Relationship of Microbial Profile With Airway Immune Response in Eosinophilic or Neutrophilic Inflammation of Asthmatics.

Jong-Sook Park, Ji-Hye Son, Jung Hyun Kim, Hun Soo Chang, Choon-Sik Park

Genome Research Center and Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Korea

Abstract

Different characteristics of airway microbiome in asthmatics may lead to differential immune responses, which in turn cause eosinophilic or neutrophilic airway inflammation. However, the relationships among these factors have yet to be fully elucidated. The numbers of operational taxonomic units were significantly higher in the mixed (n = 21) and neutrophilic (n = 23) inflammation groups than in the paucigranulocytic inflammation group (n = 19; $p < 0.05$). At the species level, *Granulicatella adiacens*, *Streptococcus parasanguinis*, *Streptococcus pneumoniae*, *Veillonella rogosae*, *Haemophilus parainfluenza*, and *Neisseria perflava* levels were significantly higher in the eosinophilic inflammation group (n = 20), whereas *JYGU_s* levels were significantly higher in the neutrophilic inflammation group compared to the other subtypes ($P < 0.05$). Additionally, IL-5 and IL-13 concentrations were correlated with the percentage of eosinophils ($P < 0.05$) and IL-13 levels were positively correlated with the read counts of *P. pasteri*. and *V. rogosae* ($P < 0.05$). IL-1β concentrations were correlated with the percentage of neutrophils ($P < 0.05$). had a tendency to be positively correlated with the read count of *JYGU_s* ($P = 0.095$), and was negatively correlated with that of *S. pneumoniae* ($P < 0.05$). Difference of microbial patterns in airways may induce distinctive endotypes of asthma, which is responsible for the neutrophilic or eosinophilic inflammation in asthma.

Background

• It has been established that the underlying pathologies of asthma are heterogenous, in terms of the inflammatory patterns seen in the airways. Neutrophilic inflammation presents in a certain proportion of severe asthmatics who are relatively resistant to corticosteroid therapy, while eosinophilic inflammation dominates in those with reversible airway obstructions.

• A recent study in a relatively large number of sputum samples demonstrated that the sputum neutrophil percentage was positively correlated with the relative abundance of *Moraxella*, and negatively correlated with the abundances of *Streptococcus I*, *Gemella*, and *Porphyromonas*. Although these data indicate that the microbiome is influenced by the different phenotypes of airway inflammation, few studies have evaluated the relationship of microbial patterns and endotypes of asthma.

Aim

• We hypothesized that eosinophilic and neutrophilic inflammation in asthmatic patients would be associated with different microbiome profiles, reflected in Th2, Th1, and Th17 expression levels, and inflammasome-dominant immune responses.

Materials & Methods

Microbes in induced sputum samples were subjected to sequence analysis of 16S rRNA. Airway inflammatory phenotypes were defined as neutrophils (>60%) and eosinophils (>3%), and inflammation endotypes were defined by levels of T helper (Th) 1 (interferon-γ), Th2 (interleukin [IL]-5 and IL-13), Th-17 (IL-17), and innate Th2 (IL-25, IL-33, and thymic stromal lymphopoietin) cytokines, inflammasomes (IL-1β), epithelial activation markers (granulocyte-macrophage colony-stimulating factor and IL-8), and Inflammation (IL-6 and tumor necrosis factor-α) cytokines in sputum supernatants was assessed by enzyme-linked immunosorbent assay.

Result

Table1. Clinical characteristics of the study subjects

Variable	Mixed	Neutrophilic	Eosinophilic	Pauci granulocytic	p-value (4 groups)	p-value (Neu vs. Eos)
No.	21	23	20	19	-	-
Sex (Male/Female)	7/14	6/17	8/12	6/13	0.812	
Age (years)	49(39-58)	61(54-70)	52(48-62)	59(37-68)	0.118	
Asthma-onset age (years)	44(30-50)	51(39.45-58)	46.5(31.5-53)	45(34-59.5)	0.530	
Smoking status (ES/NS)	4/17	7/16	6/14	3/16	0.257	
Smoking amount (pack-years)	0(0-0)	0(0-4.5)	0(0-0)	0(0-0)	0.503	
Atopy (Y/N/ND)	15/5/1	9/13/1	8/9/3	6/11/2	0.145	
Serum total IgE (IU/mL)	170(111-356)	149(30-348)	239(66-876)	78(38-225)	0.244	
Body mass index (kg/m^2)	23.2(21.1-24.5)	24.9(22.8-27.8)	23.1(21.7-25.6)	25(22.4-27.6)	0.328	
Asthma exacerbation rate*	1(0-1)	1(0-1)	1(1-1)	0(0-1)	0.375	
FVC (% predicted)	74(63-82)	70(57.5-85.5)	65(59.5-78.5)	88(81.5-99.5)	0.001	0.450
FEV1 (% predicted)	73(60-83)	67(50-94)	63.5(38.2-69.7)	93(78-107.5)	3.38E-04	0.342
FEV1/FVC (%)	74(68-81)	71(59.5-78.5)	71(58-78.25)	80(71.5-82)	0.125	
Total cell count (x10^5/mL)	1.2(0.5-4.5)	2.4(1.58-3.8)	2.62(1.4-3.98)	1.0(0.8-3.1)	0.173	
Neutrophils (%)	87.5(80.5-89.5)	89.5(84-96)	36.3(27.5-48.6)	40.5(26.1-52.2)	2.17E-13	2.13E-08
Eosinophils (%)	5.7(3.7-13.7)	0(0-1.1)	50.7(14.9-61.6)	0(0-0.4)	1.13E-14	1.49E-08
Macrophages (%)	7(2.19-9.5)	9.2(3.2-13.6)	7.1(0.3-42)	54(46.6-73.5)	4.69E-08	0.981
Lymphocytes (%)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0.466	

