaction

mRNA expression (fold change)	moMφs	moMφs + UPM	*p* value
Control (n = 7)			
IL-1β	− 1.2 (− 2.4 to 3.1)	− 1.6 (− 1.9 to 2.0)	0.71
IL-6	− 1.3 (− 1.4 to 1.1)	0.0 (− 1.5 to 1.6)	0.93
IL-8	1.3 (− 2.9 to 2.0)	− 1.0 (− 1.9 to 2.1)	1.00
MMP7	− 2.3 (− 3.0 to 6.3)	− 1.4 (− 3.4 to 5.4)	1.00
MMP9	1.3 (− 2.4 to 1.6)	− 1.2 (− 1.4 to 1.2)	0.53
TSLP	− 1.1 (− 3.9 to 1.7)	− 1.3 (− 6.6 to − 1.0)	0.84
IL-33	1.4 (− 2.5 to 3.8)	1.7 (− 8.3 to 4.0)	0.95
Asthma (n = 8)			
IL-1β	0.0 (− 1.7 to 1.8)	1.1 (− 2.2 to 2.3)	1.00
IL-6	7.5 (− 7.6 to 46.1)	1.1 (− 2.1 to 5.4)	0.90
IL-8	− 1.3 (− 1.8 to 2.1)	1.1 (− 1.8 to 1.9)	0.85
MMP7	1.2 (− 1.1 to 1.9)	− 1.4 (− 2.3 to 3.2)	0.96
MMP9	0.1 (− 1.5 to 1.4)	1.8 (− 3.6 to 2.6)	0.28
TSLP	1.7 (− 1.4 to 1.8)	− 34.2 (− 54.6 to − 5.7)	0.049
IL-33	− 0.2 (− 2.9 to 1.3)	− 1.6 (− 1.9 to 3.0)	0.95
COPD (n = 7)			
IL-1β	1.3 (− 1.5 to 1.6)	1.1 (− 2.0 to 1.6)	0.71
IL-6	1.5 (− 1.6 to 2.4)	0.0 (0.0 to 0.0)	0.001
IL-8	− 1.1 (− 1.6 to 2.0)	− 1.4 (− 4.2 to 1.1)	0.53
MMP7	− 1.6 (− 4.4 to 6.5)	− 1.4 (− 1.8 to 1.6)	0.90
MMP9	3.7 (− 1.4 to 7.0)	− 2.1 (− 2.6 to 3.0)	0.37
TSLP	0.3 (− 16.7 to 13.2)	− 2.3 (− 3.8 to 8.5)	0.89
IL-33	− 1.3 (− 2.0 to 4.6)	− 1.7 (− 11.7 to 3.0)	0.63

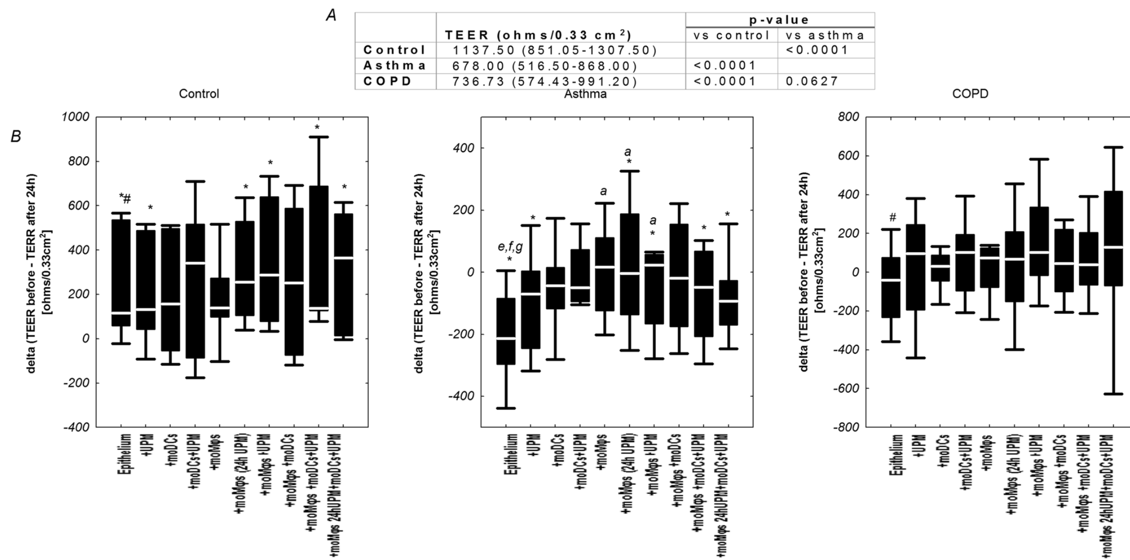
Table 3. mRNA expression of IL-1β, IL-6, IL-8, MMP7, MMP9, IL-33 and TSLP in moMφs of control, asthma and COPD patients with and without UPM treatment. The results are presented as median and IQR.

pg/ml	moMφs	moMφs + UPM	*p* value
Control (n = 7)			
IL-1β	0.0 (0.0–0.0)	1.5 (1.5–1.7)	0.04
IL-6	3.5 (0.0–10.1)	2.8 (0.0–14.0)	0.80
IL-8	812.4 (209.8–971.8)	979.6 (633.0–1101.3)	0.26
Asthma (n = 8)			
IL-1β	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.72
IL-6	0.0 (0.0–3.2)	0.0 (0.0–0.0)	0.80
IL-8	228.1 (92.8–716.5)	646.8 (39.7–1518.1)	0.72
COPD (n = 7)			
IL-1β	1.6 (0.0–2.9)	0.0 (0.0–2.6)	0.62
IL-6	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.71
IL-8	2340.0 (1422.7–2698.1)	1408.9 (950.9–1901.4)	0.05

Table 4. The level of IL-1β, IL-6, IL-8, secreted by moMφs of control, asthma and COPD patients with and without UPM treatment. The results are presented as median and IQR.

and DCs also affect epithelium during the response to UPM treatment by upregulation of expression of IL-1β, IL-6, IL-8, or downregulation of MMP7, IL-33 or TSLP. DCs are an additional source of IL-1β and IL-8 during the airway immune response, which accelerates the local inflammation. The results of this work clearly showed that DCs/epithelial interactions significantly affected the inflammatory reaction of epithelial cells after UPM exposure. We suggest that dysfunction of DCs might determine the biological reaction of airway epithelium for air pollution in asthma and COPD patients.

Our study showed that the inflammatory response of epithelial cells after UPM exposure is not mediated by interactions with moMφ, except for IL-1β and IL-6 expression in COPD. These results seem to confirm the earlier observation of Chen et al. who found that pulmonary inflammation in mice after carbon nanoparticle instillation was not stimulated by alveolar macrophages. The macrophages phagocyted the particles but did not interact with epithelial cells as long as macrophages were additionally stimulated with particles[36]. We also found qualitative and quantitative differences in the inflammatory response of respiratory epithelium after UPM stimulation between



A

	TEER (ohms/0.33 cm²)	p-value vs control	vs asthma
Control	1137.50 (851.05-1307.50)		<0.0001
Asthma	678.00 (516.50-868.00)	<0.0001	
COPD	736.73 (574.43-991.20)	<0.0001	0.0627

Figure 12. (**A**) The absolute TEER values of control, asthma, and COPD epithelial cells are shown in table. (**B**) The changes in trans epithelial electrical resistance (TEER) after 24 h of nasal epithelial cells from healthy donors, asthma and COPD cultured in air–liquid interface (ALI) conditions in multi co-culture models and UPM exposure. *p* value calculated using Mann–Whitney U test *p* value < 0.05 in comparisons * control versus asthma, # control versus COPD; a—epithelium, b—epithelium + UPM, c—epithelium + moDCs, d—epithelium + moDCs + UPM, e—epithelium + moMφs, f—epithelium + moMφs (24 h UPM), g-epithelium + moMφs + UPM, h—epithelium + moMφs + moDCs, i—epithelium + moMφs + moDCs + UPM, j—epithelium + moMφs (24hUMP) + moDCs + UPM.

healthy, asthma, and COPD groups. This reaction is probably related to pathological changes in the epithelium as well as macrophage/DC dysfunction associated with asthma or COPD. The functional abnormality of respiratory epithelium is correlated with the altered expression of various pro-inflammatory cytokines. It is interesting that numerous changes in the mRNA expression for IL-6 or IL-8 were more frequently observed in the control group than in asthma or COPD. This may suggest the impaired function of respiratory epithelium in obstructive lung diseases. The above assumption seems to be supported by the observation that air pollution worsens asthma and COPD symptoms more often than caused pulmonary dysfunction in healthy subjects[37]. The altered immune response in asthma and COPD for air pollution might be also related to disturbed biochemical and molecular signaling between immunological and structural cells. The prolonged exposure to oxidative stress in COPD indicates inherent mitochondrial impairment in macrophages, which resulted in weakened intracellular bacterial killing in respiratory infections related to COPD exacerbations and progression[38]. Our study showed that inflammatory response after UPM exposure is altered with the participation of epithelial/moMφ interactions in COPD only. Together, these facts might suggest a new therapeutic strategy that involves airway epithelium/macrophage biochemical pathways for the effective treatment of air pollution-associated COPD exacerbations.

The results of our work strongly suggest the disruption of the integrity of the epithelial barrier in asthma and as well as in COPD patients. We confirmed the observation of many authors and showed the low integrity of tight junctions of asthmatic but also less frequently studied COPD nasal epithelial cells. Using the epithelial cells from lower airways of patients with different COPD stages, Amatngalim et al. found no differences in barrier function between COPD and non-COPD bronchial epithelial cells[39]. The results of our study suggest a diverse pattern of cell–cell interaction in asthma and COPD compared to controls.

Macrophages are important cells that maintain respiratory epithelial integrity. The airways contain embryonically derived resident macrophages (airspace) and recruited macrophages, which migrate to the respiratory tract in response to inflammatory signal[40]. Macrophages in the airways are motile cells and actively participate in the lung immune response. Macrophages are distributed throughout the lung. Part of them reside in the alveoli (attached or unattached to structural cells), some of them phagocyte and are placed between the epithelial cells (submucosal), others are below the epithelial layer (interstitial) and participate in immune response together with other immune cells or migrate to lymph nodes[41]. Alveolar, submucosal and interstitial macrophages differ in terms of molecular transcriptomic profile[42]. Macrophages are important promoters of tissue repair after e.g. inflammatory lung disease, infection or toxic particles inhalation[43]. Airway macrophages impact epithelial integrity and repair airway injury by efferocytosis of apoptotic cells, interaction with structural cells, and production of growth factors (e.g. VEGF, EGF, fibroblast growth factor, heparin binding growth factor) and cytokines (e.g. GM-CSF, TNF-α, MMPs)[44–48]. In vivo study showed that submucosal macrophages promote epithelial repair and support pseudostratified epithelium growth and regeneration as alternatively activated M2 macrophages[42]. Many authors shoved that phagocytosis and efferocytosis of macrophages in asthma and COPD are impaired.

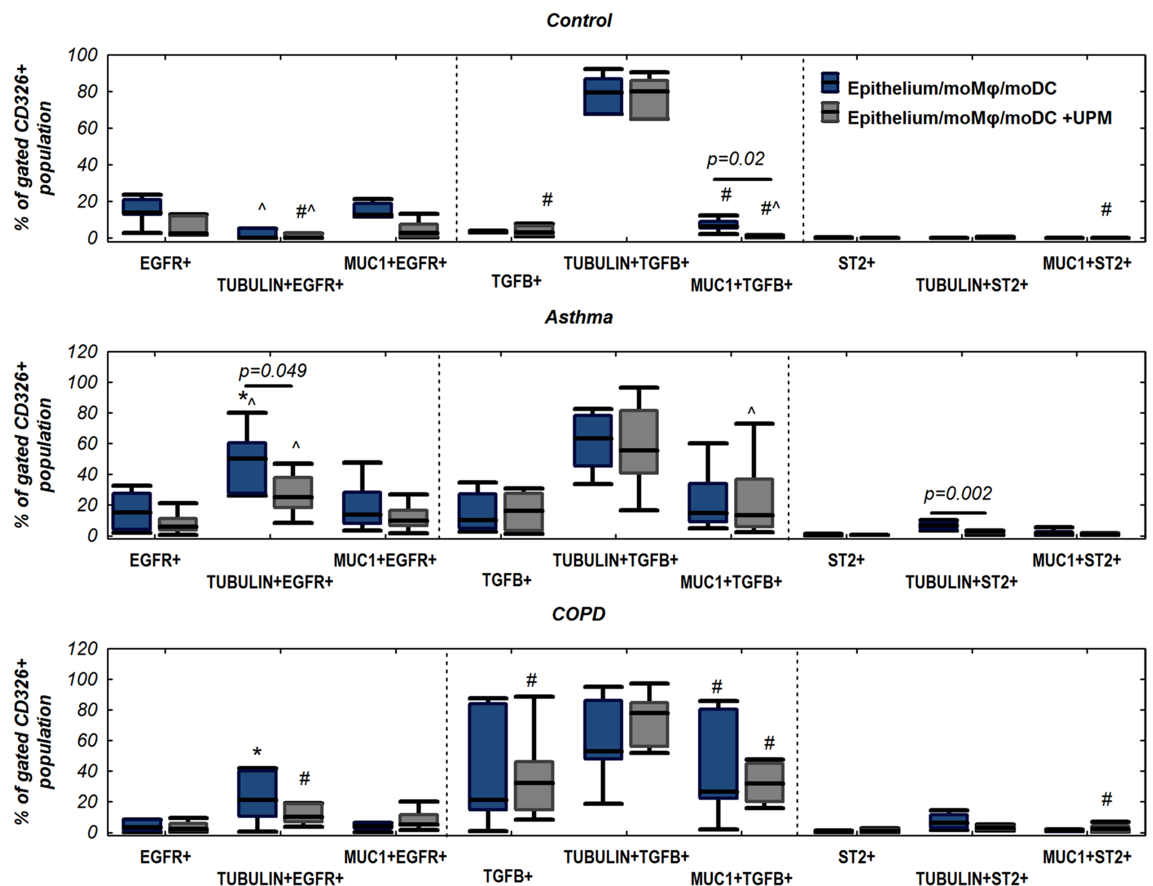


Figure 13. EGFR, TGFβ, and ST2 expression on air–liquid interface (ALI) cultured epithelium upon monocyte derived macrophages (moMφs) and monocyte derived dendritic cells (moDCs), co-cultivation with or without 24 h UPM exposure in control, asthma, or COPD groups. p value <0.05 calculated using in Mann–Whitney U test: ^ asthma versus control, # COPD versus control, *asthma versus COPD.

We found that moMφs without UPM treatment produced different cytokines in healthy people and patients with obstructive lung diseases—they do not produce IL-6 in asthma and COPD and secrete more IL-8 in COPD. The results of our study indicated that moMφs of patients with asthma and COPD vary in terms of reaction to UPM exposure from controls suggesting their changed ability for proper protective function in cooperation with epithelial cells after air pollutant response.

Our study showed that the integrity of the cellular barrier was increased in epithelium/moMφs co-cultures compared to epithelium alone of asthma patients; this effect was not UPM-depended. In other in vitro models, the addition of macrophages (THP-1 cell line) did not induce TEER values in pulmonary epithelial cells (Calu-3 cell line) co-cultured with pulmonary endothelial cells (HPMEC-ST1.6R cell line)[49]. In contrast, other authors observed significantly lower TEER values using a similar cell culture model[50]. In similar cell culture configurations as in our study, Lehman et al. found that TEER values of triple-co-cultures were lower than in epithelial monocultures[51]. We believe that our results partially confirm this observation: the addition of moDCs and/or moMφs to epithelial cells decreased TEER values in controls (with the most profound effect in triple co-cultures) but did not change TEER values in asthma and COPD suggesting that this effect is related to their pathology. The use of macrophages in the in vitro system might cause some epithelial morphological changes. The interactions of the moDCs and/or moMφs within the epithelium may elucidate the decrease of the integrity of tight junctions in control di- and triple co-cultures. The normal epithelial cells (in the control group) are probably less tightly connected to each other after moDCs and/or moMφs co-cultivation due to the development of cell–cell interactions in contrast to asthma and COPD where moDCs and/or moMφs co-cultivation with epithelial cells was able to prevent a leakage barrier. It has been shown that moDCs and/or moMφs are able to interact in the triple cell co-culture as a transepithelial network, by building cytoplasmic pseudopodia with epithelium, which might more tightly connect the cells[52]. Our study showed that the interactions between epithelium and macrophages/DCs are different in obstructive lung diseases than in the control group. We can only speculate that this effect is related to epithelial cells which were obtained from more environmental, and disease impacted origin than moDCs and moMφs (from peripheral blood). The hyperreactivity of respiratory epithelial cells in obstructive

lung diseases might explain the different effects of UPM treatment on the integrity of the cellular barrier in asthma and COPD compared to controls. The results of our work are partially compatible with other studies. Some of them showed no changes in TEER values after PM exposure with respect to the controls with a simultaneous decrease in the expression of thigh junction proteins[53,54]. Also, the type of in vitro model applied matters: Lehmann et al. found a reduction of TEER in epithelial monocultures after DEP exposure in contrast to triple co-cultures where no changes in TEER value were found[51]. On the other hand, it is known that PM disrupts the airway epithelial barrier via oxidative stress[55,56]. It should be kept in mind that chronic UPM exposure impacts the integrity of the epithelial barrier. The changes in TEER values after 24 h UPM stimulation used in our study reflect the fast changes, whereas a long-term observation could give us the whole picture of the evaluated issue, which is impossible to obtain due to the high toxicity of UPM and complexity of the in vitro model used.

Our study showed that the expression of markers of remodeling was increased in obstructive lung diseases in relation to controls, which might be related to the pathological epithelial dysfunction related to asthma and COPD biology. The components of $PM_{2.5}$ like ROS or PAH are linked to airway remodeling and EMT pathobiology[57,58]. Ambient PM can enhance the expression of ligands for EGFR and phospho-EGFR[59,60]. $PM_{2.5}$ from wood smoke-induced MUC5AC expression in NCI-H292 cell line via activation of EGFR-extracellular signal-regulated kinase (ERK) signaling[16]. EGFR activation and autocrine production of EGFR ligands are associated with IL-8 expression after DEPs exposure[61]. $PM_{2.5}$ is involved in the increase of epithelial cortical stiffness, which enables mechanical activation of TGFβ[62]. We observed fewer EGFR + and ST2 + ciliated epithelial cells in asthma and TGFβ + secretory epithelial cells in controls after UPM exposure. We can speculate that UPM stimulation causes rapid and huge production of e.g., EGFR ligands (e.g., EGF, transforming growth factor-alpha, betacellulin, and amphiregulin) with a high affinity to the receptor, which prevents binding of fluorochrome-labeled antibodies. Willmarth et al. demonstrated that EGF stimulated EGFR internalization in the normal mammary epithelial cell line MCF10A[63]. In this study, EGF stimulation resulted in significantly less mean fluorescence of EGFR in cells compared to amphiregulin stimulation. The decreased number of EGFR + in flow cytometry in our study might be the result of the receptor internalization by ligands produced by epithelial cells after UPM stimulation and be interpreted as intense receptor activity on triple co-cultured epithelial cells after UPM exposure. We can speculate that air pollution contributes to airway remodeling through EGFR or ST2 on ciliated cells in asthma patients. The reprogrammed and changed function of epithelial cells in the airways might be associated with a more intense reaction of asthmatic patients to air pollution. This observation should be investigated in more detail in the future.

Our study has some limitations. First, our cultures did not contain cells from the lower respiratory tract which are involved in pathobiological processes underlying obstructive lung diseases. This scheme is a simplified model for the evaluation of cell-to-cell interactions, only. Nasal epithelial cells are used as a functional surrogate for bronchial epithelial cells—the main effector structural cells in obstructive lung diseases. On the other hand, the non-invasive obtainment of material led us to create a unique comparison of immunological processes impacted by cell–cell interactions between healthy, asthma and COPD patients, because cells in co-culture configurations came from one patient. Secondly, we did not include healthy smokers as a better comparison for COPD. We believe that a comparison of results from COPD patients with those from non-smoking control subjects, representing a normal airway environment, provided conclusions concerning important processes impacted by UPM in respiratory epithelium. Thirdly, we did not evaluate separate components of UPM. The detailed characteristic of epithelial immunological reactions impacted by PM_{10} or $PM_{2.5}$ in relation to DCs and macrophage interactions with epithelium might be an interesting direction for future research.

