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ORIGINAL RESEARCH

Age-Specific Cytokine Profiling in Children with *Mycoplasma Pneumoniae* Infections in Post-COVID-19 Era: A Retrospective Study

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Background: In the wake of COVID-19, a resurgence of *Mycoplasma pneumoniae* pneumonia (MPP) has emerged globally since mid-2023. However, the clinical manifestations and immune responses following infection vary across different age groups of pediatric patients (infants, preschoolers and school-aged children), increasing the complexity of diagnosis and treatment.

Methods: This study retrospectively analyzed serum cytokine levels in 40 healthy children and 87 MPP patients, with additional cytokine profiling of bronchoalveolar lavage fluid (BALF) in severe cases, combining KEGG pathway analysis to investigate age-related immune patterns. SARS-CoV-2 antibody levels were further detected in these MPP patients, followed by Spearman correlation analysis to assess their correlation with cytokines in MPP children.

Results: Age-specific cytokine patterns emerged in MPP children. In 0–2 years, cytokines enriched in IL-17, TLR, and TNF pathways were upregulated in MPP groups compared to controls, while in 6–12 years, cytokines enriched in TLR, RLR, and JAK-STAT pathways were downregulated in MPP groups. Serum patterns in 3–5 years resembled those in 0–2 years, but BALF aligned with 6–12 years. SARS-CoV-2 IgG positively correlated with TWEAK, IL-22, IL-16, IL-12p40, CCL7, and CD152 in 0–2 years ($P < 0.05$), but negatively with CCL13 in 6–12 years ($P < 0.01$).

Conclusion: Overall, the immune pattern in children with MPP is age-specific and severity-dependent. Higher levels of SARS-CoV-2 IgG are associated with a more robust and mature anti-infective immune response in younger MPP patients, while in older children, besides providing immune memory against pathogens, SARS-CoV-2 IgG also appears to plant a landmine of immune exhaustion.

Keywords: age-specific, children, cytokines, immune patterns, *Mycoplasma pneumoniae*, SARS-CoV-2 antibody

Background

Mycoplasma pneumoniae (MP) is one of the most common pathogens of community-acquired pneumonia (CAP) in children, accounting for 8–40% of all CAP cases in pediatric populations.[1,2] Multiple epidemiological studies of MP in children have demonstrated significant differences in infection rates across various age groups. Specifically, the incidence of *Mycoplasma pneumoniae* pneumonia (MPP) is markedly higher in school-aged children compared to infants and toddlers.[3–5] This disparity is multifactorial. Epidemiologically, school-aged children experience greater pathogen exposure in congregate settings such as schools and after-school programs, facilitating person-to-person transmission via respiratory droplets. Historically, MP infection was indeed rare in neonates and infants. However, recent epidemiological shifts have documented increasing MPP incidence in younger children, suggesting that beyond exposure patterns, age-related immunological differences may also contribute to differential susceptibility.[6] Supporting this hypothesis, clinical presentations vary substantially across age groups, complicating diagnosis and treatment. Studies have shown that

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children <5 years often present with bronchiolitis or bronchopneumonia when infected with MP, whereas school-aged children predominantly exhibit segmental/lobar pneumonia, indicative of distinct underlying immune response patterns.[7] Cytokines, as crucial immune mediators, play a key role in the development and progression of MPP. Nevertheless, research on cytokine levels across different age groups of children with MPP remains limited.

Accumulating evidence has demonstrated that immune system maturation profoundly influences host responses to severe infections across different pediatric age groups, though specific patterns remain pathogen-dependent. Studies on viral central nervous system infections observed age-related increases in cerebrospinal fluid (CSF) interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-13 (IL-13), macrophage migration inhibitory factor (MIF), interferon gamma-induced protein 10 (IP-10), and interferon-gamma (IFN-γ) but decreases in monocyte chemoattractant protein-1 (MCP-1) among 80 children.[8] Costagliola et al attributed milder COVID-19 outcomes in children to higher naïve T cell proportions, enhanced thymic output, and potentially protective trained immunity effects.[9] Fisler et al recently reported that older children (≥5 years) with sepsis exhibited elevated granulocyte colony-stimulating factor (G-CSF), IL-6, and interleukin-8 (IL-8), levels alongside increased regulatory T cell populations.[10] These age-specific immunological differences contribute to variations in disease severity, clinical manifestations, and treatment responses across pediatric populations. However, whether similar age-dependent immune patterns exist in children with MPP, remains poorly understood. Given that MP infection exhibits marked age-related differences in both incidence and clinical presentation, elucidating the underlying age-specific immune mechanisms is of paramount importance.