The normality of the distribution was evaluated using the Shapiro-Wilk test. Comparisons of the variables among the groups were performed with Kruskal-Wallis test and post hoc Mann–Whitney U test. P values < 0.05 were considered statistically significant. Data are presented as medians (interquartile range).
ES, ex-smokers; NS, never-smokers; ND, not determined; IgE, immunoglobulin E; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.
*Asthma exacerbation rate developed in the 1st one year of follow-up.

Table 2. Comparison of normalized read counts of bacteria at the species level among the 4 inflammatory subtypes

Phylum	Genus	Species	Mixed (n = 21)	Neutrophilic (n = 23)	Eosinophilic (n = 20)	Paucigranulocytic (n = 19)	p-value (4 groups)	p-value (Neu vs. Eos)
Bacteroidetes	*Porphyromonas*	*P. pasteri*	115 (15–296)	71 (6–200)	158 (59–394)	10 (0–189)	0.049	0.093
Firmicutes	*Granulicatella*	*G. adiacens*	30 (0–212)	0 (0–74)	120 (44–281)	125 (53–243)	0.029	0.010
Firmicutes	*Streptococcus*	*JYGU_s*	0 (0–1,242)	787 (0–2,125)	0 (0–0)	0 (0–0)	0.012	0.010
Firmicutes	*Streptococcus*	*S. parasanguinis*	34 (0–187)	0 (0–62)	209 (72–408)	168 (18–339)	0.003	0.001
Firmicutes	*Streptococcus*	*S. pneumoniae*	231 (0–1916)	0 (0–514)	906 (279–2,372)	1,129 (226–2,333)	0.014	0.004
Firmicutes	*Streptococcus*	*S. sinensis*	19 (0–247)	0 (0–76)	188 (83–332)	210 (71–409)	0.004	0.004
Firmicutes	*Veillonella*	*V. rogosae*	123 (44–409)	13 (1–81)	160 (99–496)	12 (2–182)	0.001	0.001
Proteobacteria	*Haemophilus*	*H. parainfluenza*	42 (0–1647)	0 (0–57)	353 (101–1,638)	535 (63–1,055)	0.010	0.002
Proteobacteria	*Neisseria*	*N. perflava*	12 (0–73)	0 (0–1)	241 (5–348)	218 (1–1,106)	0.001	2.87E-04

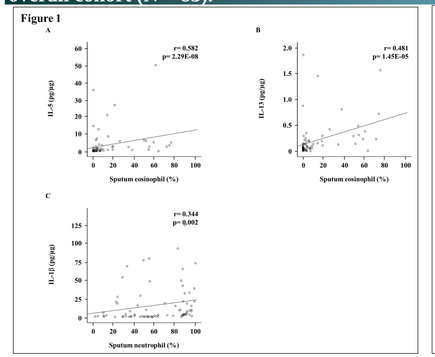
The normality of the distribution was evaluated using the Shapiro-Wilk test. Comparisons of the variables among the groups were performed with Kruskal-Wallis test and post hoc Mann–Whitney U test. P values < 0.05 were considered statistically significant. Data are presented as medians (interquartile range) of bacterial reads.