Conclusions

The inflammatory response of nasal epithelial cells to UPM stimulation is affected differently by macrophages/epithelial/DCs interactions in healthy people, asthma or COPD patients. We found a strong effect of DCs interactions on the inflammatory reaction of epithelial cells after UPM exposure and speculate that the cross-talk of these cells determinates the local airway response to air pollution. The integrity of epithelial barrier in asthma, and COPD after UPM treatment was affected differently than in controls. The inflammatory alternations after UPM treatment were more intense in patients with obstructive lung diseases than in healthy subjects. We found an increase in the integrity of the cellular barrier of asthmatic epithelium co-cultivated with moMφs not related to UPM exposure. We showed changes in the expression of EGFR + and ST2 + on epithelial cells with ciliated phenotype in asthma, and TGFβ + on epithelial cells with secretory phenotype in controls after UPM exposure. These observations suggest the possible mechanism of airway remodeling through EGFR + and ST2 + associated with air pollution in asthma. A brief summary of the results of this study is illustrated on Fig. 14. The results of our study might help in fully understanding the substantial processes associated with PMs toxicity and its impact on asthma and COPD course.

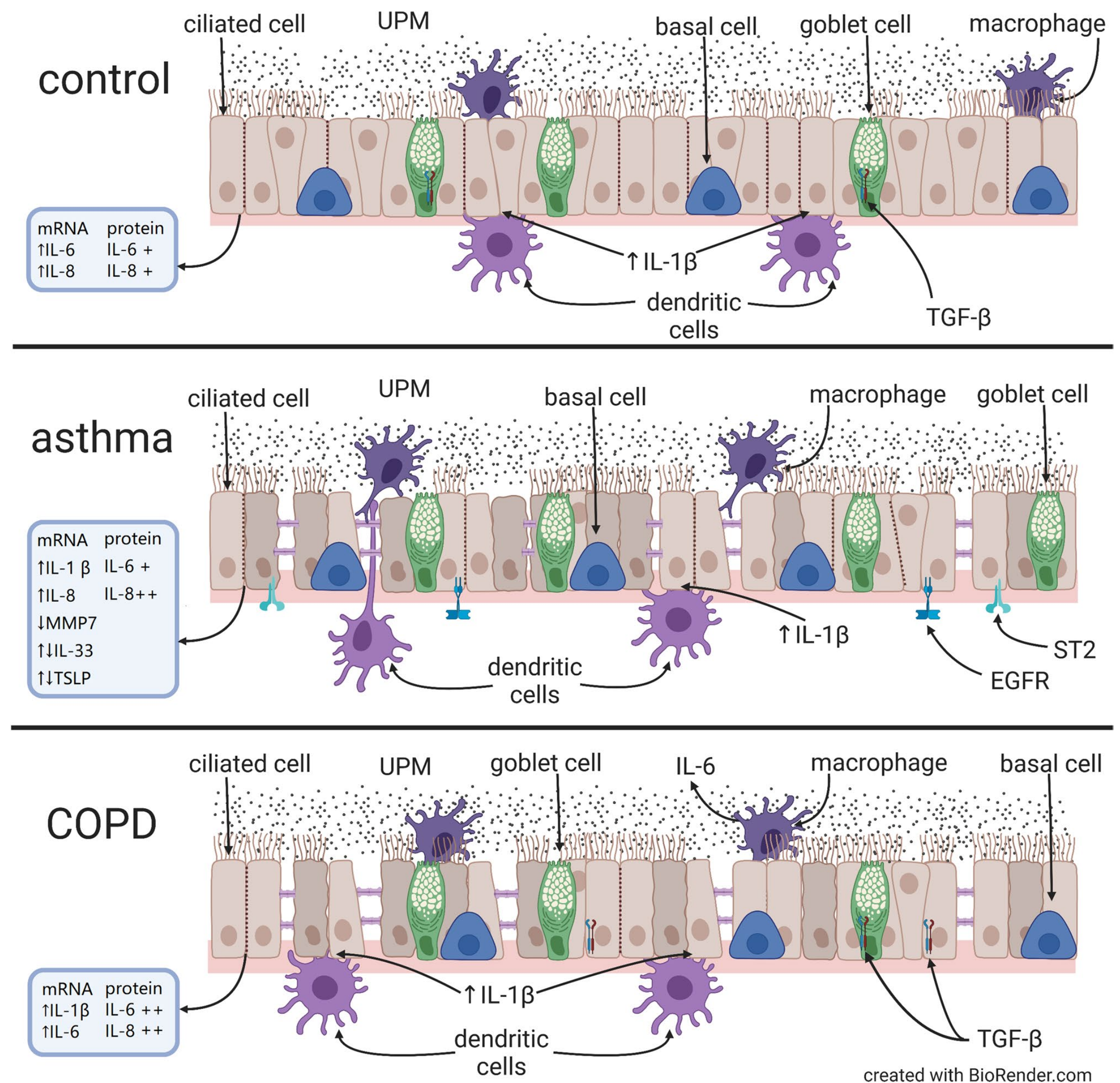


Figure 14. A schematic description of the study results.

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Author contributions
Concept and design: MPG, PMS, experiments, measurements and data acquisition: MPG, PMS, MP, PNG, EZZ, KG, data analysis: MPG, RK, drafting the manuscript for important intellectual content: MPG, PMS, RK, PNG, MP.

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Competing interests
The authors declare no competing interests.

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Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD

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Patients' characteristics

This was a prospective, cross-sectional study which involved 8 healthy controls, 10 asthma patients, and 8 patients with COPD. In all patients, the diagnosis of asthma or COPD was previously established according to current Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations, respectively [14,15]. The following examinations were performed after patient enrolment: medical history, physical examination, spirometry with flow-volume curve, airway obstruction reversibility test (when applicable, performed according to the recommendations of the European Respiratory Society (ERS)), allergy skin prick tests with a panel of fifteen aeroallergens. Disease control was assessed by Asthma Control Test (ACT) in asthmatics and by the COPD Assessment Test (CAT) in patients with COPD. The nasal brushing and peripheral blood sample were obtained from each patient. Exclusion criteria for all asthma and COPD patients were systemic or nasal steroid treatment, disease exacerbation or symptoms of respiratory tract infection in the previous 3 months. The control group consisted of smoking and non-smoking volunteers, without with normal spirometry.

Monocyte derived dendritic cell (moDC) and monocyte derived macrophages (moMφ) culture

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly drawn venous blood by Lymphoprep (Stemcell, Canada) centrifugation. The PBMC monocytes were achieved by adherence in Monocyte Attachment Medium (Promocell, Germany) for 2 hours. Monocyte derived dendritic cells (moDCs) were cultured in X-Vivo 20 medium (Lonza, Austria) supplemented with 40 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) (Stemcell, Canada) for 8 days and 20 ng/ml IL-4 (PromoKine, Germany) for 5 days and 50 ng/ml IL-1β (Stemcell, Canada) and 50 ng/ml TNF-α (Biotechne, R&D Systems, MN, USA) in the 6th day of culture. Monocyte derived macrophages (moMφs) were specialized from PBMC monocytes by stimulation with 20 ng/ml macrophage colony-stimulating factor M-CSF (Stemcell, Canada) for 10 days in Macrophage Base Medium DXF (Promocell, Germany).

The culture of epithelial cells in air liquid interface (ALI)

Epithelial cells were obtained by nasal brushing (Cytobrush Plus GT, CooperSurgical, Germany). The cells were detached from the brush by gentle agitation, centrifuged (300 g, 10 min, room temperature) and treated with Accutase (BD Biosciences, San Jose, CA, USA) for 10 min. in 37°C. The cell pellet was suspended in a total volume of 5 ml of airway epithelial growth medium (Promocell, Germany) containing antibiotics, and seeded into sterile plastic T25 bottles (Thermo Fisher Scientific, MA, USA). Cells were

incubated in a plastic dish for 24 hours at 37°C. The undetached cells were removed and the cell medium changed. The adhered cells were cultured until reached 80% of confluency. $1x10^5$ cells/cm^2 were seeded on the apical surface of 6.5 mm trans well Thin Certs with 0.4 µm pore size and placed in 24 well flat-bottomed plates (Greiner Bio-One, Austria) with epithelial growth medium (Promocell, Germany) in the basal (0.5 ml) and upper (0.2 ml) compartments. After 100% of confluence was reached, cells in the upper chamber were exposed to air, the medium in basal chamber was changed for ALI Maintenance Medium (Stemcell, Canada) and exchanged every 2 day. The ALI epithelium was generated for 21 days.

Triple Co-Culture

Each triple-cell co-culture model will contain three different cell types obtained from one patient. The fully specialized ALI epithelial cells were supplemented with moMφs and moDCs as follows: moDCs were harvested, washed, centrifuged, re-suspended in X-Vivo-20 medium and $2x10^5$ cells in 50 µl medium was added to the basal side of the inserts placed upside down. The dish with the inserts was covered and placed in the incubator for 1.5h–2h. MoMφs were harvested by washing with PBS followed by addition of Cellstripper (Corning, New York, USA) and detached with cell scrapers. A volume of 35 µl Macrophage Medium DXF (Promocell, Germany) containing $1.0x10^5$ cells was added on the apical side of the epithelial monolayer on the insert, forming the upper chamber.

RNA isolation, cDNA synthesis and real time PCR

Total RNA was isolated from the cells using Trizol (Sigma Aldrich, MO, USA). The concentration and purity of isolated RNA was measured on a DU650 spectrophotometer (Beckman Coulter, Brea, CA, USA). Eight microliters of total RNA was used for reverse transcription (Thermo Fisher Scientific, MA, USA). Real-time PCR measurements were performed with an ABI-Prism 7500 Sequence Detector System (Applied Biosystems, Thermo Fisher, MA, USA). For PCR reaction 0.8µl of cDNA was amplified in 16 µl PCR volume, containing a TaqMan master mix (Thermo Fisher Scientific, MA, USA) with 150 nM of specific primers and 100 nM of probe (Table S1 Thermo Fisher Scientific, MA, USA). Each sample was measured in duplicate. The results were expressed as relative quantification units (fold change). Relative quantification values were calculated by the $2^{-\Delta\Delta CT}$ method. 18S rRNA was applied for each sample as an internal control in order to normalize gene expression levels. The mean ΔCT of unstimulated epithelial cells from controls was used as a calibrator for all groups.

Table S1 Sequence of primers used in PCR.

	Forward primer	Reverse primer		Product size
18s rRNA	Hs99999901_s1			187
IL-1β	Hs01555410_m1			91
MMP7	Hs01042796_m1			64
IL-6	CCGGGAACGAAAGAGAAGCT	GCGCTTGTGGAGAAGGAGTT	TCTCCCCTCCAGGAGCCCAGCTA	67
IL-8	GAGCACTCCATAAGGCACAAACT	ATCAGGAAGGCTGCCAAGAG	CCAGGAAGAAACCACCGGAAGGAACC	149
MMP9	GCTCACCTTCACTCGCGTG	CGCGACACCAAACTGGATG	ACAGCCGGGACGCAGACATCG	61
TSLP	CCACTGGTGTTTATAGGGTTCTGA	TCTTGAATTCCCGCTGCAA	TCTCCCCTCCAGGAGCCCAGCTA	79

IL-33	GCCTAGATGAGACACC GAATTAACA	CCAGGGTCAGAAGGG ATGCT	AACTGACTGTCCCTCATG TCCATGGCC	86

Flow cytometry

Human TruStain FcX (Biolegend, San Diego, CA, USA) (5 µl per 100 µl of sample) was added to block non-specific bindings. Cells were stained with antibodies against the surface binding molecules, for epithelial cells: CD326, CD45, EGF, MUC1 (BD Biosciences, San Jose, CA, USA), ST2 (Biotechne, R&D Systems, MN, USA) (Table S2) in BD Horizon Brilliant Stain Buffer (BD Biosciences, San Jose, CA, USA), and incubated for 20 minutes in the dark at room temperature. After washing away the reagents, the cells were fixed and permeabilizated using lysis buffer and permeabilizations solution 2 (BD Biosciences, San Jose, CA, USA), then stained with intracellular marker (TGF-β1, β-tubulin (BD Biosciences, San Jose, CA, USA)) for 20 minutes in the dark.

Cells were analysed by flow cytometry using a FACSCelesta instrument (BD Biosciences, San Jose, CA, USA) equipped with blue (488-nm), violet (405-nm), and red (640-nm) lasers. Unstained cells and compensation beads (BD Biosciences, San Jose, CA, USA) were used to set voltages and create single stain negative and positive controls. Compensation was set to account for spectral overlap between the seven fluorescent channels used in the study. Samples were examined by side scatter area (SSC-A) versus forward scatter area (FSC-A), then using forward scatter height (FSC-H) versus FSC-A to select single cells, eliminating debris and clumped cells from the analysis. At least 50 000 cells in the target gate were collected.

Table S2. The characterisation of fluorochrome-conjugated antibodies for epithelial cells used in flow cytometric analysis

Target	Fluorochrome conjugate	Emission Max (nm)	Clone	Supplier	Catalogue number	Quantity per sample
EGF	BV421	421	EGFR1	BD Biosciences	566254	3 µl
β-TUBULIN	ALEXA FLUOR 488	519	TUJ1	BD Biosciences	560381	7 µl
TGF	PE	578	TW4-9E7	BD Biosciences	562339	3 µl
MUC1	BV605	602	HMFG2	BD Biosciences	747654	3 µl
ST2	APC	660		Biotechne	FAB5231A	7 µl
CD326	BB700	693	EBA-1	BD Biosciences	745841	3 µl
CD45	APC-H7	785	2D1	BD Biosciences	641399	3 µl

Table S3. IL-1β, IL-6, IL-8, MMP7, MMP9, TSLP, IL-33 mRNA expression in air–liquid interface (ALI) cultured nasal epithelium after 24h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients.

		Control (n=8)	Asthma (n=10)	COPD (n=8)
	IL-1β mRNA expression (fold change)			
a	Epithelium	-1.1 (-2.3-1.3)	3.7 (2.3-15.0)	2.4 (-1.2-8.3)
b	Epithelium+UPM	-1.4 (-4.3-6.0)	16.1 (5.1-45.8)	5.5 (-0.7-27.9)
c	Epithelium+DC	2.0 (-1.4-38.5)	8.0 (2.4-21.3)	0.1 (-14.1-7.0)
d	Epithelium+DC+UPM	4.5 (-1.5-89.7)	25.9 (13.0-36.2) [f]	3.2 (-1.4-37.6)
e	Epithelium+Mφ	-2.7 (-9.4 – -1.6)	4.1 (-1.7-26.5)	0.2 (-3.0-3.8)
f	Epithelium+Mφ (UPM24h)	-1.6 (-3.2 – -1.1)	3.5 (0.5-9.5) [d]	1.6 (-1.8-3.2)
g	Epithelium+Mφ+UPM	0.0 (-8.3-14.5)	10.2 (-5.6-19.4)	9.3 (1.6-29.7) h
h	Epithelium+Mφ+DC	-2.4 (-6.4 – -1.7)	5.2 (2.2-8.9)	-1.5 (-28.1-2.9) g
i	Epithelium+Mφ+DC+UPM	8.2 (-1.8-46.7)	9.8 (7.4-32.2)	3.4 (-0.2-18.2)
j	Epithelium+Mφ (UPM24h)+DC+UPM	5.6 (-1.6-42.7)	4.3 (1.9-12.0)	1.9 (-0.4-4.4)
	IL-6 mRNA expression (fold change)			
a	Epithelium	-1.3 (-1.7-2.3)	3.7 (-2.1-22.5)	1.4 (-2.1-2.8) [d]
b	Epithelium+UPM	1.1 (-6.5-2.6) d	1.3 (-5.1-16.7)	3.5 (-0.1-8.5) [e,h]
c	Epithelium+DC	-1.4 (-2.7-22.2)	8.1 (-7.2-83.5)	1.6 (-3.6-3.6)
d	Epithelium+DC+UPM	4.0 (3.5-121.1) [b,e,f]	7.7 (-4.5-194.5)	7.0 (2.9-12.2) [a,e,h]
e	Epithelium+Mφ	-3.7 (-16.7– -2.0) [d,i]	1.5 (-9.4-14.9)	-2.4 (-7.0-1.1) [b,d,g]
f	Epithelium+Mφ (UPM24h)	-2.0 (-13.0-2.3) [d]	-0.1 (-19.2-15.9)	2.3 (-4.8-4.0)
g	Epithelium+Mφ+UPM	-5.1 (-30.4-6.4)	1.3 (-1.1-5.2)	9.8 (0.4-29.1) [e,h]
h	Epithelium+Mφ+DC	-2.5 (-33.4-3.0)	4.4 (-1.7-23.2)	-0.3 (-7.6-2.3) [b,d,g]
i	Epithelium+Mφ+DC+UPM	3.6 (-1.2-25.1) [e]	9.0 (-1.3-171.3)	3.8 (1.4-22.3)
j	Epithelium+Mφ (UPM24h)+DC+UPM	1.9 (-1.0-45.9)	1.2 (-3.2-22.6)	-0.3 (-6.1-9.1)
	IL-8 mRNA expression (fold change)			
a	Epithelium	1.2 (-4.9-1.6) [j]	2.7 (-1.9-4.4)	-2.5 (-8.6-0.3)
b	Epithelium+UPM	-2.1 (-4.8 – -1.4) [d,i,j]	1.6 (-3.1-3.8)	-1.8 (-3.5– -0.1)
c	Epithelium+DC	1.5 (-3.0-30.2)	1.6 (-1.2-8.2)	-0.1 (-5.9-1.9)
d	Epithelium+DC+UPM	3.4 (1.7-7.8) [b,e,g]	6.4 (3.4-11.5) [h,j]	-1.3 (-1.8-1.7)
e	Epithelium+Mφ	-2.2 (-3.5-2.0) [d,j]	-1.4 (-3.5-3.3)	-1.7 (-7.0 – -1.1)
f	Epithelium+Mφ (UPM24h)	1.6 (-1.8-2.1)	-1.1 (-1.8-3.4)	-2.1 (-3.1– -0.1)
g	Epithelium+Mφ+UPM	-2.7 (-3.4 – -1.7) [d,j]	2.0 (-4.1-2.5)	-1.4 (-2.5-1.3)
h	Epithelium+Mφ+DC	-2.2 (-5.2-1.5)	1.5 (1.1-3.6) [d]	-1.8 (-8.0-0.1)
i	Epithelium+Mφ+DC+UPM	1.5 (1.3-7.2) [b]	3.5 (1.8-5.7) [j]	-1.5 (-2.4-1.7)