The global outbreak of the novel coronavirus pneumonia (COVID-19) in 2019 has introduced new challenges to MP infections in children. Since July 2023, a notable resurgence of *Mycoplasma pneumoniae* has been reported in our country, with positive cases reaching up to 50%.[8] This observed increase in the number of MP infection cases aligns with the surveillance data from the United States and Europe, which indicating the first signs of an outbreak detected in June 2023, peaking by December 2023.[9,10] Further fundamental research has revealed that COVID-19 exerts long-term effects on the immune system, particularly through persistent molecular and cellular alterations.[11] These enduring changes within the immune system may influence subsequent immune responses to pathogens or vaccines, thereby impacting their development of clinical symptoms.[12–14] However, there is currently limited knowledge regarding whether COVID-19 facilitates the clinical manifestations and immune imbalance of MPP in the aftermath by affecting the host immune system. Therefore, exploring the correlation between COVID-19 antibody levels and cytokine responses in MPP is of great significance for uncovering the underlying mechanisms.

This study aims to analyze the differences in immune response patterns by detecting cytokine profiles in serum and bronchoalveolar lavage fluid (BALF) from children with MPP across different age groups, including infants and toddlers (0–2 years), preschooler (3–5 years), and school-aged children (6–12 years). Additionally, combining with the detection of COVID-19 antibody levels in those children with MPP, it is aimed to elucidate the potential impact of previous COVID-19 infection on current MPP immune patterns in the post-COVID-19 era. This represents the first systematic, dual-compartment (systemic versus local pulmonary) immune profiling study across the full pediatric age spectrum in MPP, and uniquely investigates the potential modulation of MPP immune responses by prior SARS-CoV-2 exposure in the post-pandemic era. The results of this study will provide new insights and theoretical foundations for optimizing current diagnostic and therapeutic strategies.

Methods

Patients and Controls

This study recruited hospitalized patients aged 0–12 years with MPP, who were admitted to the Children's Hospital, Zhejiang University School of Medicine, from January to February 2024. The diagnosis of MPP followed the Diagnosis and Treatment of Pediatric MPP Guidelines (2023 version), full definition of MPP and severe MPP (SMPP) were listed in Supplementary Methods.

Healthy control samples were obtained from individuals undergoing health examinations at the Children's Hospital, Zhejiang University School of Medicine. Inclusion criteria for healthy control and exclusion criteria for all participants were listed in Supplementary Methods. This study was approved by the Ethics Committee of the Children's Hospital,

Zhejiang University School of Medicine (No. 2024-IRB-0171-P-01) and was conducted in accordance with the Declaration of Helsinki. Given the retrospective nature of this study using residual serum and BALF samples that were originally collected during routine clinical care, the Ethics Committee waived the requirement for individual informed consent. All samples were obtained for standard diagnostic purposes and subsequently stored according to institutional biobank protocols. No patient received any invasive procedure solely for research purposes. The confidentiality of patient information was strictly protected, with individual identifiers removed during data collection and replaced with serial code numbers.

Specimen Collection

Venous blood samples were collected within 24 hours of patient admission. The indications and procedures for bronchoscopy adhered to the Chinese Pediatric Flexible Bronchoscopy Guidelines (2018 version).[15] Following the insertion of the bronchoscope into the target bronchus, lavage was performed with 37°C physiological saline (1 mL/kg per wash, maximum 20 mL), followed by negative pressure suction to obtain BALF. Both serum and BALF samples were centrifuged to extract the supernatant, which was subsequently stored at −80°C.

Detection of Cytokines and Chemokines

The levels of 50 cytokines and chemokines in the serum and BALF of participants were quantified using ABplex Human 50-Plex Custom Panel (Catalog No.: RK04378, ABclonal Technology, Wuhan, China). The experiment and instrument calibration were conducted according to the manufacturer's instructions. For a comprehensive list of the 50 biomarkers, including their abbreviations, full names, and alternative designations, see Supplementary Table 1. Samples, quality controls, and standards were each tested in duplicate. The detected cytokine concentrations in BALF specimen were further converted to the total cytokine amount in lung lesions using the following formula: cytokine amount (pg) = original concentration (pg/mL) × saline volume (mL).