Table 3. Comparison of cytokine levels among the 4 inflammatory subtypes

Cytokine	Mixed (n = 21)	Neutrophilic (n = 23)	Eosinophilic (n = 20)	Paucigranulocytic (n = 19)	p-value (4 groups)	p-value (Neu vs. Eos)
IL-5 (pg/μg)	1.346(0.51-2.94)	0.133(0.094-0.453)	2.611(0.835-5.908)	0.15(0.102-0.233)	1.50E-04	0.001
IL-13 (pg/μg)	0.111(0.057-0.236)	0.051(0.028-0.092)	0.246(0.141-0.463)	0.085(0.042-0.147)	0.004	0.001
GM-CSF (pg/μg)	0.048(0.021-0.106)	0.049(0.04-0.071)	0.091(0.043-0.224)	0.064(0.041-0.251)	0.056	
IFNγ (pg/μg)	0.008(0.002-0.035)	0.007(0.003-0.015)	0.006(0.002-0.026)	0.018(0.004-0.032)	0.370	
IL-17A (pg/μg)	0.009(0.002-0.023)	0.021(0.01-0.045)	0.006(0.001-0.033)	0.026(0.007-0.048)	0.144	
IL-6 (ng/μg)	0.005(0.001-0.01)	0.004(0.001-0.018)	0.002(0.001-0.009)	0.005(0.003-0.017)	0.622	
GRO (ng/μg)	0.669(0.39-1.419)	0.905(0.48-1.256)	0.742(0.52-1.178)	0.934(0.71-1.731)	0.378	
IL-8 (ng/μg)	0.445(0.347-0.587)	0.728(0.461-1.014)	0.371(0.341-0.583)	0.561(0.393-0.775)	0.062	
TNFα (ng/μg)	0.002(0-0.009)	0.004(0.001-0.016)	0.002(0.001-0.005)	0.01(0.002-0.019)	0.249	
IL-1β (ng/μg)	0.005(0.003-0.02)	0.013(0.003-0.024)	0.001(0-0.007)	0.002(0.001-0.025)	0.006	0.001
IL-33 (pg/μg)	0.747(0.294-1.323)	0.942(0.464-1.331)	0.633(0.274-1.427)	1.075(0.294-2.048)	0.874	
IL-25 (ng/μg)	0.007(0.004-0.017)	0.01(0.004-0.016)	0.008(0.004-0.02)	0.014(0.009-0.023)	0.343	
TSLP (ng/μg)	0.005(0.003-0.008)	0.004(0.003-0.006)	0.005(0.003-0.007)	0.006(0.004-0.01)	0.552	

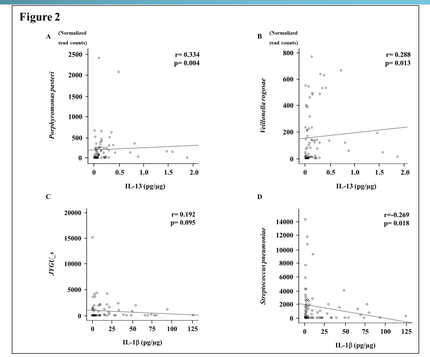
The normality of the distribution was evaluated using the Shapiro-Wilk test. Comparisons of the variables among the groups were performed with Kruskal-Wallis test and post hoc Mann–Whitney U test. P values < 0.05 were considered statistically significant. Data are presented as medians (interquartile range).
IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; GRO-γ, chemokine (C-X-C motif) ligand 3; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

Figure 1. Correlations between cytokine levels and the percentages of eosinophils and neutrophils in the sputum for the overall cohort (N = 83).



The interleukin (IL)-5, IL-13, and IL-1β values were normalized to protein levels in the sputum. The normality of the data distribution was assessed with the Shapiro-Wilk test and statistical significance was evaluated with the Spearman's rho test. Values are presented as correlation coefficients (r) with p-values.

Figure 2. Correlations between cytokine levels and bacterial normalized read counts for the overall cohort (N = 83).



The normality of the data distribution was assessed using the Shapiro-Wilk test and statistical significance was evaluated with the Spearman's rho test. Values are presented as correlation coefficients (r) and with p-values.

Summary

The level of *JYGU_s* was significantly higher in the neutrophilic group, whereas the levels of the other species were significantly higher in the eosinophilic group, compared to the other groups. The percentage of eosinophils had a significant positive correlation with IL-5 and IL-13 levels, and was also correlated with the numbers of *V. rogosae* and *P. pasteri*. The percentage of neutrophils had a positive correlation with IL-1β level.

Conclusion

The present study demonstrated that *V. rogosae* and *P. pasteri* may be related to eosinophilic inflammation via the production of IL-13, and that *JYGU_s* may be related to neutrophilic inflammation via the production of IL-1β. Thus, the difference in microbial patterns in the airways may induce distinct endotypes of asthma, which is responsible for neutrophilic or eosinophilic inflammation in asthma. Our study may contribute to understanding the relationship between microbiome and the type of airway inflammation, which is fundamental knowledge to control inflammation in asthma by targeting candidate microbes and to develop novel drugs and treatment strategies for uncontrolled asthma.

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