j	Epithelium+Mφ (UPM24h)+DC+UPM	3.0 (1.7-25.4) [a,b,e,g]	-0.1 (-2.8-1.7) [d,i]	-1.8 (-3.1 – -0.1)
	MMP7 mRNA expression (fold change)			
a	Epithelium	-1.8 (-3.1-1.6)	5.6 (3.3-11.5) [j]	4.4 (-4.9-24.2)
b	Epithelium+UPM	-1.6 (-4.6-1.0)	0.1 (-4.2-12.2)	-1.2 (-6.7-9.5)
c	Epithelium+DC	1.6 (-1.8-33.7)	2.2 (-2.6-9.4)	9.4 (-4.7-22.0)
d	Epithelium+DC+UPM	-1.8 (-4.8-8.5)	1.9 (-1.4-9.6)	0.2 (-7.7-6.4)
e	Epithelium+Mφ	-2.4 (-5.3-2.3)	6.1 (1.6-14.3)	11.2 (-2.9-23.2)
f	Epithelium+Mφ (UPM24h)	-3.0 (-3.3-1.8)	2.1 (-5.5-7.4)	0.0 (-7.4-5.5)
g	Epithelium+Mφ+UPM	-2.2 (-4.3 – -1.1)	-1.0 (-2.2-8.5)	2.0 (-5.9-6.5)
h	Epithelium+Mφ+DC	-2.9 (-4.8-1.0)	2.2 (1.5-5.3)	1.2 (-5.0-11.0)
i	Epithelium+Mφ+DC+UPM	-1.4 (-3.3-3.1)	-1.3 (-2.0-12.5)	0.8 (-12.4-7.2)
j	Epithelium+Mφ (UPM24h)+DC+UPM	-2.2 (-3.0-1.1)	1.0 (-6.3-2.6) [a]	-0.7 (-6.3-5.2)
	MMP9 mRNA expression (fold change)			
a	Epithelium	1.2 (-2.0-1.4)	4.9 (2.3-8.8) [c]	-2.0 (-4.7 – -1.4)
b	Epithelium+UPM	-3.4 (-7.3-1.0)	-1.4 (-2.5-2.0)	-3.6 (-6.6 – -1.8)
c	Epithelium+DC	-3.7 (-5.8-6.5)	1.2 (-2.4-1.4) [a]	-4.7 (-6.6 – -2.2)
d	Epithelium+DC+UPM	2.1 (-2.9-5.5)	1.8 (-1.5-3.8)	-3.0 (-14.3-2.3)
e	Epithelium+Mφ	-1.1 (-7.9 – -1.1)	2.7 (1.4-14.7)	-1.5 (-3.3-2.2)
f	Epithelium+Mφ (UPM24h)	-2.0 (-5.3--1.2)	-1.5 (-2.6-5.9)	-2.1 (-7.7--0.2)
g	Epithelium+Mφ+UPM	-2.1 (-2.9-1.1)	-1.1 (-2.6-6.8)	-2.0 (-4.2-2.1)
h	Epithelium+Mφ+DC	-2.8 (-6.1-1.3)	2.2 (-1.6-4.5)	-1.8 (-2.4-0.1)
i	Epithelium+Mφ+DC+UPM	0.0 (-1.5-5.8)	1.6 (-1.2-7.5)	-2.2 (-6.3-0.9)
j	Epithelium+Mφ (UPM24h)+DC+UPM	-1.0 (-4.8-35.0)	2.9 (1.0-6.5)	-3.1 (-23.1-1.3)
	IL-33 mRNA expression (fold change)			
a	Epithelium	-1.0 (-3.0-1.0)	11.6 (2.9-45.5) [j]	6.1 (2.3-35.9)
b	Epithelium+UPM	-2.3 (-5.1– -1.7)	1.3 (-1.2-2.5)	8.3 (1.8-20.5)
c	Epithelium+DC	-1.8 (-5.2-5.4)	2.3 (1.3-4.7) [j]	9.5 (2.3-49.2)
d	Epithelium+DC+UPM	-1.8 (-2.8 – -1.0)	4.2 (1.5-6.0) [j]	4.1 (2.3-15.4)
e	Epithelium+Mφ	-1.4 (-4.1-2.7)	4.4 (-1.1-63.1)	3.9 (2.0-53.7)
f	Epithelium+Mφ (UPM24h)	-1.8 (-3.0-10.2)	2.1 (-0.1-13.2)	9.3 (0.0-44.6)
g	Epithelium+Mφ+UPM	-3.4 (-10.7-4.1)	-1.7 (-4.8-1.6)	5.5 (1.5-18.8)
h	Epithelium+Mφ+DC	-4.5 (-12.1-5.3)	1.3 (-2.4-2.2)	6.4 (3.6-22.8)
i	Epithelium+Mφ+DC+UPM	-2.4 (-2.8-1.4)	1.8 (-1.9-3.1)	3.1 (1.3-27.1)
j	Epithelium+Mφ (UPM24h)+DC+UPM	-2.1 (-2.9-30.0)	-1.1 (-2.8-1.2) [a,c,d]	2.4 (1.4-36.5)
	TSLP mRNA expression (fold change)			
a	Epithelium	1.3 (-4.0-3.0)	7.1 (2.9-22.1) [c,d,i,j]	3.7 (-3.6-10.2)
b	Epithelium+UPM	-5.2 (-12.8 – -2.3) [d]	3.0 (-1.7-6.9)	4.3 (-6.4-14.3)
c	Epithelium+DC	-6.3 (-12.7-11.9)	0.7 (-2.3-4.2) [a]	3.8 (0.9-12.5)
d	Epithelium+DC+UPM	1.4 (1.1-2.9) [b]	1.8 (-1.3-2.4) [a]	8.0 (-0.3-29.7)

e	Epithelium+Mφ	-1.8 (-4.7-2.8)	3.1 (-1.4-5.4)	2.5 (-2.4-13.2)
f	Epithelium+Mφ (UPM24h)	-1.4 (-4.9-1.1)	2.1 (0.3-3.1)	10.1 (-6.0-13.2)
g	Epithelium+Mφ+UPM	-3.3 (-41.5-1.5)	1.7 (-1.6-8.5)	5.4 (-0.1-14.8)
h	Epithelium+Mφ+DC	-8.9 (-34.7-1.5)	2.0 (-2.0-4.5)	2.2 (-2.8-4.6)
i	Epithelium+Mφ+DC+UPM	-2.2 (-2.8-3.1)	-0.1 (-2.0-2.3) **[a]**	3.6 (-4.3-21.8)
j	Epithelium+Mφ (UPM24h)+DC+UPM	-2.1 (-9.5-355.9)	1.3 (-2.5-2.8) **[a]**	3.3 (-10.5-21.3)

The results are presented as median and IQR, p-value calculated using Mann–Whitney U test. The p-value<0.05 in comparison to: a - epithelium, b - epithelium+UPM, c - epithelium+moDCs, d – epithelium+moDCs+UPM, e - epithelium+moMφs, f - epithelium+moMφs (24h UPM), g- epithelium +moMφs+UPM, h - epithelium+moMφs+moDCs, i - epithelium+moMφs+moDCs+UPM, j - epithelium +moMφs (24hUMP)+moDCs+UPM

Table S4. IL-1β, IL-6, IL-8 secretion by air–liquid interface (ALI) cultured nasal epithelium after 24h UPM exposure in multi co-culture schemes in control subjects, asthma and COPD patients.

		Control (n=8)	Asthma (n=10)	COPD (n=8)
	IL-1β (pg/ml)			
a	Epithelium	0.8 (0.0-2.3) **[c,d,h,i,j]**	0.0 (0.0-3.5) **[c,d,h,i,j]**	0.8 (0.0-2.6) **[c,d,f,i,j]**
b	Epithelium+UPM	1.7 (0.0-2.3) **[c,d,h,i,j]**	2.2 (0.9-7.0)**[d,e,f,h,i,j]**	0.0 (0.0-7.3) **[c,d,h,i,j]**
c	Epithelium+DC	15.6 (7.1-22.9) **[a,b,e,f,g]**	6.8 (4.1-8.3) **[a,e,f,g]**	12.6 (7.4-18.3)**[a,b,e,f,g]**
d	Epithelium+DC+UPM	12.8 (8.3-18.0) **[a,b,e,f,g]**	6.8 (6.2-10.4) **[a,b,e,f,g]**	12.3 (7.5-14.2)**[a,b,e,f,g]**
e	Epithelium+Mφ	0.0 (0.0-1.8) **[c,d,h,i,j]**	0.0 (0.0-0.0) **[b,c,d,h,i,j]**	0.0 (0.0-0.0) **[c,d,h,i,j]**
f	Epithelium+Mφ (UPM24h)	0.0 (0.0-0.0) **[c,d,f,i,j]**	0.0 (0.0-0.0) **[b,c,d,h,i,j]**	0.0 (0.0-0.0) **[c,d,h,i,j]**
g	Epithelium+Mφ+UPM	0.0 (0.0-0.0) **[c,d,h,i,j]**	0.8 (0.0-3.2) **[c,d,h,i,j]**	0.8 (0.0-2.6) **[c,d,h,i,j]**
h	Epithelium+Mφ+DC	9.2 (5.0-20.9) **[a,b,e,f,g]**	6.2 (4.1-9.3) **[a,b,e,f,g]**	11.4 (6.8-18.4) **[a,b,e,f,g]**
i	Epithelium+Mφ+DC+UPM	11.2 (7.2-20.7) **[a,b,e,f,g]**	7.6 (5.0-13.5) **[a,b,e,f,g]**	16.6 (13.4-28.5) **[a,b,e,f,g]**
j	Epithelium+Mφ (UPM24h)+DC+UPM	12.2 (4.9-17.7) **[a,b,e,f,g]**	8.3 (6.6-12.0) **[a,b,e,f,g]**	14.1 (8.0-19.0) **[a,b,e,f,g]**
	IL-6 (pg/ml)			
a	Epithelium	3.4 (1.1-11.9) **[d,i,j]**	4.7 (3.5-6.0)	2.7 (1.2-66.5)
b	Epithelium+UPM	11.0 (2.3-88.7)	34.3 (5.8-57.3) **[h]**	15.8 (9.9-92.7)
c	Epithelium+DC	14.4 (9.5-42.6)	10.5 (1.1-29.0)	15.5 (8.8-63.8)
d	Epithelium+DC+UPM	18.0 (12.7-21.1) **[a,e,f]**	19.4 (4.8-36.5)	31.3 (11.8-102.1)
e	Epithelium+Mφ	2.2 (0.0-10.5) **[d,i,j]**	4.0 (1.9-19.6)	9.8 (3.3-20.6) **[g,i,j]**
f	Epithelium+Mφ (UPM24h)	3.1 (2.1-7.9) **[d,i,j]**	19.3 (5.1-51.3)	14.6 (9.5-28.0) **[g,j]**
g	Epithelium+Mφ+UPM	8.9 (0.0-18.0)	25.8 (4.5-68.6)	55.6 (31.9-76.0) **[e,f]**
h	Epithelium+Mφ+DC	10.2 (4.5-19.1)	5.6 (2.9-11.7) **[b,j]**	16.1 (9.2-55.8)
i	Epithelium+Mφ+DC+UPM	16.0 (13.3-37.1) **[a,e,f]**	32.6 (3.9-65.2)	54.5 (19.8-117.4) **[e]**
j	Epithelium+Mφ (UPM24h)+DC+UPM	15.2 (12.3-19.1) **[a,e,f]**	34.6 (6.0-54.3) **[h]**	54.4 (18.4-85.5) **[e,f]**
	IL-8 (pg/ml)			

a	Epithelium	168.6 (143.2-196.5)	184.9 (115.9-321.9) [d,g,j]	311.3 (165.5-1330.6)
b	Epithelium+UPM	180.3 (171.3-184.6)	358.4 (262.5-566.4)	417.8 (255.0-1009.7)
c	Epithelium+DC	249.9 (194.2-264.0) [g]	276.4 (187.0-533.6) [d,j]	461.5 (410.0-1017.3)
d	Epithelium+DC+UPM	227.8 (219.6-297.5) [f,g]	607.7 (442.9-804.5) [a,c,e,h]	431.0 (320.8-544.5)
e	Epithelium+Mφ	199.8 (132.4-232.8)	288.4 (182.3-405.6) [d,j]	403.5 (237.3-549.7) [j]
f	Epithelium+Mφ (UPM24h)	105.2 (84.9-199.0) [d,i]	310.4 (199.9-506.2) [j]	368.5 (247.6-1121.7)
g	Epithelium+Mφ+UPM	163.1 (110.5-194.0) [c,d,i]	407.1 (308.8-507.8) [a]	423.1 (308.3-1643.6)
h	Epithelium+Mφ+DC	203.8 (148.9-244.9)	315.9 (168.3-500.1) [d,j]	482.1 (350.0-1161.0)
i	Epithelium+Mφ+DC+UPM	238.2 (229.6-247.9) [f,g]	363.7 (283.9-592.4)	593.2 (455.0-1539.2)
j	Epithelium+Mφ (UPM24h)+DC+UPM	226.2 (152.0-289.5)	614.4 (425.4-764.8) [a,c,e,f,h]	708.1 (533.6-921.5) [e]

The results are presented as median and IQR, p-value calculated using Mann–Whitney U test. The p-value<0.05 in comparison to: a - epithelium, b - epithelium+UPM, c - epithelium+moDCs, d – epithelium+moDCs+UPM, e - epithelium+moMφs, f - epithelium+moMφs (24h UPM), g- epithelium +moMφs+UPM, h - epithelium+moMφs+moDCs, i - epithelium+moMφs+moDCs+UPM, j - epithelium +moMφs (24hUMP)+moDCs+UPM

Table S5 The comparison (Mann-Whitney test) of IL-1β protein concentration with or without UPM stimulation in nasal epithelial cells cultured in air liquid interference (ALI) alone or co-cultured with monocyte derived macrophages (moMφs) and/or monocyte derived dendritic cells (moDCs) in control group

control	Epithelium	Epithelium +UPM	Epithelium +DC	Epithelium +DC +UPM	Epithelium +Mφ	Epithelium +Mφ(24h UPM)	Epithelium +Mφ +UPM	Epithelium +Mφ +DC	Epithelium +Mφ +DC +UPM	Epithelium +Mφ (24hUMP) +DC +UPM
Epithelium		0.837	0.001	0.0002	0.299	0.227	0.335	0.0003	0.001	0.00003
Epithelium +UPM	0.837		0.022	0.017	0.259	0.209	0.295	0.014	0.035	0.007
Epithelium +DC	0.0008	0.022		0.505	0.001	0.001	0.002	0.573	0.699	0.553
Epithelium +DC+UPM	0.0002	0.017	0.505		0.001	0.001	0.001	0.694	0.836	0.711
Epithelium +Mφ	0.299	0.259	0.001	0.001		0.805	0.836	0.0003	0.001	0.00004
Epithelium +Mφ (24h UPM)	0.227	0.209	0.001	0.001	0.805		0.945	0.0003	0.001	0.00004
Epithelium +Mφ+UPM	0.335	0.295	0.002	0.001	0.836	0.945		0.001	0.002	0.0001
Epithelium +Mφ+DC	0.0003	0.014	0.573	0.694	0.0003	0.000	0.001		0.950	0.970
Epithelium +Mφ+DC +UPM	0.001	0.035	0.699	0.836	0.001	0.001	0.002	0.950		0.750
Epithelium+Mφ (24hUMP)+DC+ UPM	0.00003	0.007	0.553	0.711	0.00004	0.00004	0.0001	0.970	0.750	

Results of pairwise comparisons between groups. P-values from Mann-Whitney test are given above the diagonal. Proportion of means (group given in a column name divided by group given in a row name) are given below the diagonal.

Table S6 The comparison (Mann-Whitney test) of IL-1β protein concentration with or without UPM stimulation in nasal epithelial cells cultured in air liquid interference (ALI) alone or co-cultured with monocyte derived macrophages (moMφs) and/or monocyte derived dendritic cells (moDCs) in asthma group

asthma	Epithelium	Epithelium +UPM	Epithelium +DC	Epithelium +DC +UPM	Epithelium +Mφ	Epithelium +Mφ(24h UPM)	Epithelium +Mφ +UPM	Epithelium +Mφ +DC	Epithelium +Mφ +DC +UPM	Epithelium +Mφ (24hUMP) +DC +UPM
Epithelium		0.382	0.003	0.0003	0.328	0.328	0.798	0.001	0.0003	0.00002
Epithelium +UPM	0.382		0.130	0.038	0.038	0.021	0.328	0.045	0.021	0.016
Epithelium +DC	0.003	0.130		0.505	0.0003	0.0003	0.005	1.000	0.505	0.238
Epithelium +DC+UPM	0.0003	0.038	0.505		0.0002	0.0002	0.0003	0.417	0.959	0.528
Epithelium +Mφ	0.328	0.038	0.0003	0.0002		0.959	0.234	0.00001	0.0002	0.00001
Epithelium +Mφ (24h UPM)	0.328	0.021	0.0003	0.0002	0.959		0.195	0.000003	0.0002	0.00001
Epithelium +Mφ+UPM	0.798	0.328	0.005	0.0003	0.234	0.195		0.001	0.002	0.0001
Epithelium +Mφ+DC	0.001	0.045	1.000	0.417	0.00001	0.000003	0.001		0.417	0.184
Epithelium +Mφ+DC +UPM	0.0003	0.021	0.505	0.959	0.0002	0.0002	0.002	0.417		0.928
Epithelium+Mφ (24hUMP)+DC+ UPM	0.00002	0.016	0.238	0.528	0.00001	0.00001	0.0001	0.184	0.928	

Results of pairwise comparisons between groups. P-values from Mann-Whitney test are given above the diagonal. Proportion of means (group given in a column name divided by group given in a row name) are given below the diagonal.

Table S7 The comparison (Mann-Whitney test) of IL-1β protein concentration with or without UPM stimulation in nasal epithelial cells cultured in air liquid interference (ALI) alone or co-cultured with monocyte derived macrophages (moMφs) and/or monocyte derived dendritic cells (moDCs) in COPD group

COPD	Epithelium	Epithelium +UPM	Epithelium +DC	Epithelium +DC +UPM	Epithelium +Mφ	Epithelium +Mφ(24h UPM)	Epithelium +Mφ +UPM	Epithelium +Mφ +DC	Epithelium +Mφ +DC +UPM	Epithelium +Mφ (24hUMP) +DC +UPM
Epithelium		0.721	0.002	0.002	0.328	0.328	1.000	0.002	0.001	0.0001
Epithelium +UPM	0.721		0.038	0.050	0.645	0.645	0.721	0.023	0.010	0.011
Epithelium +DC	0.002	0.038		0.721	0.001	0.003	0.001	0.834	0.442	0.697
Epithelium +DC+UPM	0.002	0.050	0.721		0.001	0.005	0.002	0.928	0.105	0.490
Epithelium +Mφ	0.328	0.645	0.001	0.001		0.959	0.328	0.001	0.0003	0.0001
Epithelium +Mφ (24h UPM)	0.328	0.645	0.003	0.005	0.959		0.328	0.001	0.001	0.0002
Epithelium +Mφ+UPM	1.000	0.721	0.001	0.002	0.328	0.328		0.001	0.0003	0.00003
Epithelium +Mφ+DC	0.002	0.023	0.834	0.928	0.001	0.001	0.001		0.264	0.515
Epithelium +Mφ+DC +UPM	0.001	0.010	0.442	0.105	0.0003	0.001	0.0003	0.264		0.417
Epithelium+Mφ (24hUMP)+DC+ UPM	0.0001	0.011	0.697	0.490	0.0001	0.0002	0.00003	0.515	0.417	

Results of pairwise comparisons between groups. P-values from Mann-Whitney test are given above the diagonal. Proportion of means (group given in a column name divided by group given in a row name) are given below the diagonal.

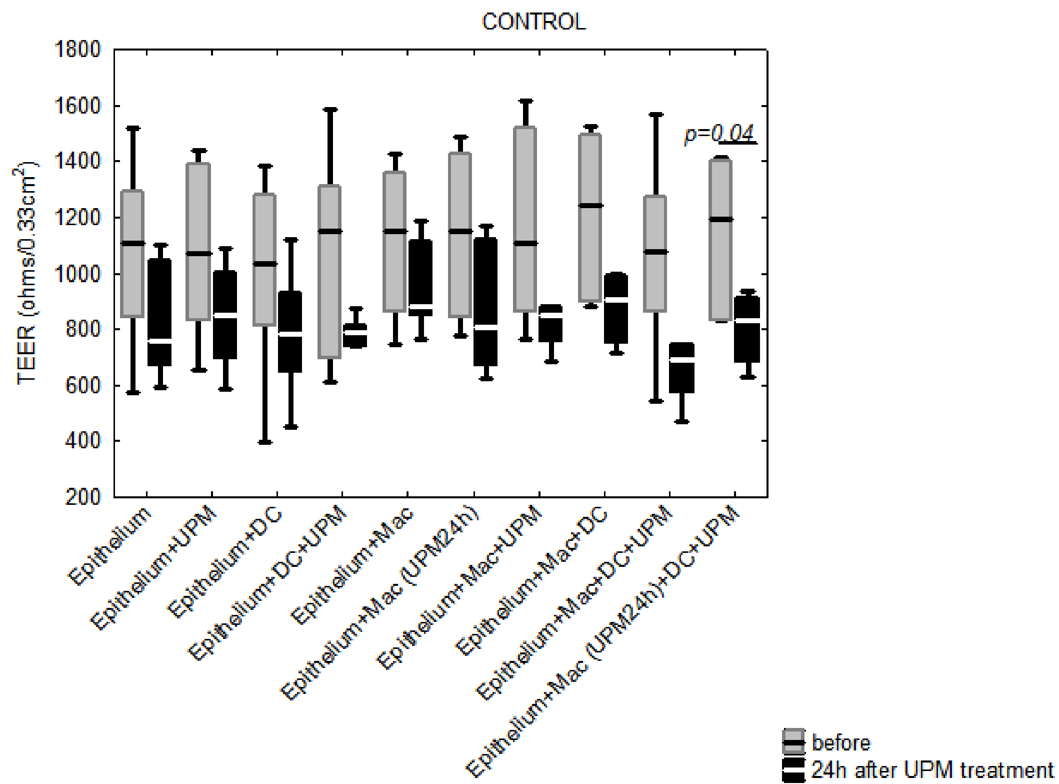

Figure S1 Transepithelial electrical resistance (TEER) after 24h of nasal epithelial cells from healthy donors cultured in air–liquid interface (ALI) conditions in multi co-culture models and stimulated with UPM.