Detection of SARS-CoV-2 Antibody

For IgG and IgM antibody detection against SARS-CoV-2, all serum specimen were applied to iFlash-SARS-CoV-2 IgM/IgG Antibody Test (Catalog No.: C86095G/M, Shenzhen YHLO Biotech Co., China). The results were obtained by iModules-G CLIA analyzers (Shenzhen YHLO Biotech Co.) in Children's Hospital, Zhejiang University School of Medicine. Antibody level ≤20 is defined as negative as per manufacturer's guide.

Statistical Analysis

Data were analyzed using SPSS 29.0.1.0 and GraphPad Prism 10.2.0. Continuous variables with non-normal distribution were expressed as median (interquartile range, IQR), where IQR represents the 25th to 75th percentiles [Q1-Q3]. Normally distributed data were presented as mean±SD. Group differences were compared using One-way ANOVA, Kruskal–Wallis test, or Two-Way ANOVA, followed by Tukey's post hoc test. Chi-square or Fisher's exact test was used for categorical variables. Spearman correlation analysis was employed to examine the relationship between paired serum and BALF cytokine levels from the same SMPP patients in each age group. Additionally, the association between SARS-CoV-2 IgG and serum cytokines in children with MPP was explored also using Spearman correlation analysis. A p-value <0.05 was considered statistically significant. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was conducted using the OmicShare tool.[16]

Results

Demographic Information

In total, 40 healthy controls (HC group) and 87 patients with MPP (MPP group) were enrolled in this study. The demographic characteristics of the participants were well-matched between the two groups, with no significant differences observed in age, sex, or weight (Supplementary Table 2).

Patient Characteristics

Given the substantial variation in clinical manifestations in different ages, the MPP patients were further divided into three groups based on age: 1) MPP Group A (0–2 years), comprising 12 general MPP (GMPP) and 5 severe MPP (SMPP); 2) MPP Group B (3–5 years), including 12 GMPP and 12 SMPP; 3) MPP Group C (6–12 years), consisting of 23 GMPP and 23 SMPP. Baseline characteristics were summarized in Table 1.

Serum Cytokine Profiling in MPP Patients is Age-Specific and Severity-Dependent

This study detected a total of 50 cytokines in serum specimen from both healthy children and those with MPP. A heatmap combined with cluster analysis was utilized to display the comprehensive serum cytokine profiles in children classified into healthy, GMPP and SMPP (Supplementary Figure 1). However, the data reveals considerable variability in cytokine levels within each group, with particularly pronounced differences in SMPP. Given the fact that a single perspective

Table 1 Clinical Characteristics of MPP Patients with Serum Cytokine Detection

	0-2Y (n=17) (12 GMPP, 5 SMPP)	3-5Y (n=24) (12 GMPP, 12 SMPP)	6-12Y (n=46) (23 GMPP, 23 SMPP)	p-value
Duration of fever, Median (Q1, Q3)	4 (2.5–8.5)[b, c]	8 (6–11)[a]	8(6–11)[a]	0.0068**
Hospitalization days, Median (Q1, Q3)	7 (5.5–9)	6.5 (5.25–8)	7 (6–9)	0.8523
Pulmonary complications, n(%)				
Hypoxemia	3 (17.65)	8 (33.33)	6 (13.04)	0.1245
Atelectasis	10 (58.82)	12 (50.00)	22 (47.83)	0.7761
Consolidation	9 (35.29)	17 (70.83)	34 (73.91)	0.2886
Pleural effusion	4 (16.67)	10 (41.67)	23 (50.00)	0.1894
Lung necrosis	0 (0)	0 (0)	1 (2.17)	0.9999
Extrapulmonary complications, n(%)				
Digestive system	4 (16.67)	8 (33.33)	10 (21.74)	0.638
Toxic encephalopathy	0 (0)	0 (0)	0 (0)	0.9999
Skin manifestations	0 (0)	1 (4.17)	5 (10.87)	0.3923
Circulatory system	2 (11.76)	3 (12.50)	7 (15.22)	0.9999
Hematologic system	0 (0)	0 (0)	0 (0)	0.9999
Uninary system	0 (0)	0 (0)	0 (0)	0.9999
Laboratory findings, Median (Q1, Q3)				
WBC (x10E9/L)	8.14 (6.51–11.65)	6.925 (5.75–11.31)	8.02 (6.05–9.87)	0.9212
Neutrophil count (%)	53.1 (45.95–66.15)[c]	63.8 (58.8–77.18)	70.75 (64.23–77.2)[a]	0.0007***
Lymphocyte count (%)	38.6 (25.85–43.15)[b, c]	27.45 (16.63–32.78)[a]	21.15 (14.98–27.6)[a]	0.0002***
RBC (x10E12/L)	4.3 (1.08–4.55)	4.45 (4.16–4.62)	4.39 (4.01–4.66)	0.5781
HGB (g/L)	117 (115–126.5)[c]	126 (116.3–129)	125.5 (117.8–133.3)[a]	0.0384*
PLT (x10E9/L)	376 (262.5–458.5)	348.5 (271.3–416.8)	311.5 (225–381.8)	0.1772
CRP (mg/L)	8.9 (5.33–19.16)	17.82 (6.54–22.67)	15.86 (9.71–37.11)	0.1435
PCT (ng/mL)	0.08 (0.035–0.178)	0.118 (0.071–0.179)	0.111 (0.064–0.2)	0.2099
ALB (g/L)	41.9 (41.55–45.55)[c]	41.8 (39–44.18)[c]	39.85 (37.55–42.23)[a, b]	0.0038**
ALT (U/L)	17 (12.5–30)	14.5 (11.25–24)	15 (11–24.25)	0.5602
AST (U/L)	40 (35–56.5)[c]	33 (27–40.75)	29 (24.75–47.5)[a]	0.038*
LDH (U/L)	350 (312.5–586)	328 (281.8–466.3)	389 (281.3–527.5)	0.7579
Urea (mmol/L)	3.52 (3.3–4.09)	4.15 (3.13–4.50)	4.285 (3.43–5.09)	0.134
D-D (mg/L)	0.47 (0.31–0.59)	0.61 (0.39–1.22)	0.73 (0.43–2.23)	0.1215