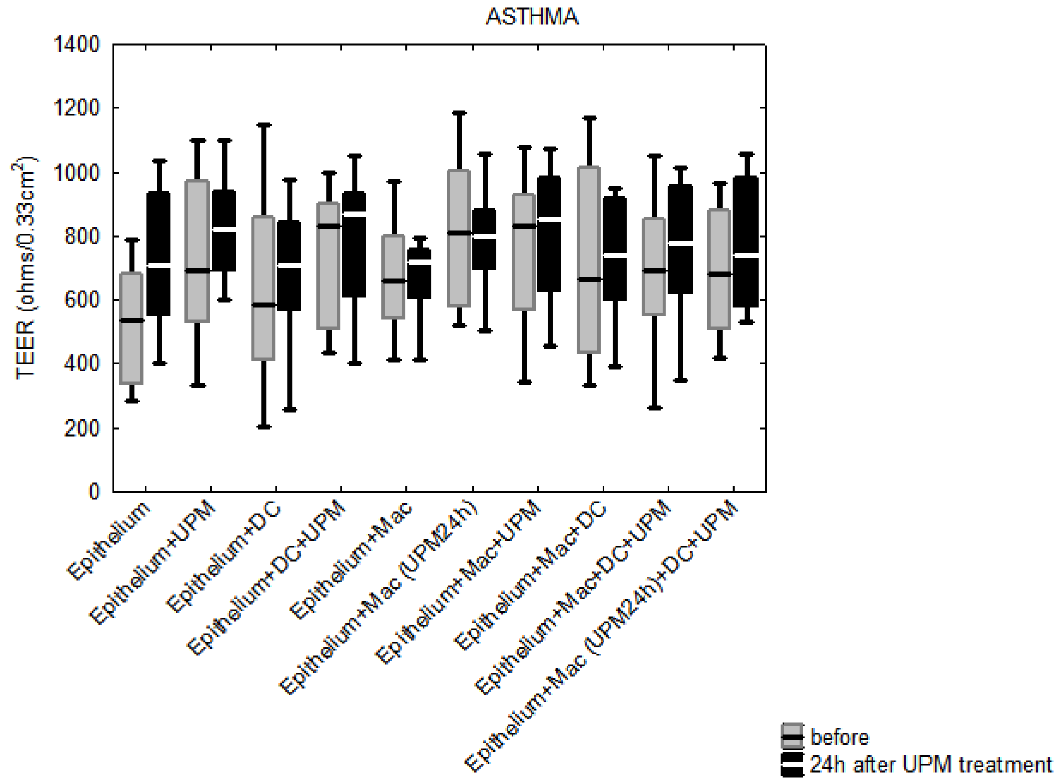

Figure S2. Transepithelial electrical resistance (TEER) after 24h of nasal epithelial cells from asthma patients cultured in air–liquid interface (ALI) conditions in multi co-culture models and stimulated with UPM.

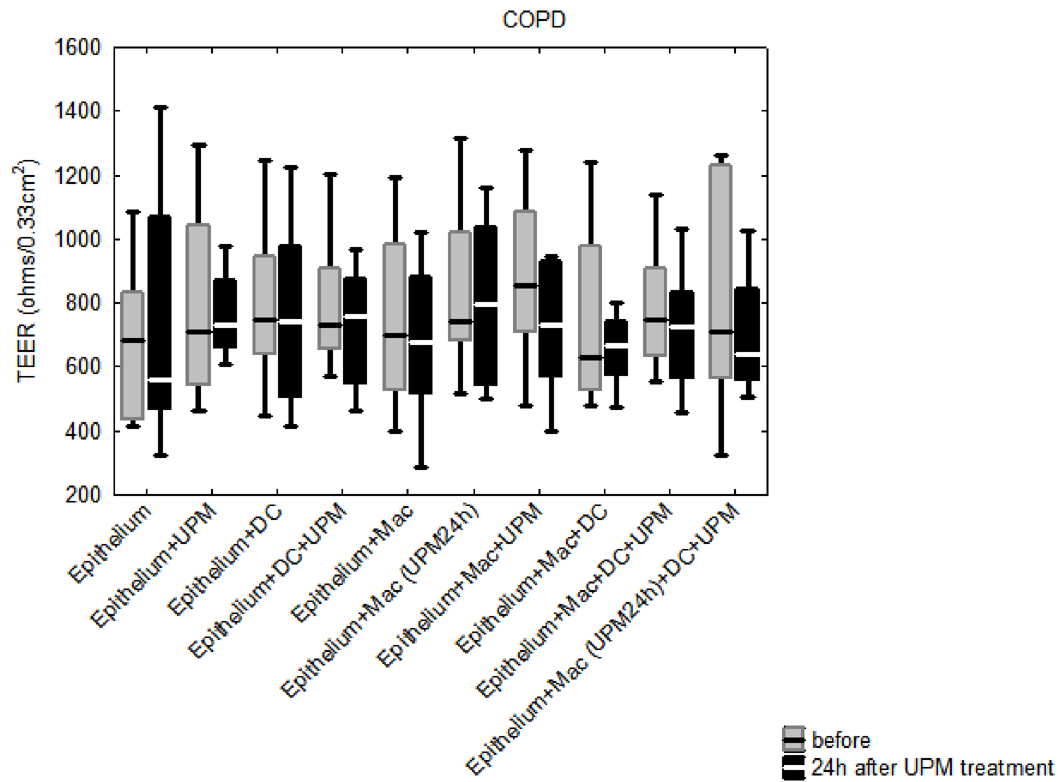

Figure S3. Transepithelial electrical resistance (TEER) after 24h of nasal epithelial cells from COPD patients cultured in air–liquid interface (ALI) conditions in multi co-culture models and stimulated with UPM.

III. RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases

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RNA-Seq Analysis of UPM-Exposed Epithelium Co-Cultivated with Macrophages and Dendritic Cells in Obstructive Lung Diseases

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Abstract: Background. Elevated concentrations of airborne pollutants are correlated with an enlarged rate of obstructive lung disease morbidity as well as acute disease exacerbations. This study aimed to analyze the epithelium mRNA profile in response to airborne particulate matter in the control, asthma, and COPD groups. Results. A triple co-culture of nasal epithelium, monocyte-derived macrophages, and monocyte-derived dendritic cells obtained from the controls, asthma, and COPD were exposed to urban particulate matter (UPM) for 24 h. RNA-Seq analysis found differences in seven (CYP1B1, CYP1B1-AS1, NCF1, ME1, LINC02029, BPIFA2, EEF1A2), five (CYP1B1, ARC, ENPEP, RASD1, CYP1B1-AS1), and six (CYP1B1, CYP1B1-AS1, IRF4, ATP1B2, TIPARP, CCL22) differentially expressed genes between UPM exposed and unexposed triple co-cultured epithelium in the control, asthma, and COPD groups, respectively. PCR analysis showed that mRNA expression of BPIFA2 and ENPEP was upregulated in both asthma and COPD, while the expression of CYP1B1-AS1 and TIPARP was increased in the epithelium from COPD patients only. Biological processes changed in UPM exposed triple co-cultured epithelium were associated with epidermis development and epidermal cell differentiation in asthma and with response to toxic substances in COPD. Conclusions. The biochemical processes associated with pathophysiology of asthma and COPD impairs the airway epithelial response to UPM.

Keywords: epithelium; urban particulate matter; UPM; asthma; COPD

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1. Introduction

Ambient air pollution is one of the key issues of public health. According to the WHO, one-in-nine deaths globally is caused by harmful air pollutants emitted from anthropogenic sources [1]. Airborne pollution is a mixture consisting of several gaseous components and a load of particulate matter (PM). The PM fractions are described according to an aerodynamic diameter (AD) of particles—AD $\leq$ 10 µm, AD $\leq$ 2.5 µm, and AD $\leq$ 0.1 µm, respectively, referred to as PM_{10}, $PM_{2.5}$, and UFP (ultrafine particles) [2]. Airborne pollution in highly populated areas is characterized by an increased content of fine particles originating from vehicle exhaust [3] and the coarse fraction emitted from household heating systems, particularly during the heating season [4]. Urban particulate matter (UPM) is a mixture of liquid and solid particles, which contains carcinogenic chemicals including inorganic ions, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) characteristic for local urban, industry, and household errands [5]. The physical and chemical diversity of the inhaled compounds included in airborne pollution as well as the complex interplay and multiple interactions occurring within the airway epithelium result in various harmful effects.

Characteristics of airway epithelium including tightly clustered cells, a diversity of cell types with a large number of ciliated and mucus secreting cells as well as the production of a surfactant film contribute to human body protection not only against air pollutants, but also against different respiratory pathogens: viruses and bacteria. The respiratory epithelium actively regulates the local inflammatory response through interactions with the macrophages and dendritic cells (DCs). In our previous study, we found that active interactions between the epithelial cells and DCs are important components for the proper response of airways for air pollution exposure [6]. The in vitro effect of ambient air pollutants has been a subject of intense investigation. The exposure to air pollution resulted in the decreased antimicrobial properties of the airway epithelium and impacted the innate immunity [7]. Other effects of ambient air pollution on the respiratory epithelium were found to be associated with its toxicity [8], oxidative stress [9], and inflammation [10].

Pathophysiology of asthma and chronic obstructive pulmonary disease (COPD) is associated with respiratory epithelium dysfunction and destruction. Ongoing inflammation and remodeling significantly affect the airway microenvironment in asthma and COPD [11]. The metabolic reprogramming as well as the structural changes associated with these diseases modulate the epithelial response to airborne pollutants. Due to the loss of proper epithelium function and barrier integrity disruption, asthma and COPD patients are more susceptible to hazardous outcomes of airborne particulate matter. Elevated levels of harmful substances in the air are correlated with the increased prevalence of asthma and COPD as well as acute disease exacerbations [12,13]. However, detailed knowledge of the impact of the pathophysiology of obstructive lung diseases on the epithelial response to UPM exposure remains elusive. Therefore, we undertook a study aimed to evaluate the airway epithelium mRNA profile in response to airborne particulate matter. An in vitro triple cell co-culture model based on cells derived from healthy subjects and obstructive lung disease patients was used in this study.

2. Results

2.1. RNA-Seq Data Analysis

The RNA-Seq data analysis showed 68 differentially expressed genes (DEGs) between the mono- and triple co-cultures in the control group, 1396 DEGs between the mono- and triple co-cultures in asthma, and only three DEGs between the mono- and triple co-cultures in the COPD group (according to adjusted p-value $p < 0.05$). UPM exposure resulted in seven (CYP1B1, CYP1B1-AS1, NCF1, ME1, LINC02029, BPIFA2, EEF1A2), five (CYP1B1, ARC, ENPEP, RASD1, CYP1B1-AS1), and six (CYP1B1, CYP1B1-AS1, IRF4, ATP1B2, TIPARP, CCL22) DEGs between the UPM exposed and unexposed triple co-cultures in the control, asthma, and COPD groups, respectively (according to adjusted p-value $p < 0.05$) (Figure 1A). Considering the triple co-cultures exposed to UPM, we observed 5256 DEGs between the asthma and control group, 2297 DEGs between the COPD and control group, and 7591 DEGs between the COPD and asthma group (Figure 1B).

Volcano plots were used to visualize the detailed analysis of DEGs between the epithelium from the triple co-cultures exposed to UPM from the COPD and asthma group as well as between those groups and the control group separately (Figure 2A–C).

2.2. GO and KEGG Pathway Enrichment Analysis

The GO annotation revealed significantly changed (up- and downregulated) genes associated with the carbohydrate biosynthetic process (GO:0016051) in a comparison between the UPM stimulated and unstimulated epithelium from a triple co-culture of the controls. Several terms were also strongly related to lipid biosynthesis and metabolism. The differentiating genes after UPM stimulation in the control epithelium from the triple co-cultures (Figure 3A) were associated with the sterol biosynthetic process (GO:0016126), cholesterol biosynthetic process (GO:0006695), steroid biosynthetic process (GO:0006694), and steroid metabolic process (GO:0008202).

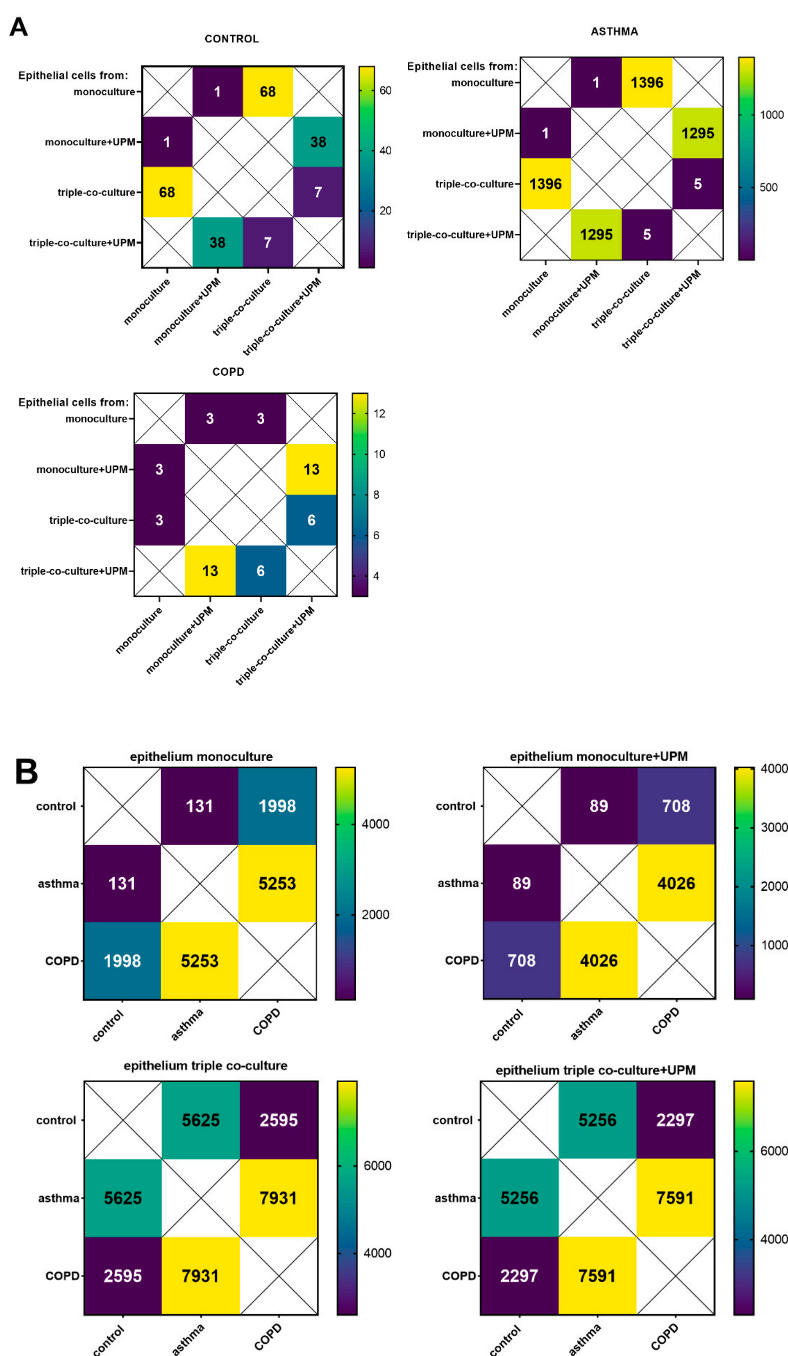

Figure 1. The heat map of the number of differentially expressed genes (DEGs) within the control, asthma, and COPD groups related to UPM exposure (**A**) and the DEGs between the control, asthma, and COPD groups in the same stimulation model (**B**). The values represent the number of DEGs (according to adjusted *p*-value, $p < 0.05$) in a comparison between the groups: epithelium from the monoculture, epithelium from the monoculture exposed to UPM for 24 h, epithelium from the triple co-culture, and epithelium from the triple co-culture exposed to UPM for 24 h, UPM—urban particulate matter.

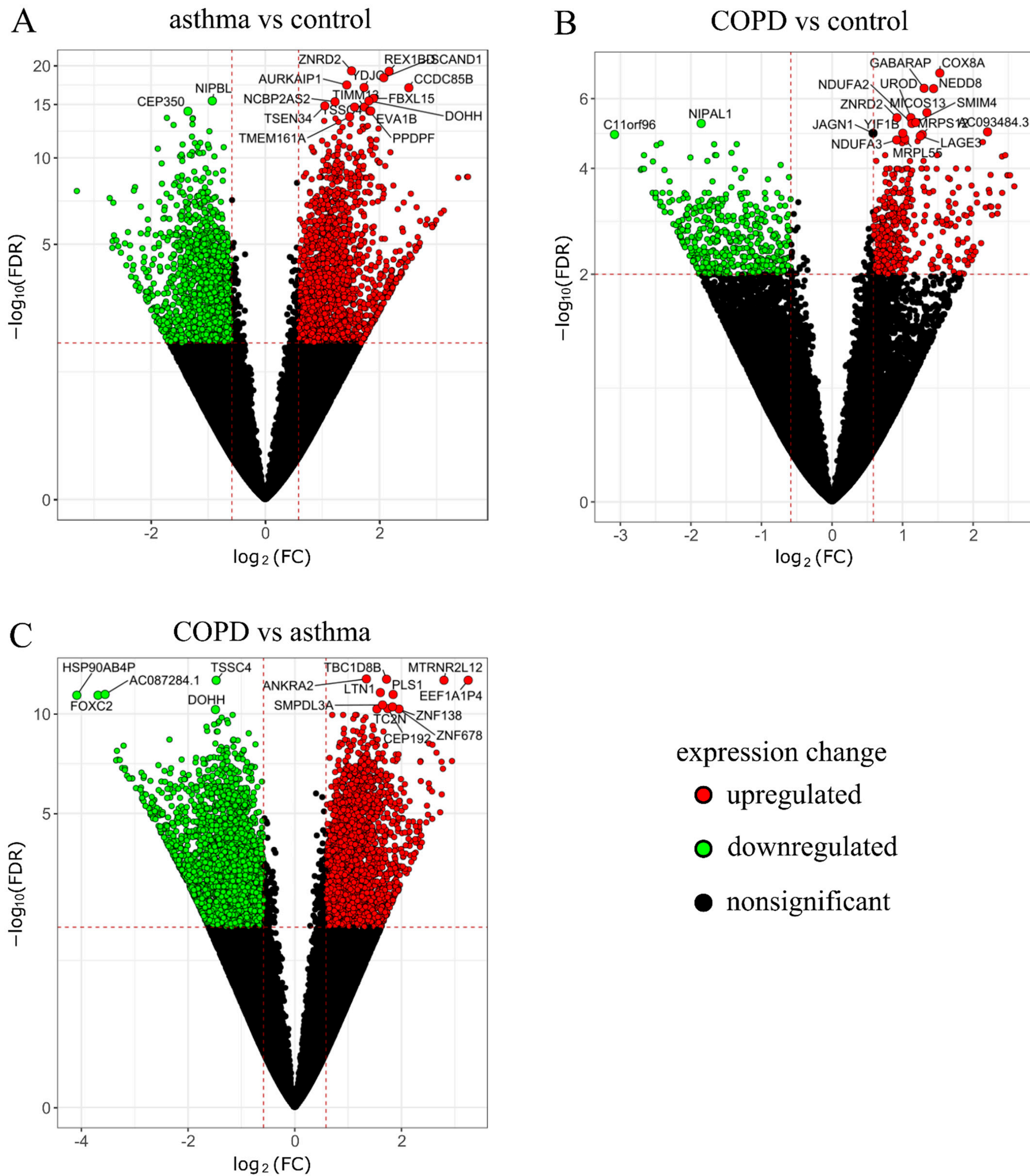

Figure 2. Volcano plots for the comparison between the UPM exposed epithelium (from triple co-cultures) in the asthma and control group (**A**), the COPD and control group (**B**), the COPD and asthma group (**C**). Genes are colored by fold change (FC). The *x*-axis illustrates the fold change (FC) (log-scaled) while the *y*-axis indicates the false discovery rate (FDR) adjusted *p*-values (log-scaled). Red points represent increased (upregulated) genes, green points stand for decreased (downregulated) genes, and black points show non-significantly deregulated genes.

Figure 3. Gene Ontology (GO) analysis of the most significantly up- and downregulated terms (categories among the top 5% of genes) according to the biological process in the epithelium (from triple co-culture) after UPM exposure (**A**) and in a comparison between the control, asthma, and COPD groups (**B**). Analysis according to the adjusted *p*-value.

The GO analysis of genes differentiating the UPM exposed epithelium from triple co-culture in the asthma group and corresponding unexposed epithelium co-culture revealed an association with epidermis development (GO:0008544) and epidermal cell differentiation (GO:0009913) with 35 and 26 DEGs, respectively. Moreover, this comparison also demonstrated a significant enrichment in genes associated with the humoral immune response (GO:0006959) and granulocyte chemotaxis (GO:0071621) (Figure 3A).

Genes differentiating the UPM-exposed COPD epithelium (triple co-cultured) from the corresponding unstimulated one were associated with response to toxic substance (GO: 0009636) with 40 DEGs. Furthermore, the GO analysis in this comparison identified up- and downregulated genes assigned to terms involved in the migration of leukocytes (GO:0050900), granulocytes (GO:0097530), neutrophils (GO:1990266), and the reactive oxygen species metabolic process (GO:0072593) (Figure 3A).

Comparison between the asthmatics and controls in terms of the GO analysis of the UPM exposed epithelium from triple co-cultures revealed up- and downregulated genes associated with ribonucleoprotein complex biogenesis (GO:0022613), ncRNA processing (GO:0034470), and mitochondrial translational termination (GO:0070126) (Figure 3B), while a comparison between the control and COPD (triple co-cultured epithelium) showed GO terms associated with mitochondrial translational elongation (GO:0070125) and termination (GO:0070126), oxidative phosphorylation (GO:0006119), mitochondrial gene expression (GO:0140053), and cellular protein complex disassembly (GO:0043624) (Figure 3B).

In addition, the KEGG pathway analysis revealed six significantly overrepresented pathways in the controls after UPM stimulation (Figure 4A). Significant associations with rheumatoid arthritis and viral protein interaction with cytokine and cytokine receptors among genes differentiating the UPM stimulated asthmatic epithelium compared to the unstimulated one were observed (Figure 4B). In the COPD group, UPM exposure resulted in the enrichment of pathways especially associated with steroid hormone biosynthesis, cytokine–cytokine receptor interaction, and viral protein interaction with cytokine and cytokine receptors compared to the unstimulated group (Figure 4C). Additionally, we observed 16 enriched pathways in the COPD group after UPM exposure compared to the control group (Figure 4D). No significant differences after UPM stimulation were established in the KEGG analysis between the epithelium from the asthma and control groups as well as between the COPD and asthma groups.

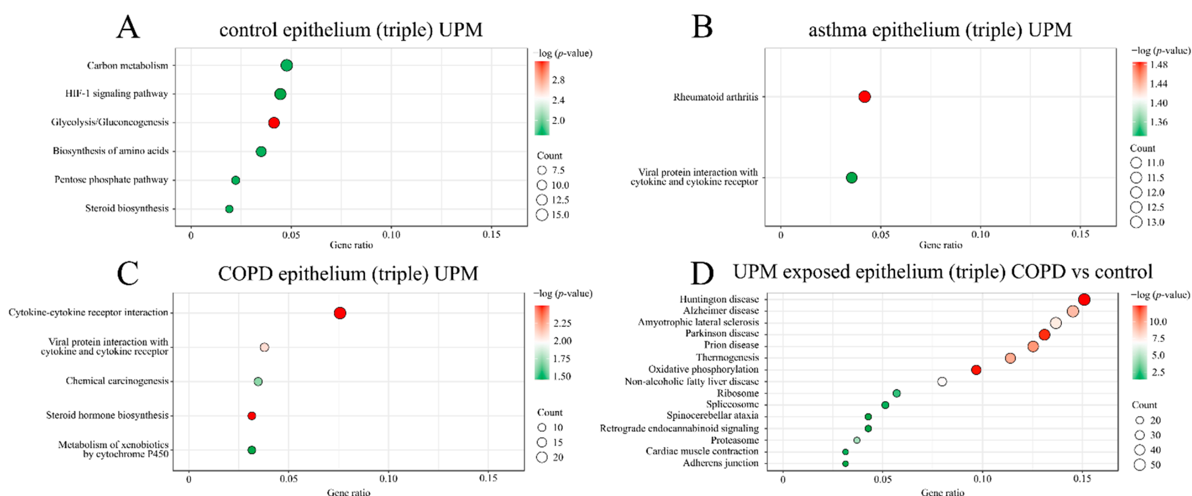

Figure 4. The Kyoto Encyclopedia of Genes and Genomes (KEGG) (categories among the top 5% of genes) significantly enriched pathways after UPM exposure in the control (**A**), asthma (**B**), and COPD (**C**) epithelium from the triple co-culture and in the UPM-exposed COPD epithelium compared to the control (**D**). Analysis according to the adjusted *p*-value.

2.3. RT-qPCR Analysis

The genes selected from RNA-Seq analysis with a significant and the highest fold change of mRNA expression were chosen for qRT-PCR verification (Figure 5A,B). Genes with *p*-value (corrected) lower than 0.1 in comparison between the UPM exposed and nonexposed epithelial cells from monoculture or triple co-culture in the control, asthma, and COPD groups were selected for verification. Differences in expression observed in RNA-Seq for the selected genes are illustrated in Figure 5 as follows:

(a) Fold change of mRNA expression between the UPM exposed and nonexposed epithelial cells from the triple co-culture (Figure 5A, *Y*-axis) was compared to the fold change of mRNA expression between the UPM and no UPM exposed epithelial cells from the monoculture (Figure 5A, *X*-axis). A separate plot (and gene selection) was prepared for each group (control/asthma/COPD).

(b) Fold change of mRNA expression between the UPM exposed and nonexposed epithelial cells from the triple co-culture in one of the clinical group was plotted against the same value in other clinical groups (three panels: asthma–control, COPD–control, COPD–asthma).

The detailed list of 17 tested candidate genes (AHRR, ARC, ATP1B2, BPIFA2, CCL22, CYP1B1, CYP1B1-AS1, EDC3, EEF1A2, ENPEP, LINC02029, IRF4, ME1, NCF1, RASD1, RMDN2-AS1, TIPARP) is described in Appendix A, Table A1. The results obtained in qRT-PCR were close to these obtained by the RNA-Seq measurements. In most cases, UPM exposure upregulated gene expression or remained unchanged except for CCL22 and RMDN2-AS1 for all groups and CYP1B1 and LINC02029 for the controls and asthma, which were suppressed in almost all tested combinations. qRT-PCR confirmed several significant changes in the mRNA expression in the epithelium after UPM exposure. The results of the PCR analysis showed that the expression of the evaluated mediators in the epithelial cells co-cultivated with moMφs and moDCs was much stronger than in the epithelial cells cultured alone. The overall *p*-values of the selected genes' mRNA expression comparisons between the control, asthma, and COPD groups in the UPM exposed epithelium from mono- and triple co-cultures are presented in Appendix A, Table A2. PCR analysis highlighted the differences in the epithelial response after UPM stimulation between the controls and patients with obstructive lung diseases. We found that the mRNA expression of BPIFA2 and ENPEP was upregulated after UPM treatment, most potently in triple co-cultures of the asthma and COPD patients only. Additionally, it seems that the epithelial cells from COPD patients highly activated CYP1B1-AS1, ME1, TIPARP mRNA expression after UPM stimulation in contrast to the control and asthma group, where these changes were not observed (Figure 6).

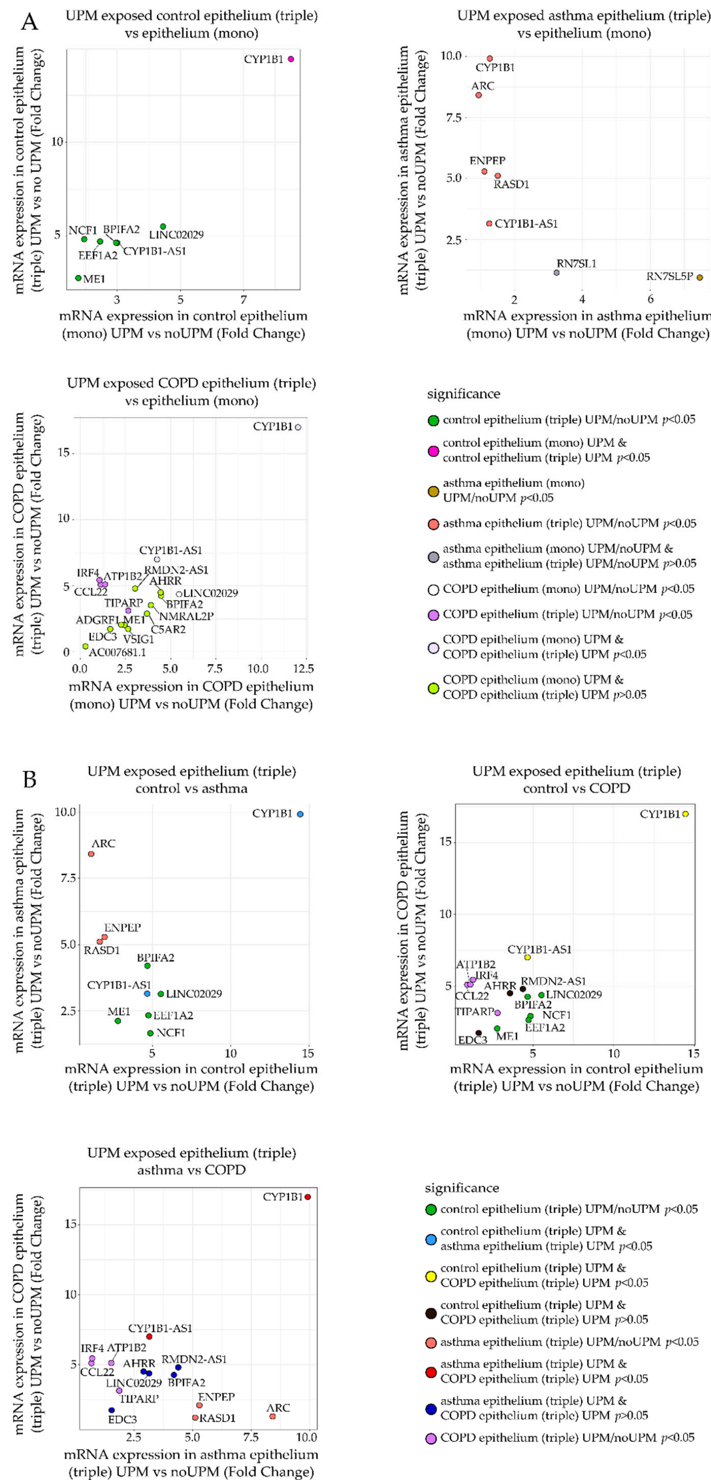

Figure 5. A comparison of the mRNA expression (fold change) (**A**) in the epithelium after UPM exposure from mono- and triple co-cultures and (**B**) between the UPM-exposed triple co-cultures in the control, asthma, and COPD groups.

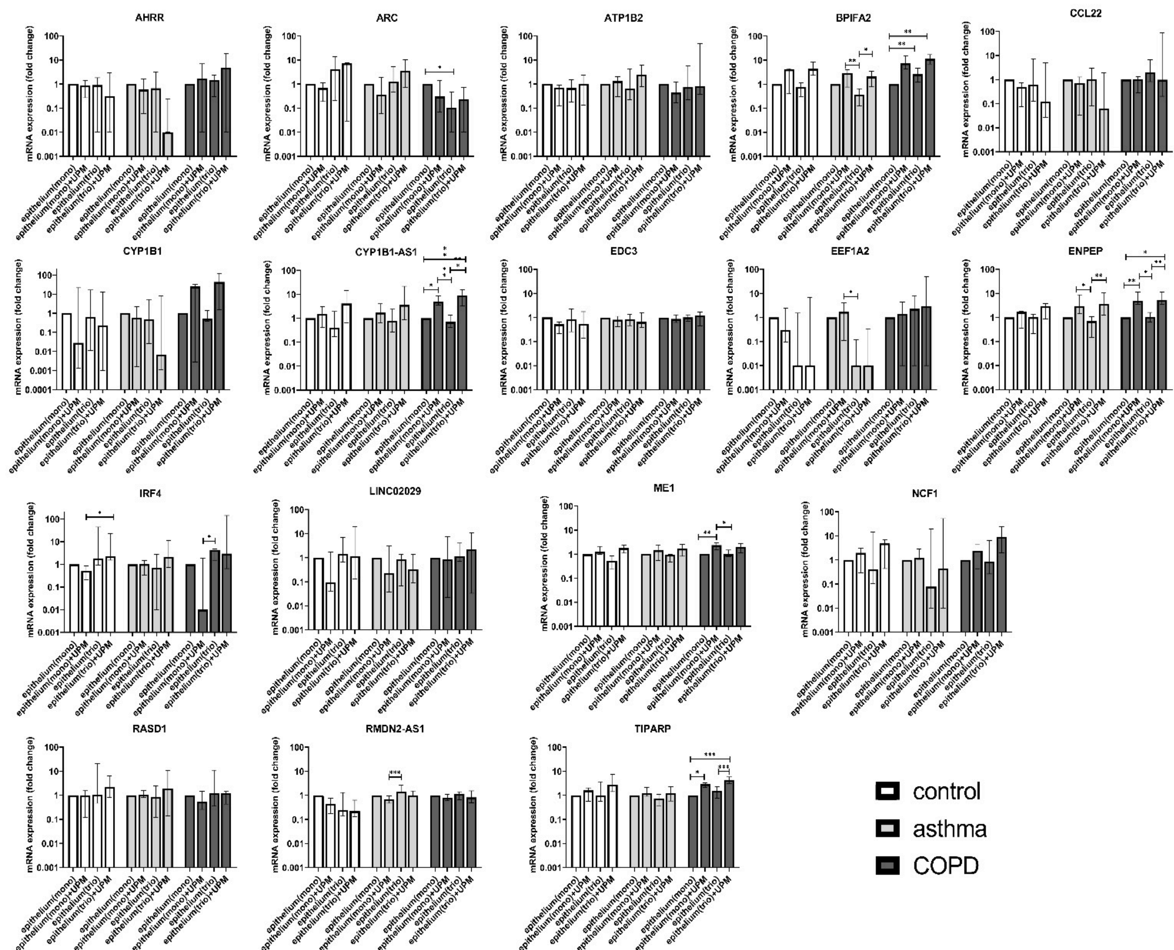

Figure 6. The mRNA expression of selected markers in the epithelium after 24 h of UPM exposure in the mono- and triple co-cultures in the control subjects, asthma, and COPD patients. The data are shown as interquartile range (whiskers), and median (column); *p*-value was calculated using the Kruskal–Wallis test followed by Dunn's post hoc test. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

3. Discussion

Obstructive lung diseases carry a particular risk of serious consequences of air pollution exposure. Our study, which used an advanced triple cell co-culture model, showed a distinct pattern of transcriptomic changes after UPM exposure in the epithelium from triple co-cultures in healthy controls, asthma, and COPD patients. Pre-existing obstructive lung diseases were associated with considerable changes in gene expression in the UPM-exposed epithelium, as we observed 5256 DEGs in a comparison between the asthma and control group, 2297 DEGs between the COPD and control group, and 7591 DEGs between the COPD and asthma group. Our results revealed the genes and biological processes apparently involved in response to UPM exposure. We showed that the most potently activated genes after air pollution exposure in the triple co-cultured epithelium of asthma and COPD patients were BPIFA2 and ENPEP, while CYP1B1-AS1 and TIPARP were upregulated in the COPD epithelium only. Here, for the first time, we present a comprehensive characterization of the molecular processes taking place in the respiratory epithelium after UPM stimulation in the in vitro model, which considers the impact of the interaction of the epithelial–macrophage–dendritic cells in the healthy controls, asthma, and COPD patients.

The effect of airborne PM on the monocultured airway epithelium has been well-characterized. The exposure of the ALI cultured epithelium resulted in toxic effects associated with oxidative stress, pro-inflammatory response as well as enhanced cytotoxicity [14]. Our study, which showed the upregulation of genes associated with response to toxic substances, is in line with those earlier observations. CYP1B1-AS1 is a member of the long-noncoding antisense RNAs located on chromosome 2 [15] and is considered to be a positive regulator (i.e., enhancer) of CYP1B1 transcription [16]. CYP1B1 belongs to the cytochrome family and is linked to xenobiotic metabolism. Our study demonstrated that the epithelium of COPD patients was more susceptible to UPM exposure than the asthmatic as well as control epithelium and showed the highest expression of CYP1B1-AS1 in both the mono- and triple co-cultures. Earlier studies showed the upregulation of CYP1B1-AS1 expression in oral masticatory mucosa from cigarette smokers compared to never smokers [17] and in A549 cells exposed to cigarette smoke extract [18]. The expression measured in our study was also significantly elevated in the UPM-exposed COPD epithelium cultures. As the COPD patients included in our study were active smokers, it can be supposed that epithelium pre-exposure to cigarette smoke manifests as increased vulnerability to UPM stimulation. Bioinformatic analysis of our RNA-Seq results revealed significant upregulation of epithelial CYP1B1 expression within all tested groups after 24 h of UPM exposure but PCR revealed an insignificant elevation of CYP1B1 expression in the COPD group only. CYP1B1 as well as CYP1A1 are aryl hydrocarbon receptor-dependent (AHR) and are considered as the markers of AHR activation [19]. AHR is involved in xenobiotic metabolism, especially aromatic hydrocarbons (PAHs), compounds embedded on airborne PM [20]. AHR can be repressed by the aryl hydrocarbon receptor repressor (AHRR). Previous study showed that wood smoke, urban fine particulate matter, and PAHs increased the AHRR expression in the airway epithelial cells [21]. Our data showed that AHRR expression tended to be decreased in asthma while it was increased in the COPD triple co-cultured epithelium after UPM exposure. Cell AHR signaling is mediated by TCDD-inducible poly-ADP-ribose polymerase (TIPARP) expression [22], or by TIPARP via ADP-ribosylation [23]. Increased expression of TIPARP was shown to decrease not only AHR activity but also AHR-associated genes, suggesting its role in the negative regulation of the AHR-pathway [24]. Our results showed that upregulation of TIPRAP mRNA expression after UPM stimulation in the epithelium from COPD patients. As in previous murine studies, the downregulation of TIPARP expression increased the sensitivity to TCDD-dependent toxicity [25], and we suggest that increased TIPARP expression in the UPM-exposed COPD epithelium is associated with enlarged UPM toxicity within this group. Our study may imply that air pollution highly elevates oxidative stress in the airway epithelium, especially for COPD patients, which can cause disease exacerbation or other pathological processes such as carcinogenesis through the downregulation of protective defense biochemical mechanisms of the airway epithelium. Thus, combining the results of the current study and a common knowledge of the impairment of antioxidant defense in COPD, a preventive antioxidant treatment for COPD patients susceptible to air pollution seems to be a reasonable approach.

Our study revealed an increased expression of ENPEP in the asthma and COPD epithelium triple co-cultures exposed to UPM. Glutamyl aminopeptidase (ENPEP) is a mammalian type II integral membrane zinc-containing endopeptidase belonging to the aminopeptidase family. ENPEP is a crucial regulatory factor of blood pressure, taking part in blood vessel remodeling [26]. The exact role of ENPEP in respiratory physiology is not explained yet, but recent studies suggest a strong correlation between ENPEP and angiotensin converting enzyme 2 (ACE2) expression [27]. The possible mediatory role of ENPEP in UPM-induced epithelial changes in patients with obstructive respiratory diseases is still a novel aspect to elucidate. We can only speculate that due to its aminopeptidase activity that this marker might be associated with cell activation, signal transduction, and cell-matrix adhesion in the asthmatic and COPD airways after air pollution exposure.

Another important mediator of UPM induced epithelial response revealed by our study is BPIFA2, a member of the palate, lung, and nasal epithelium clone (PLUNC) protein family, encoded by the gene cluster located on chromosome 20 [28]. Proteins included in this family are associated with local antibacterial responses in the nose, mouth, and upper respiratory tract [29]. Kang et al. found an upregulation of BPIFA2 after PM_{10} exposure in several cell lines such as normal lung epithelial cells (BEAS-2B), human lung carcinoma (A549), and human bronchiolar carcinoma (NCI-H358) [30]. The activation of BPIFA2 after UPM exposure in asthma and COPD epithelium from the triple co-cultures in our study suggest the upregulation of innate immune response in the respiratory tract of patients with obstructive lung diseases after air pollution exposure.

The GO terms and KEGG pathways analysis in our study demonstrated that biological processes in epithelial cells from patients with obstructive lung diseases exposed to UPM differed considerably compared to the healthy individuals. In healthy subjects, UPM treatment altered pathways associated with lipid metabolism and glucose catabolism. Similar effects have already been reported in several previous studies [31,32]. Our results showed upregulated NADH regeneration and glucose catabolic processes, which reflects a preserved regenerative capacity of healthy epithelium. In contrast, the exposure to hazardous airborne material such as cigarette smoke was shown to downregulate glucose metabolism and increase fatty acid oxidation (FAO) with simultaneous enzymatic pathway alterations in the lung alveolar cells [33]. These processes caused cellular damage and surfactant deficiency, leading to impaired lung function in the smokers and COPD patients [34]. Likewise, in our study, the UPM-exposed COPD epithelium from triple co-cultures showed the upregulation of pathways associated with response to toxic substances, mitochondrial associated pathways, oxidative stress as well as ROS metabolism. Interestingly, Leclercq et al. found that despite an enhanced expression of genes involved in the metabolism of harmful substances, the COPD epithelium showed a decreased response capacity to air-pollution-derived hazardous compounds [35]. Other authors have reported that free radicals formed in cells resulted in expanded oxidative stress, causing the increased transcription of pro-inflammatory genes via the NF-kB pathway such as IL-8 as well as epigenetic changes such as histone acetylation, further increasing DNA unfolding and the transcription of pro-inflammatory genes [36]. Furthermore, COPD patients revealed increased PM_{10}-induced genotoxicity compared to the healthy subjects [37]. Importantly, our results found that UPM exposure of the COPD epithelium also resulted in the increased expression of genes associated with the migration of granulocytes, especially neutrophils. It is known that the long-lasting presence and activity of neutrophils in the airways leads to the release of cytotoxic and profibrotic agents, resulting in local tissue remodeling by injury and fibrosis. We also observed the deregulation of genes associated with cellular protein complex disassembly in the UPM-exposed COPD epithelium compared with the healthy one. It has been reported that the exposure to airborne pollutants induce methionine oxidation, resulting in protein-misfolding and endoplasmic reticulum stress in chronic lung diseases [38,39]. We suggest that UPM-induced protein reorganization is associated with highly activated oxidative stress, and disturbed COPD epithelium layer structure and function, which may accelerate the COPD exacerbations induced by air pollution.

In contrast to COPD, the UPM-exposed asthma epithelium was characterized by a deregulation of distinct pathways, especially biological processes associated with humoral immune response and granulocyte chemotaxis. Our results are consistent with other studies. Using a murine asthma model, Huang et al. showed that the exposure to airborne PM supported the intensified neutrophil recruitment and induction of Th1-related cytokine synthesis (TNF-α and IFN-γ) and resulted in allergic-like immune responses including increased eosinophil influx and upregulated Th2-cell mediated cytokine production (IL-5 and IL-13) [40]. Eosinophils are important sources of transforming growth factor β (TGF-β), which mediates the induction of structural changes in asthmatic airways associated with subepithelial fibrosis, myocyte hyperplasia, and hypertrophy, disruption of epithelial integrity, goblet cell metaplasia, and vascular permeability [41]. Our results also showed a

deregulated pathway associated with the retinoid metabolic process in the UPM-exposed epithelium. It has been demonstrated that long-lasting interactions of retinoids with their overexpressed receptors on asthmatic bronchial epithelium enable an aberrant tissue repair and rebuilding via TGF-β1 synthesis [42]. Moreover, results of our study showed UPM dependent regulation of epidermis development and epidermal cell differentiation pathways. These biological processes contribute to the deregulation of epithelial tight junction integrity, wound healing, tissue repair, and by stimulating cell proliferation [28]. Our study revealed that the main biological processes activated by air pollution exposure in asthmatic airways are associated with inflammation, especially granulocyte chemotaxis, humoral immune response, and the disruption of epithelial integrity, which as a consequence result in the loss of cellular defense mechanisms.

Our study had some limitations. First, a small but statistically optimized group of healthy subjects, asthmatics, and COPD patients was included in the study. Verification of RNA-Seq results by the qRT-PCR measurements was performed in extended groups. Second, our model contained nasal epithelium, a non-invasively obtained functional substitute of bronchial epithelial cells. Our unique cell co-culture model certainly did not mimic all of the pathophysiological processes involved in obstructive lung diseases, but it seems useful to determine the impact of cell–cell interactions on the epithelium transcriptome after exposure to UPM between healthy subjects and patients with asthma and COPD. Third, our results were obtained by a full transcriptome analysis using RNA-Seq. Although this method did not allow us to provide detailed information concerning the UPM impact on specific epithelium cell types constituting the epithelium layer, it let us recognize the wide picture of transcriptome differences caused by UPM exposure among all of the tested groups. As the epithelium layer consists of several cell types, further investigation in this field of study using single-cell RNA (sc-RNA) sequencing might be a promising approach to reduce the information noise associated with epithelial cell heterogeneity.

4. Materials and Methods

4.1. Patient Characteristics

The study involved 10 asthma patients, eight patients with COPD, and eight healthy subjects. The diagnosis of asthma and COPD was established according to the current GINA and GOLD reports, respectively [43,44]. The control group consisted of healthy individuals with no airway obstruction confirmed by normal spirometry results. The exclusion criteria were as follows: treatment with systemic or nasal steroids, asthma, or COPD exacerbation within 3 months from sampling, and symptoms of respiratory tract infection in the preceding 3 months. Peripheral blood samples and nasal brushing were collected from each participant. RNA-Seq analysis was performed in a group of 12 subjects (four controls, four asthma, four COPD) (Table 1). The COPD patients were heavy smokers, in contrast to the asthma patients and healthy controls. The clinical characteristics of all patients and controls recruited to the study are summarized in Appendix A, Table A3.

Table 1. The patient characteristics.

	Control n **= 4**	**Asthma** n **= 4**	**COPD** n **= 4**	**Overall** p**-Value**
Age (years)	36 (27–44.5)	61 (38–71)	67 (62–72.5)	0.06
Gender (F/M)	4/0	1/3	2/2	0.09
BMI (kg/m^2)	22.4 (20.3–23.1)	27.2 (26–30.1)	28 (25.8–30.9)	0.025 *
Atopy (n)	2	3	0	0.03
Smoking exposure (pack-years)	0 (0–3.5)	0 (0–0.75)	25 (20–52)	0.015 *
FEV_1 (% predicted)	105.5 (101–109.5)	84 (81–100)	53 (47–61)	0.018 *
FEV_1/VC (%)	100.5 (98.5–106.5)	76.3 (70–80.8)	53 (47–61)	0.013 *

Table 1. *Cont.*

	Control n **= 4**	**Asthma** n **= 4**	**COPD** n **= 4**	**Overall** p**-Value**
FeNO (ppb)	9.3 (9.3–9.3)	47.5 (29.6–67.7)	22.4 (13.9–37.3)	0.124
ACT (points)	N.A.	19 (10–22)	N.A.	N.A.
ICS treatment (n)	N.A.	2	0	N.A.
CAT (points)	N.A.	N.A.	11 (8–17)	N.A.
mMRC (points)	N.A.	N.A.	3 (1–3)	N.A.

Data are presented as median (IQR) or n. BMI—body mass index, FEV_1—forced expiratory volume at first second, VC—vital capacity, FeNO—fractional exhaled nitric oxide, ACT—asthma control test, ICS- inhaled corticosteroids, CAT—COPD assessment test, mMRC—modified Medical Research Council, N.A.—not applicable. * control vs. COPD.

The study protocol was approved by the Ethics Committee of the Medical University of Warsaw (KB/37/2020) and written informed consent was obtained from all participants.

4.2. Flow Cytometry Analysis

Cells were stained with antibodies against the surface binding molecules CD45 (APC-H7), CD326 (PerCP-Cy5-5), MUC1 (BV605) (BD Biosciences, San Jose, CA, USA), and incubated for 20 min in the dark at RT. After washing away the reagents, the cells were fixed and permeabilizated using lysis buffer and permeabilization solution 2 (BD Biosciences, San Jose, CA, USA), then stained with intracellular markers β-tubulin (Alexa fluor 488) and cytokeratin (BV510) (BD Biosciences, San Jose, CA, USA) in BD Horizon Brilliant Stain Buffer (BD Biosciences, San Jose, CA, USA) for 20 min in the dark. Cells were analyzed by flow cytometry using the FACS Celesta instrument (BD Biosciences, San Jose, CA, USA) equipped with blue (488-nm), violet (405-nm), and red (640-nm) lasers. Unstained cells and compensation beads (BD Biosciences, San Jose, CA, USA) were used to set voltages and create single stain negative and positive controls. Compensation was set to account for the spectral overlap between the seven fluorescent channels used in the study. Cells with a basal phenotype were identified as CD45-CD326 + cytokeratin+, with the secretory phenotype as CD45-CD326 + MUC1+, with the ciliated phenotype as CD45-CD326 + β-tubulin+.

4.3. Hematoxylin and Eosin (H&E) Staining

Cultures were fixed with 10% neutral buffered formalin, pre-embedded in the desired orientation in premelted 1% agarose (Sigma Aldrich, St. Louis, MO, USA), processed on paraffin blocks with the standard protocol, cut into 5 µm sections, and mounted on positively charged glass slides (Leica, Wetzlar, Germany). Slides were dried in 60 °C for 30 min for paraffin melting and further dewaxed and hydrated with xylene and decreasing concentrations of alcohols. Sections were then stained with hematoxylin and eosin (H&E) and analyzed under light microscope equipped with a digital camera PrimoStar with AxioCam ERc5s (Zeiss, Oberkochen, Germany).

4.4. Cell Culture and Scheme of the Study

The nasal epithelial cells were isolated, cultivated, and specialized as previously described [45]. In brief, nasal epithelial cells were isolated from brush swabs (Cytobrush Plus GT, CooperSurgical, San Ramon, CA, USA) sampled at the interior surface of both nostrils. Then, the cells were cultured in the air–liquid interface (ALI) for 21 days in PneumaCult-ALI medium (StemCell, Vancouver, BC, Canada). Macrophages and DCs were isolated from a peripheral blood sample by Lymphoprep (StemCell, Vancouver, BC, Canada) centrifugation. The PBMC were frozen and thawed for specialization. Monocyte derived macrophages (moMφs) were specialized by 20 ng/mL M-CSF (StemCell, Vancouver, BC, Canada) stimulation for 10 days and monocyte derived DCs (moDCs) by cultivation in combination with 40 ng/mL GM-CSF (StemCell, Vancouver, BC, Canada), 20 ng/mL IL-4

(StemCell, Vancouver, BC, Canada) for 8 days, 50 ng/mL TNF-α (Bio-Techne, Minneapolis, MN, USA), and 50 ng/mL IL-1β (StemCell, Vancouver, BC, Canada) on the sixth day of specialization.

Fully differentiated ALI cultures of epithelial cells were cultured in a two-chamber system (Greiner Bio-One, Kremsmünster, Austria) (Figures 7 and 8). The triple co-cultures were prepared as previously described [46]. Briefly, the triple-co-cultures contained nasal epithelial cells cultured in ALI conditions for 21 days, fully differentiated moMφs (cultured for 10 days before the experiment) located on the top of the epithelium, and subepithelial moDCs (in eighth day of their specialization). moMφs and moDCs in triple co-cultures were suspended in media without supplements. The co-cultures were autologous (i.e., for each co-culture, the epithelial cells, macrophages and moDC were obtained from the same individual). UPM was added to the epithelial cells after the cells were combined in a triple-co-culture. Epithelial cells were cultured with or without stimulation with 100 μg/mL UPM (10 μL of UPM stock solution added on the top of the epithelial cells) for 24 h in a scheme as follows:

(1) Epithelial cells (monoculture);
(2) Epithelial cells + moMφs + moDCs (triple co-culture).

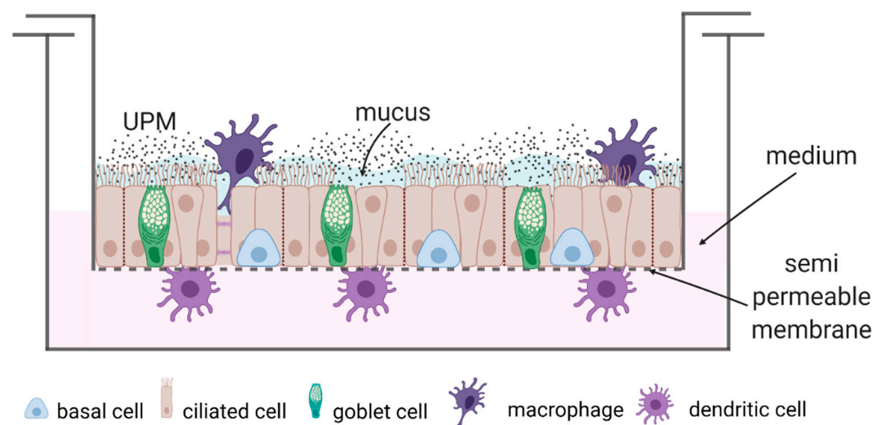

Figure 7. A schematic drawing of the triple co-culture stimulated with UPM.

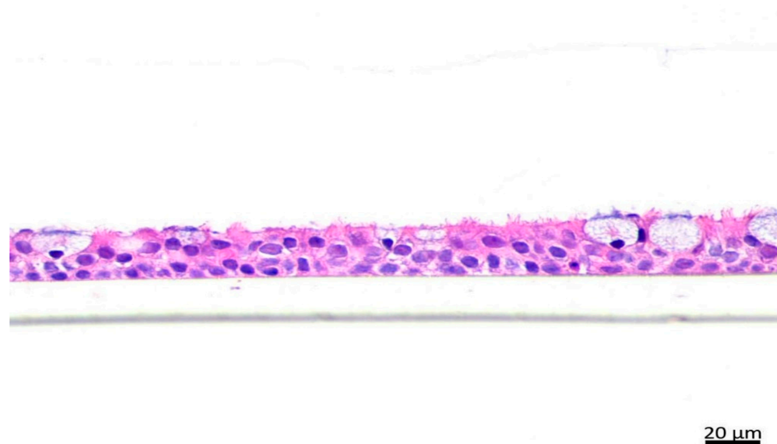

Figure 8. The hematoxylin and eosin (H&E) staining of the differentiated nasal epithelial cells cultured in ALI conditions for 21 days (original magnification ×40).

The epithelial cells cultured in ALI conditions contained a domination of cells with the ciliated and secretory phenotype, and less basal epithelial cells (Appendix A, Figure A1). The co-cultivation of epithelial cells with moMφs and moDCs did not change the proportion of epithelial subpopulations Appendix A Figure A2).

After 24 h, the cells were harvested, moMφs and moDCs were rinsed off, and the epithelium was used in the RNA-Seq analysis.

4.5. Particle Preparation

The filters with urban particulate matter were provided by the Silesian University of Technology. The samples were collected with a low-volume PM sampler type PNS-15 (Atmoservice, Poland) 1.5 m above the ground level, at a flow rate of 2.3 m^3/h, according to the PN-EN 12341:2006 standard [47] in Zabrze, Gliwice, and Żory during the heating season as published previously [6]. These cities are located in the Upper Silesia Region, which, compared with other EU countries as well as other Polish regions, is characterized by relatively high levels of PM. Airborne particulate matter was collected on high-purity quartz (SiO_2) microfiber filters (QM-A, Whatman, Little Chalfont, Buckinghamshire, UK). The heavy metal content in UPM was previously described [6]. The particles were detached from the filters by sonication and filtrated through strainers with 70 μm pores (Corning, Corning, NY, USA). The sediment of the particles was dried at 96 °C to dry mass, weighted, resuspended in PBS into stock solution containing 10 μg/μL UPM, and autoclaved.

4.6. RNA Isolation

After 24 h of incubation, the UPM DCs and macrophages were washed out. The attached cells were collected, the total RNA was isolated by the TRI reagent (Sigma-Aldrich, St. Louis, MA, USA) method, and further purified with NucleoSpin RNA (Machery&Nagel, Düren, Germany) using the protocol including DNA digestion. The concentration and quality of the isolated RNA were determined using Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and validated by Agilent Bioanalyzer 2100 with an RNA 6000 Pico Kit (Agilent, Santa Clara, CA, USA). The control of possible mycoplasma contamination was analyzed using the MycoSPY Kit (Biontex, Mainz, Germany).

4.7. RNA-Seq Analysis

The mRNA sequencing was performed in four asthmatics, four COPD patients, and four control samples. Libraries for the RNA-Seq measurements were prepared according to the manufacturer's protocol for the KAPA mRNA HyperPrep Kit (Roche, Basel, Switzerland). A total of 250 ng of intact total RNA was subjected to heat fragmentation (94 °C, 5 min or 85 °C, 6 min for partially degraded samples) and TruSeq Unique dual index adapters (Illumina, San Diego, CA, USA) were used. Twelve cycles of library amplification were applied. The size distribution of the final libraries were validated using Agilent Bioanalyzer 2100 and a High Sensitivity DNA Kit (Agilent, Santa Clara, CA, USA). The final library concentration was determined by qPCR using a Kapa Library Quantification Kit (Roche, Basel, Switzerland). Sequencing was performed using Illumina NovaSeq 6000 with the NovaSeq 6000 S1 Reagent Kit (Illumina, San Diego, CA, USA), generating 2×100 pair-end reads using the manufacturer's standard protocols. High quality output data were obtained (more than 98% of data with quality exceeding Phred Score Q37) in an amount of 36–55 MR/sample.

4.8. Bioinformatic Analysis

Raw sequences were trimmed according to quality using Trimmomatic [48] (version 0.39) with default parameters, except MINLEN, which was set to 50. Trimmed sequences were mapped to the human reference genome provided by ENSEMBL, (version grch38_snp_tran) using Hisat2 [49] with default parameters. Optical duplicates were removed using the Mark Duplicates tool from the GATK [50] package (version 4.1.2.0) with default parameters, except with OPTICAL_DUPLICATE_PIXEL_DISTANCE set to 12,000.

Reads that failed to map to the reference were extracted using Samtools [51] and mapped to the Silva meta-database of rRNA sequences [52] (version 119) with Sortmerna [53] (version 2.1b) using the "–best 1" option. Mapped reads were associated with transcripts from the GRCh38 database [54] (Ensembl, version 77) using HTSeq-count [55] (version 0.9.1) with the default parameters except with the stranded set to "reverse". Differentially expressed genes were selected using the DESeq2 package [56] (version 1.16.1). Fold change was corrected using apeglm [57]. *p*-values were corrected for the multiple hypothesis test with the Benjamini–Hochberg algorithm. To provide equal power during testing overrepresentation of the Gene Ontology (GO) [58] terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) [59] categories, the same proportion of genes was selected for each comparison. The top 5% of genes (according to *p*-value) were tested for overrepresentation vs. the whole set of genes with detectable expression. The assessment was carried out with cluster profiler package [60]. The RNA-Seq data were uploaded to GEO Omnibus (reference no. GSE175541). https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE175541, accessed on 6 August 2022.

4.9. qRT-PCR Measurements

cDNA synthesis was conducted using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher Scientific, USA). Quantitative real-time PCR was performed to assess the mRNA expression of AHRR, ARC, ATP1B2, BPIFA2, CCL22, CYP1B1, CYP1B1-AS1, EDC3, EEF1A2, ENPEP, IRF4, LINC02029, ME1, NCF1, RASD1, RMDN2-AS1, TIPARP, and 18s rRNA in the epithelial cells. The quantitative real-time PCR analysis was performed on an ABI-Prism 7500 Sequence Detector System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The primer specification is shown in Appendix A, Table A4. Relative quantification values were calculated by the 2-ΔΔCT method and 18s rRNA was applied for each sample as an internal control in order to normalize the gene expression levels. The unstimulated epithelial cells from monocultures of each individual were used as a calibrator.

4.10. Statistical Analysis

Statistical analysis was performed with the use of Statistica 13.3 software package (StatSoft Inc., Tulsa, Ok, USA), GraphPad (version 9.3.1 GraphPad Software, Inc., San Diego, CA, USA. https://www.graphpad.com/, accessed on 27 October 2021), or the R environment (version 4.1.0, https://cran.r-project.org/, accessed on 18 May 2021). The Kruskal–Wallis test, followed by the Dunn's post hoc test, was used to assess the differences between the continuous variables in the three study groups. The Mann–Whitney U test was applied for pairwise comparisons. The Pearson Chi-square test was used to compare the inter-group differences between the categorical variables. The results are given as the median and interquartile range (IQR). Differences were considered statistically significant at $p < 0.05$.

5. Conclusions

The response of the asthma and COPD epithelium to UPM stimulation was distinct from the healthy subjects. Our study strongly suggests a cellular genetic reprogramming after UPM exposure in the asthma and COPD patients compared to the healthy subjects, which was associated with ongoing pathophysiological processes in obstructive lung diseases. Based on the presented results, we propose selected genes as potential markers of progressive epithelial cell activity and possible cell damage associated with UPM exposure in patients with asthma and COPD. Further research focused on the suggested target genes and affected biochemical processes might contribute to the development of novel therapeutic approaches for the effective treatment of UPM-associated exacerbations of obstructive lung diseases.

Author Contributions: Funding acquisition: M.P.-G.; Concept and design: M.P.-G. and P.M.-S.; Experiments, measurements, and data acquisition: P.M.-S., M.P.-G., E.Z.-Z., M.M.; RNA-Seq analysis: D.A. and M.S.; Data analysis: K.G. and M.P.-G.; Writing original draft: P.M.-S. and M.P.-G.; Writing-reviewing and editing: M.P.-G. and R.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This work received approval for research ethics from the Medical University of Warsaw Review Board (KB/37/2020) and proof/certificate of approval is available upon request.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available in the Gene Expression Omnibus repository, accession number GSE175541. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE175541, accessed on 6 August 2022.

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Conflicts of Interest: RK reports personal fees and other from Boehringer Ingelheim, personal fees and other from Chiesi, personal fees and other from AstraZeneca, and personal fees from Polpharma, outside of the submitted work. The other authors declare no conflict of interest.

Appendix A

Table A1. The list of genes chosen for qPCR validation.

Acronym	Full Gene Name
18s rRNA	18s ribosomal RNA
AHRR	aryl-hydrocarbon receptor repressor
ARC	activity regulated cytoskeleton associated protein
ATP1B2	ATPase Na+/K+ transporting subunit beta 2
BPIFA2	BPI fold containing family A member 2
CCL22	C-C motif chemokine ligand 22
CYP1B1	cytochrome P450 family 1 subfamily B member 1
CYP1B1-AS1	CYP1B1 antisense RNA 1
EDC3	enhancer of mRNA decapping 3
EEF1A2	eukaryotic translation elongation factor 1 alpha 2
ENPEP	glutamyl aminopeptidase
IRF4	interferon regulatory factor 4
LINC02029	Long non-coding RNA
ME1	malic enzyme 1
NCF1	neutrophil cytosolic factor 1
RASD1	ras related dexamethasone induced 1
RMDN2-AS1	RMDN2 antisense RNA 1
TIPARP	TCDD inducible poly(ADP-ribose) polymerase

Table A2. The overall *p*-value of the comparisons between the control, asthma, and COPD groups of mRNA expression of the selected genes in the UPM-exposed epithelium from the mono- and triple co-cultures.

	Epithelium (mono) + UPM	Epithelium (trio) + UPM
AHRR	0.640	0.144
ARC	0.957	0.047 #
ATP1B2	0.154	0.597
BPIFA2	0.025 #	0.006 #
CCL22	0.214	0.109
CYP1B1	0.224	0.167
CYP1B1-AS1	0.023 *	0.435
EDC3	0.065	0.655
EEF1A2	0.745	0.318
ENPEP	0.028 *	0.150
IRF4	0.407	0.913
LINC02029	0.777	0.530
ME1	0.093	0.868
NCF1	0.806	0.353
RASD1	0.394	0.663
RMDN2-AS1	0.091	0.040
TIPARP	0.049	0.029 #

Differences between groups were tested by the Kruskal–Wallis test followed by Dunn's post hoc test. * control vs. COPD, # asthma vs. COPD.

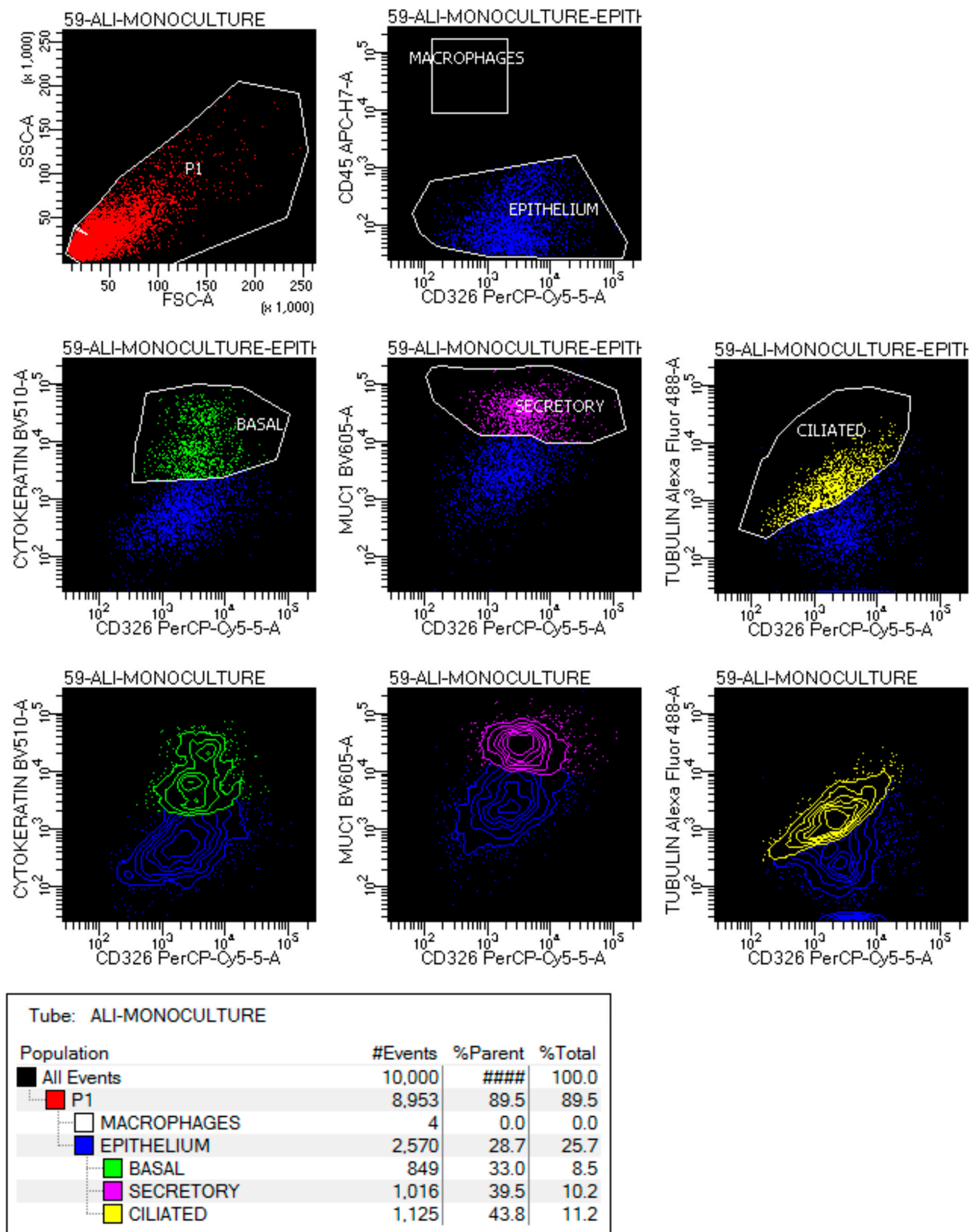
Table A3. The characteristics of the subjects (n = 26) used in the PCR analysis.

	Control n = 8	Asthma n = 10	COPD n = 8	Overall p-Value &	Pairwise p-Value *		
					Asthma vs. Control	COPD vs. Control	Asthma vs. COPD
Age (years)	38.5 (32.5–48)	55 (38–62)	62 (59.5–72.5)	0.005	0.138	0.0002	0.138
Gender (F/M)	6/2	3/10	5/3		0.046		
BMI (kg/m^2)	22.1 (20.7–24.1)	26.9 (26–27.7)	28 (25.4–30.3)	0.002	0.002	0.0003	0.696
Atopy (n)	3	8	2	0.03			
Smoking exposure (pack-years)	0 (0–0)	0 (0–4)	32.5 (22.5–50)	0.0002	0.277	0.0002	0.0003
FEV_1 (% predicted)	103 (81–111)	81 (75–94)	61.5 (51.5–76.5)	0.006	0.043	0.004	0.034
FEV_1/VC (%)	106 (81.8–112)	82 (75–86)	54 (50–68)	0.0003	0.02	0.0006	0.0004
FeNO (ppb)	11.0 (9.3–12.6)	52.3 (31.3–77.6)	17.4 (12.6–26.1)	0.0047	0.03	0.333	0.003
ACT (points)	N.A.	20.5 (17–25)	N.A.	N.A	N.A.	N.A.	N.A.
ICS treatment (n)	N.A.	6	1	N.A	N.A.	N.A.	N.A.
CAT (points)	N.A.	N.A.	10.5 (8–15)	N.A	N.A.	N.A.	N.A.
mMRC (points)	N.A.	N.A.	1.5 (1–3)	N.A	N.A.	N.A.	N.A.

Data are presented as the median (IQR) or n. BMI—body mass index, FEV_1—forced expiratory volume at first second, VC—vital capacity, FeNO—fractional exhaled nitric oxide, ACT—asthma control test, ICS- inhaled corticosteroids, CAT—COPD assessment test, Mmrc—modified Medical Research Council, N.A.—not applicable. & Kruskal Wallis or Chi square test, * Mann–Whitney U test.

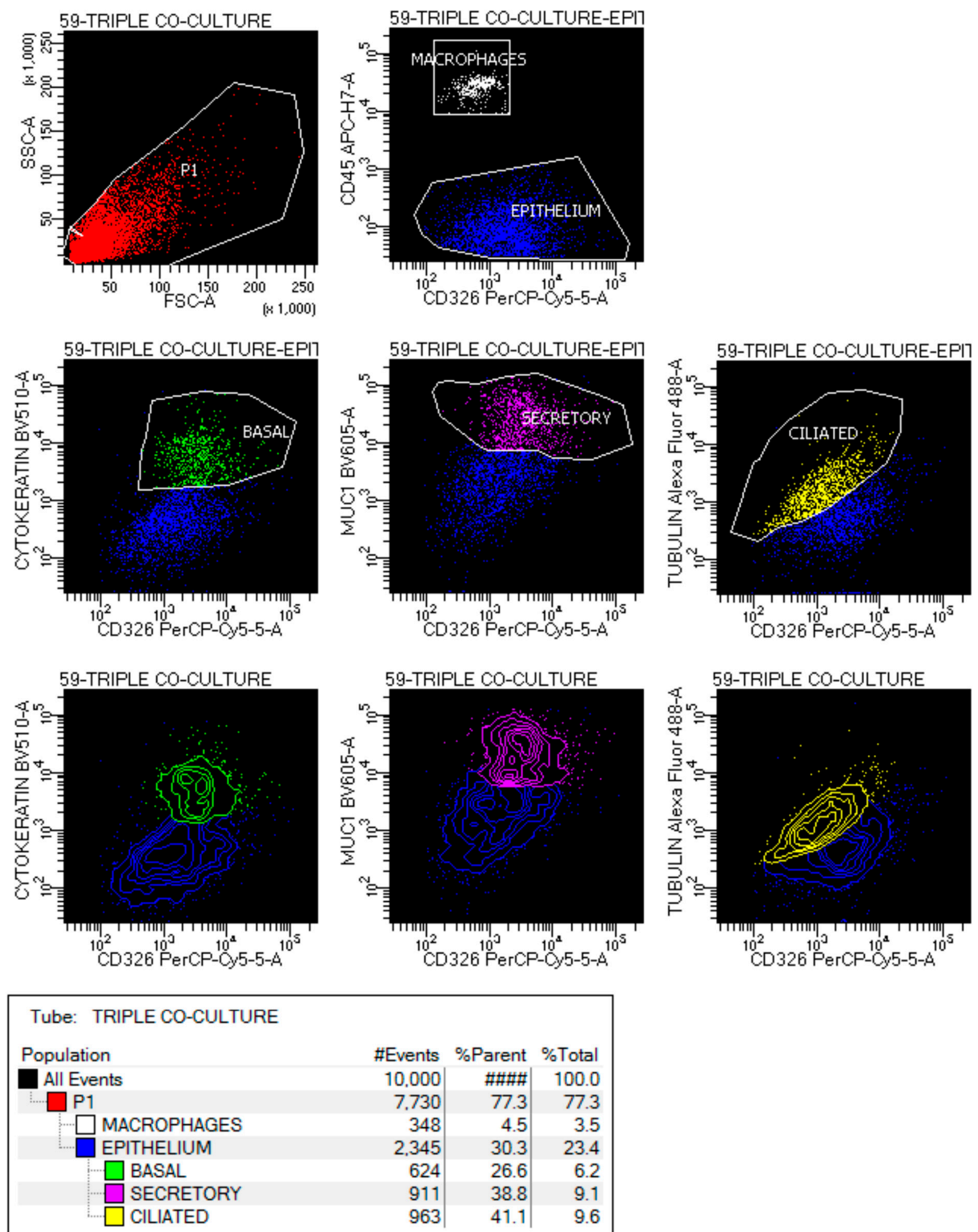
Table A4. The sequence of primers used in the PCR.

Gene Symbol	Forward Primer	Reverse Primer	Probe	Product Size
LINC02029	**TGCCCCCACG AGGTACAC**	**CAGGACCCAAA GAAGGAATGAT**	**6-FAM-TCCCGGGA AACAAA-MGB**	**58**
Gene symbol		Entrez Gene ID		
18s rRNA		Hs99999901_s1		187
AHRR		Hs01005075_m1		98
ARC		Hs01045540_g1		92
ATP1B2		Hs01020302_g1		81
BPIFA2		Hs00395980_m1		68
CCL22		Hs01574247_m1		88
CYP1B1		Hs00164383_m1		118
CYP1B1-AS1		Hs00381672_m1		80
EDC3		Hs00257810_m1		122
EEF1A2		Hs00951278_m1		80
ENPEP		Hs00989749_m1		70
IRF4		Hs00180031_m1		88
ME1		Hs00159110_m1		73
NCF1		Hs00165362_m1		113
RASD1		Hs02568415_s1		159
RMDN2-AS1		Hs04409587_s1		86
TIPARP		Hs00296054_m1		80



Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	8,953	89.5	89.5
MACROPHAGES	4	0.0	0.0
EPITHELIUM	2,570	28.7	25.7
BASAL	849	33.0	8.5
SECRETORY	1,016	39.5	10.2
CILIATED	1,125	43.8	11.2

Figure A1. The characterization of mono-ALI-cultured epithelial cells.



Tube: TRIPLE CO-CULTURE

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	7,730	77.3	77.3
MACROPHAGES	348	4.5	3.5
EPITHELIUM	2,345	30.3	23.4
BASAL	624	26.6	6.2
SECRETORY	911	38.8	9.1
CILIATED	963	41.1	9.6

Figure A2. The characterization of the triple- ALI- co-cultured epithelial cells.

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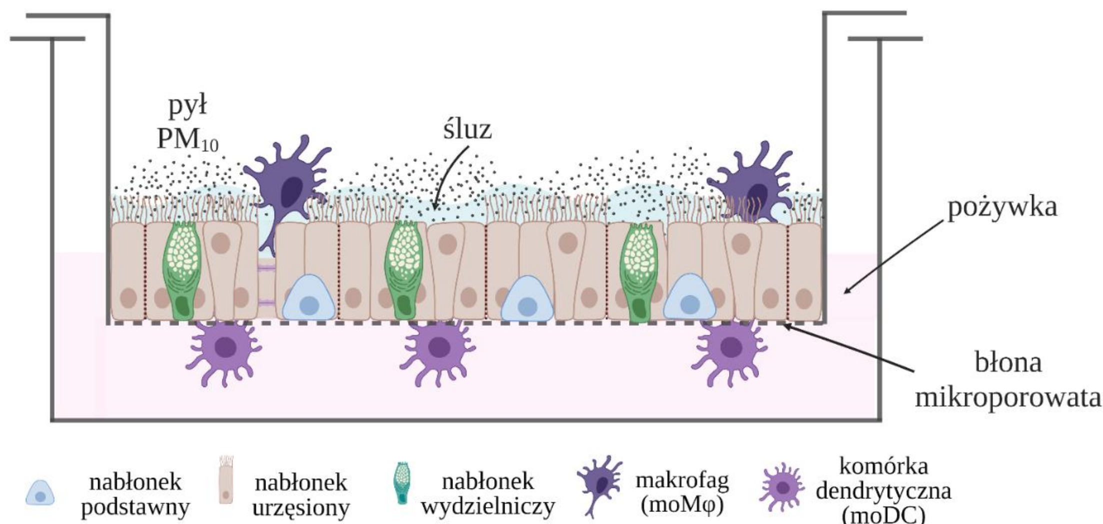
7. Podsumownie

Przedstawiony w rozprawie doktorskiej cykl publikacji zawiera szeroką analizę wpływu cząstek pyłu PM_{10} na nabłonek dróg oddechowych. Zastosowany model *in vitro* składał się z komórek nabłonka uzyskanych z wymazów szczoteczkowych błony śluzowej nosa, hodowanych na granicy faz powietrze-ciecz (*air-liquid interface,* ALI). Metodyka ta pozwala na uzyskanie wyspecjalizowanej hodowli komórkowej zawierającej wiele typów komórek nabłonkowych, w tym komórki podstawne, urzęsione i wydzielnicze, stanowiącej zaawansowany model *in vitro* nabłonka dróg oddechowych. Użyte przez nas w doświadczeniach komórki dendrytyczne oraz makrofagi izolowane i wyspecjalizowane z monocytów krwi obwodowej (*monocyte derived dendritic cells,* moDC; *monocyte derived macrophages*, moMφ) są komórkami modelowymi, będącymi odpowiednikami napływowych komórek immunologicznych dróg oddechowych. Każda z hodowli wielowarstwowych składała się z trzech typów komórek (makrofagi, nabłonek, komórki dendrytyczne) pozyskanych od indywidualnego dawcy (Ryc. 2) i została poddana 24-godzinnej ekspozycji na zawiesinę PM_{10} o stężeniu 100 µg/ml. Hodowle wielokomórkowe zastosowane w niniejszej pracy pozwalają na określenie wpływu cząstek PM_{10} na nabłonek, uwzględniając wpływ sygnalizacji i prostych interakcji międzykomórkowych, które mogą modulować tkankową odpowiedź immunologiczną. Wykorzystany przez nas model hodowli nie odzwierciedla w pełni złożoności komunikacji międzykomórkowej występującej w organizmie człowieka, która jest kształtowana przez zespół różnorodnych komórek napływowych i strukturalnych w miejscu reakcji immunologicznej, jednak jest jednym z najbardziej zaawansowanych modeli oceny procesów biologicznych *in vitro.* Poznanie zróżnicowania mechanizmów patofizjologicznych po ekspozycji na zanieczyszczenia powietrza w obturacyjnych chorobach płuc może przyczynić się do opracowania bardziej dostosowanych do potrzeb i stanu pacjenta metod terapii astmy i POChP.



Rycina 2. Schemat modelu hodowli wielokomórkowej eksponowanej na PM_{10} wykorzystanej w niniejszej pracy. moMφ- makrofag wyspecjalizowany z monocytu krwi obwodowej, moDC- komórka dendrytyczna wyspecjalizowana z monocytu krwi obwodowej. [Zaadaptowano z publikacji Misiukiewicz-Stępień et al. "*RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases*"]

W pracy *Biological effect of PM_{10} on airway epithelium-focus on obstructive lung diseases* szczegółowo scharakteryzowałyśmy frakcję PM_{10} pyłu zawieszonego w powietrzu. PM_{10} stanowi mieszaninę cząstek frakcji $PM_{2,5}$ oraz dodatkowych, większych i bardziej zróżnicowanych składników. Dlatego też charakteryzuje się odmiennym od frakcji $PM_{2,5}$ składem chemicznym i działaniem toksycznym, które wykazuje szerszy wpływ na zdrowie niż sama frakcja $PM_{2,5}$. W kolejnych podrozdziałach pracy opisałyśmy mechanizmy, za pośrednictwem których pył PM_{10} oddziałuje niekorzystnie na nabłonek dróg oddechowych. Zaliczane są do nich modulacja odporności wrodzonej, odpowiedzi zapalnej oraz indukcja stresu oksydacyjnego, których szczegółowe procesy zależne są od składu chemicznego pyłu z zanieczyszczenia powietrza atmosferycznego. W niniejszej pracy, uwzględniając wyniki badań epidemiologicznych oraz doświadczalnych, scharakteryzowałyśmy wpływ uszkodzeń i dysfunkcji nabłonka po ekspozycji na PM_{10} na rozwój i zaostrzenia chorób obturacyjnych.

W badaniu *Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD* wykazaliśmy, że hodowle nabłonka pozyskanego od chorych na astmę i POChP charakteryzują się istotnie obniżoną szczelnością połączeń międzykomórkowych, wyrażonych jako niższe wartości przeznabłonkowego oporu elektrycznego (TEER)

w porównaniu do hodowli od osób zdrowych. Na szczególną uwagę zasługuje fakt, że komórki nabłonka astmatycznego współhodowane z makrofagami wykazywały wyższe wartości TEER w porównaniu do hodowli mononabłonkowej, co może świadczyć o uszczelnieniu bariery nabłonkowej przez makrofagi. Zaproponowana przez nas, relatywnie krótka, 24-godzinna ekspozycja na PM_{10} nie spowodowała istotnych zmian w szczelności połączeń pomiędzy komórkami nabłonka we wszystkich badanych grupach. Analiza cytometryczna wykazała najwyższy odsetek komórek eksprymujących receptor dla nabłonkowego czynnika wzrostu (*epithelial growth factor receptor,* EGFR) wśród urzęsionych komórek nabłonka od osób z astmą. Co ciekawe, 24-godzinna stymulacja PM_{10} obniżyła ekspresję EGFR oraz receptora dla IL-33, ST-2. Nabłonek od chorych na POChP charakteryzował się najsilniejszą odpowiedzią zapalną: produkował najwyższe stężenia białek cytokinowych IL-6 i IL-8 po ekspozycji na PM_{10}. Wykazaliśmy także istotny, modulujący efekt współhodowli nabłonka z komórkami dendrytycznymi. Odpowiedź zapalna nabłonka współhodowanego z komórkami dendrytycznymi oraz makrofagami na stymulację PM_{10} była silniejsza niż hodowli mononabłonkowych. Wyniki naszych doświadczeń sugerują, na brak udziału IL-1β w mechanizmie odpowiedzi nabłonka na działanie zanieczyszczeń atmosferycznych, stymulacja jej produkcji zależna była wyłącznie od współhodowli z komórkami dendrytycznymi nie zaś od stymulacji PM_{10}.

W pracy *RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases* przenalizowaliśmy transkryptom nabłonka współhodowanego z makrofagami i komórkami dendrytycznymi w grupie kontrolnej (osoby zdrowe) oraz chorych na astmę lub POChP po ekspozycji na PM_{10}. W wyniku analizy zidentyfikowaliśmy 7 genów (*CYP1B1, CYP1B1-AS1, NCF, ME1, LINC02029, BPIFA2, EEF1A2*) o zróżnicowanej ekspresji mRNA w porównaniu do nabłonka niepoddanego ekspozycji w grupie kontrolnej, 5 genów różnicujących (*CYP1B1, ARC, ENPEP, RASD1, CYP1B1-AS1*) w grupie astmy oraz 6 genów różnicujących (*CYP1B1, CYP1B1-AS1, IRF4, ATPB1, TRIAPR, CCL22*) w grupie POChP. W toku dalszych prac z wykorzystaniem metody RT-qPCR wykazaliśmy, że najsilniej aktywowanymi genami po ekspozycji na zanieczyszczenia powietrza w nabłonku chorych na astmę i POChP z hodowli wielokomórkowych były *BPIFA2* i *ENPEP*, podczas gdy ekspresja *CYP1B1-AS1* i *TIPARP* była podwyższona tylko w POChP. Gen *BPIFA2* koduje ludzkie białko wydzielnicze ślinianek przyusznych (*BPI fold-containing family A member 2*), które należy do rodziny białek klonu

podniebienia, płuc i nabłonka (*palate, lung and nasal epithelium clone proteins*, PLUNC) związanych z lokalną odpowiedzią przeciwbakteryjną w górnych drogach oddechowych. Zwiększenie ekspresji *BPIFA2* po ekspozycji na PM_{10} w nabłonku chorych na astmę i POChP wskazuje na pobudzenie wrodzonej odpowiedzi immunologicznej w drogach oddechowych osób z obturacyjnymi chorobami płuc narażonych na zanieczyszczenia powietrza. Szczegółowa rola endopeptydazy ENPEP (*glutamyl aminopeptidase*) w fizjologii układu oddechowego nie jest dotychczas opisana. Badania innych autorów sugerują dodatnią korelację pomiędzy ekspresją tego białka, a ekspresją enzymu konwertującego angiotensynę typu 2 (*angiotensin-converting enzyme 2*, ACE2), oraz jego aktywność aminopeptydazową, co sugeruje rolę ENPEP w aktywacji komórek, transdukcji sygnałów oraz przyleganiu komórek do błony podstawnej.

Należy zwrócić uwagę na fakt, że analiza transkryptomiczna nabłonka współhodowanego z makrofagami i komórkami dendrytycznymi wykazała podwyższoną ekspresję mRNA dla genów związanych z reakcją na toksyczne substancje po ekspozycji na PM_{10}, *CYP1B1-AS1 (cytochrome P450 B1 antisense RNA 1)* i *TIPARP (TCDD Inducible Poly (ADP-Ribose) Polymerase),* jedynie w grupie POChP. *CYP1B1-AS1* należy do długich, niekodujących antysensownych RNA i jest uważany za wzmacniacz ekspresji genu kodującego izoenzym cytochromu P450 (*cytochrome P450 B1*) *CYP1B1* związany z metabolizmem ksenobiotyków. Sugerujemy, że wcześniejsza ekspozycja na dym papierosowy u chorych na POChP zwiększa wrażliwość i odpowiedź komórek nabłonka na szkodliwe działanie pyłu zawieszonego w powietrzu. Analiza RNA-Seq wykazała istotny wzrost ekspresji mRNA *CYP1B1* w POChP, jednak wynik ten nie został potwierdzony w dodatkowej analizie metodą RT-qPCR. Warto jednak zauważyć, że ekspresja *CYP1B1* może być zależna od aktywacji receptorów węglowodorów aromatycznych (*aryl hydrocarbon receptor,* AHR), które z kolei są hamowane przez represor AHR (*aryl hydrocarbon receptor represor*, AHRR). W naszym badaniu pokazaliśmy, że po ekspozycji na PM_{10} w nabłonku astmatycznym ekspresja mRNA *AHRR* obniża się, natomiast w przypadku POChP ulega podwyższeniu. Niższa ekspresja mRNA *AHRR* sugeruje aktywną ekspresję AHR w nabłonku chorych na astmę i aktywację *CYP1B1* za pośrednictwem tego mechanizmu. Jednak długotrwała aktywność AHR może prowadzić do zwiększenia stężenia szkodliwych metabolitów pośrednich ksenobiotyków, a przez to ich toksycznego działania na komórki. W POChP po ekspozycji na PM_{10} zaobserwowaliśmy podwyższoną ekspresję mRNA *TIPARP*, która negatywnie reguluje aktywność receptora AHR oraz genów od niego zależnych. Może to

prowadzić do hamowania ścieżek sygnałowych zależnych od tego receptora i skutkować zwiększonym efektem toksycznym po narażeniu na zanieczyszczenie powietrza w drogach oddechowych chorych na POChP.

Analiza ontologii genów (GO) ujawniła, że stymulacja zdrowego nabłonka pyłem PM_{10} aktywuje szereg mechanizmów regeneracyjnych głównie genów zaangażowanych w regenerację NADH (GO:0006735) i katabolizm glukozy (GO:0006007). Należy podkreślić fakt, że ekspozycja na PM_{10} aktywowała odmienne ścieżki sygnałowe w nabłonku osób z chorobami obturacyjnymi układu oddechowego w porównaniu do osób zdrowych. W przypadku astmy nadreprezentowane były ścieżki związane m.in. z hemotaksją granulocytów (GO:0071621) i metabolizmem retinoidów (GO:0001523), a w grupie POChP te związane z odpowiedzią na toksyczne substancje (GO:0009636), komórkową odpowiedzią na stres oksydacyjny (GO:0034599) oraz migracją leukocytów (GO:0050900). Wyniki te sugerują aktywację odmiennych odpowiedzi biologicznych i różnych mechanizmów naprawczych nabłonka, po ekspozycji na zanieczyszczenia powietrza w drogach oddechowych chorych na astmę i POChP.

8. Wnioski

1. Przegląd literatury światowej szeroko opisuje toksyczne działanie pyłu PM_{10} i jego istotny wpływ na patofizjologię astmy i POChP. Podwyższone stężenia zanieczyszczeń w powietrzu atmosferycznym, w tym PM_{10}, należy traktować jako potencjalny czynnik zaostrzający objawy u chorych na astmę i POChP.

2. Nabłonek dróg oddechowych chorych na obturacyjne choroby płuc, a w szczególności chorych na POChP, charakteryzował się wyższą niż u osób zdrowych produkcją cytokin prozapalnych w odpowiedzi na ekspozycję PM_{10}. Ekspresja IL-6 i IL-8 po stymulacji PM_{10} była większa w nabłonku współhodowanym z komórkami dendrytycznymi i makrofagami w porównaniu do hodowli mononabłonkowych, co sugeruje istotny wpływ komórek napływowych na odpowiedź zapalną nabłonka dróg oddechowych.

3. 24-godzinna ekspozycja na pył PM_{10} nie zmieniła integralności połączeń międzynabłonkowych we wszystkich analizowanych grupach, jednak wpłynęła na ekspresję EGFR i ST2 na nabłonku od chorych na astmę.

4. Analiza transkryptomiczna nabłonka dróg oddechowych wykazała, że miejscowa odpowiedź biologiczna po ekspozycji na zanieczyszczenia powietrza w astmie i POChP jest odmienna niż u osób zdrowych. W zastosowanym przez nas modelu *in vitro* stymulacja pyłem frakcji PM_{10} u osób z chorobami obturacyjnymi pobudziła odpowiedź immunologiczną, w szczególności rekrutację leukocytów, dodatkowo w astmie wpływała na procesy przebudowy komórek strukturalnych, zaś w POChP modulację odpowiedzi antyoksydacyjnej. Natomiast u osób zdrowych, ekspozycja na PM_{10} aktywowała potencjał regeneracyjny komórek nabłonkowych.

9. Opinia komisji bioetycznej

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61
02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

KB/37/2020

Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym
w dniu 09 marca 2020 r. po zapoznaniu się z wnioskiem:

Dr hab. n. med. Katarzyna Górska
Katedra i Klinika Chorób Wewnętrznych, Pneumonologii i Alergologii
ul. Banacha 1a, 02-097 Warszawa

dotyczącym: wyrażenia opinii w sprawie badania pt.: „Wpływ miejskich pyłow zanieczyszczenia powietrza na odpowiedź zapalną nabłonka nosowego z uwzględnieniem interakcji pomiędzy komórkami nabłonka, makrofagami i komórkami dentrycznymi w chorobach obturacyjnych płuc."

wyraża następującą
opinię

- stwierdza, że jest ono dopuszczalne i zgodne z zasadami naukowo-etycznymi*.
- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.*~~

Uwagi Komisji – *verte*

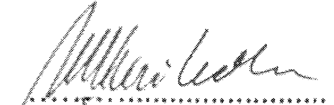
Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
Komisja działa zgodnie z zasadami GCP .

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma-Kozakiewicz

*niepotrzebne skreślić

strona podpisowa do uchwały Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym nr KB/........37........ z dnia 09 marca 2020r.



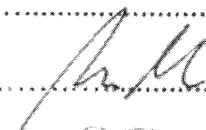
1. Prof. dr hab. n.med. Magdalena Kuźma –Kozakiewicz ....

2. Dr hab. n. med. Tomasz Grzela ....

3. Dr hab. n. med. Andrea Horvath-Stolarczyk ....

4. Prof. dr hab. n. med. Paweł Piątkiewicz ....

5. Dr hab. n. med. Marek Postuła ....



6. Prof. dr hab. n. med. Marcin Ufnal ....

7. Dr hab. n. farm. Sylwia Flis ....

8. Dr n. med. Agnieszka Serafin ....A.Serafin....

9. Ks. Paweł Śmierzchalski ....

10.Mec.Danuta Lewandowska ....

11. Prof. Joanna Domagała-Kulawik ....

12. Dr hab. n.med. Barbara Grzechocińska ....

13. Dr hab. n. med. Michał Grąt ....

14.Dr n. med. Joanna Drozd-Sokołowska ....

10. Oświadczenia współautorów publikacji

mgr inż. Paulina Misiukiewicz-Stępień

Warszawa, 09.11.2022

OŚWIADCZENIE

Jako współautor pracy ***Biological effect of PM_{10} on airway epithelium-focus on obstructive lung diseases. Clinical Immunology 2021;227;108754*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował pracę nad tekstem manuskryptu oraz ostateczne poprawki. Mój udział w przygotowaniu publikacji określam jako 60%.

Jako współautor pracy ***Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD. Scientific Reports 2021;11;13259*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował opracowanie koncepcji i planu doświadczeń, wykonanie oznaczeń laboratoryjnych oraz ostateczne poprawki. Mój udział w przygotowaniu publikacji określam jako 40%.

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował opracowanie koncepcji i planu doświadczeń, wykonanie oznaczeń laboratoryjnych, pracę nad tekstem manuskryptu oraz ostateczne poprawki. Mój udział w przygotowaniu publikacji określam jako 50%.

(podpis oświadczającego)

dr hab. n. med. Magdalena Paplińska-Goryca

Warszawa, 09.11.2022

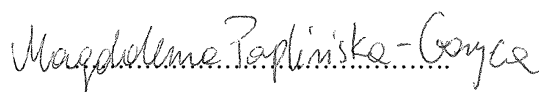
OŚWIADCZENIE

Jako współautor pracy ***Biological effect of PM_{10} on airway epithelium-focus on obstructive lung diseases. Clinical Immunology 2021;227;108754*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował opracowanie koncepcji i ogólnego planu manuskryptu, pracę nad tekstem manuskryptu oraz merytoryczne poprawki. Mój udział w przygotowaniu publikacji określam jako 40%.

Jako współautor pracy ***Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD. Scientific Reports 2021;11;13259*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował opracowanie koncepcji i planu doświadczeń, wykonanie oznaczeń laboratoryjnych, analizę uzyskanych wyników, pracę nad pierwszą wersją manuskryptu oraz ostateczne poprawki. Mój udział w przygotowaniu publikacji określam jako 45%.

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował pozyskanie źródła finansowania publikacji, opracowanie koncepcji i planu doświadczeń, wykonanie oznaczeń laboratoryjnych, analizę uzyskanych wyników, pracę nad tekstem manuskryptu oraz merytoryczne poprawki. Mój udział w przygotowaniu publikacji określam jako 25%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w prac jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.



(podpis oświadczającego)

prof. dr hab. n. med. Rafał Krenke

Warszawa, 07.11.2022

OŚWIADCZENIE

Jako współautor pracy ***Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD. Scientific Reports 2021;11;13259*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował analizę uzyskanych wyników oraz merytoryczne poprawki. Mój udział w przygotowaniu publikacji określam jako 3%.

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował pracę nad ostateczną wersją manuskryptu oraz merytoryczne poprawki. Mój udział w przygotowaniu publikacji określam jako 3%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w prac jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.

....

(podpis oświadczającego)

dr hab. inż. Elwira Zajusz-Zubek

Gliwice, 09.11.2022

OŚWIADCZENIE

Jako współautor pracy ***Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD. Scientific Reports 2021;11;13259*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował pozyskanie materiału środowiskowego do doświadczeń oraz wykonanie oznaczeń laboratoryjnych. Mój udział w przygotowaniu publikacji określam jako 3%.

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Jednocześnie wyrażam zgodę na wykorzystanie w/w prac jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.

...Elwira Zajusz-Zubek...

(podpis oświadczającego)

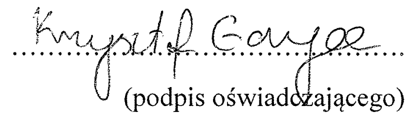
dr Krzysztof Goryca

Warszawa, 24.10.2022

OŚWIADCZENIE

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował analizę uzyskanych wyników oraz merytoryczne poprawki. Mój udział w przygotowaniu publikacji określam jako 8%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.



....

(podpis oświadczającego)

mgr inż. Dorota Adamska

Warszawa, 24.10.2022

OŚWIADCZENIE

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował wykonanie oznaczeń laboratoryjnych oraz ostateczne poprawki. Mój udział w przygotowaniu publikacji określam jako 6%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.

(podpis oświadczającego)

dr hab. n. med. Patrycja Nejman-Gryz

Warszawa, 09.11.2022

OŚWIADCZENIE

Jako współautor pracy ***Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD. Scientific Reports 2021;11;13259*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował wykonanie oznaczeń laboratoryjnych oraz merytoryczne poprawki. Mój udział w przygotowaniu publikacji określam jako 3%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.

.....Patrycja.....Nejman-Gryz

(podpis oświadczającego)

mgr Małgorzata Proboszcz

Warszawa, 09.11.2022

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.

Małgorzata Proboszcz ....

(podpis oświadczającego)

dr hab. n.med. Katarzyna Górska

Warszawa, 10.11.2022

OŚWIADCZENIE

Jako współautor pracy ***Interactions of nasal epithelium with macrophages and dendritic cells variously alter urban PM-induced inflammation in healthy, asthma and COPD. Scientific Reports 2021;11;13259*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował rekrutację pacjentów oraz pozyskiwanie materiału biologicznego do doświadczeń. Mój udział w przygotowaniu publikacji określam jako 3%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.

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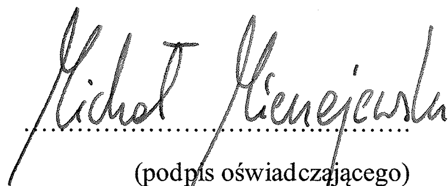
lek. Michał Mierzejewski

Warszawa, 24.10.2022

OŚWIADCZENIE

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował rekrutację pacjentów oraz pozyskiwanie materiału biologicznego do doświadczeń. Mój udział w przygotowaniu publikacji określam jako 3%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.



....
(podpis oświadczającego)

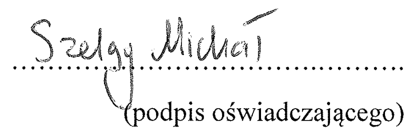
mgr Michał Szeląg

Warszawa, 24.10.2022

OŚWIADCZENIE

Jako współautor pracy ***RNA-Seq analysis of UPM-exposed epithelium co-cultivated with macrophages and dendritic cells in obstructive lung diseases. International Journal of Molecular Sciences 2022;23;9125*** oświadczam, że mój wkład merytoryczny w powstanie tej pracy obejmował wykonanie oznaczeń laboratoryjnych. Mój udział w przygotowaniu publikacji określam jako 2%.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako części rozprawy doktorskiej mgr inż. Pauliny Misiukiewicz-Stępień.



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(podpis oświadczającego)

11. Piśmiennictwo do rozdziałów w języku polskim

Piśmiennictwo dotyczące opublikowanych prac stanowi integralną część odpowiednich publikacji.

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