Notes: Data are presented as median (IQR, 25th-75th percentiles) for continuous variables with non-normal distribution, and n (%) for categorical variables. The Kruskal–Wallis test was used for non-normally distributed variables. Tukey's multiple comparisons test was further applied for post hoc analysis to pinpoint specific group differences. Chi-square test was performed for classified variables. *P < 0.05; **P < 0.01; ***P < 0.001. [a]represents P<0.05 compared to group 0–2Y. [b]represents P<0.05 compared to group 3–5Y. [c]represents P<0.05 compared to group 6–12Y.

Abbreviations: ALB, albumin; ALT, alanine transferase; AST, aspartate transferase; CRP, C-reactive protein; D-D, d-dimer; HGB, hemoglobin; LDH, lactate dehydrogenase; PCT, procalcitonin; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

cannot adequately characterize the immune patterns underlying MPP in children across different age stages, we further analyzed the cytokine profiles for each age group separately.

MPP Group A (0–2 Years)

Compared to the other two groups, 9 cytokines were significantly elevated in SMPP, including CHI3L1, IL-15, CD40, S100A12, MMP9, CXCL11, S100A8, G-CSF, and CCL20 (Figure 1A–I). Notably, S100A8 (P=0.0454), G-CSF (P=0.0969), and CCL20 (P=0.4183) in GMPP were also much higher than that in the control group. KEGG pathway enrichment analysis revealed that these cytokines are predominantly linked to the IL-17 signaling pathway, Toll-like receptor (TLR) and tumor necrosis factor (TNF) signaling pathway (Figure 1J). Intriguingly, levels of IL-12P70 and IL-22 were both reduced in GMPP relative to healthy controls, but reverted to normal levels in SMPP (Figure 1K and L).

MPP Group B (3–5 Years)

In SMPP, 13 cytokines (CXCL10, CCL11, IL-6, CXCL9, TNF-α, TSLP, IL-2, CD40L, S100A12, IFN-γ, CCL2, TNFR2 and CFD) were markedly increased in comparison with the healthy controls and GMPP (Figure 2A–M). Among these, S100A12 (P=0.1424), IFN-γ (P=0.2491), CCL2 (P=0.6773), TNFR2 (P=0.3972), and CFD (P=0.9043) also exhibited modest rises in GMPP, although these differences did not reach statistical significance (Figure 2I–M). KEGG analysis showed that these cytokines were primarily associated with the IL-17 signaling pathway, compared to innate immune pathways *eg* the TLR and TNF signaling pathway (Figure 2N). The combined elevation of cytokines such as CCL11, IL-6, TSLP, S100A12, CCL2, TNFR2, and CFD points to a gradual emergence of humoral immunity, as seen in both GMPP and SMPP.

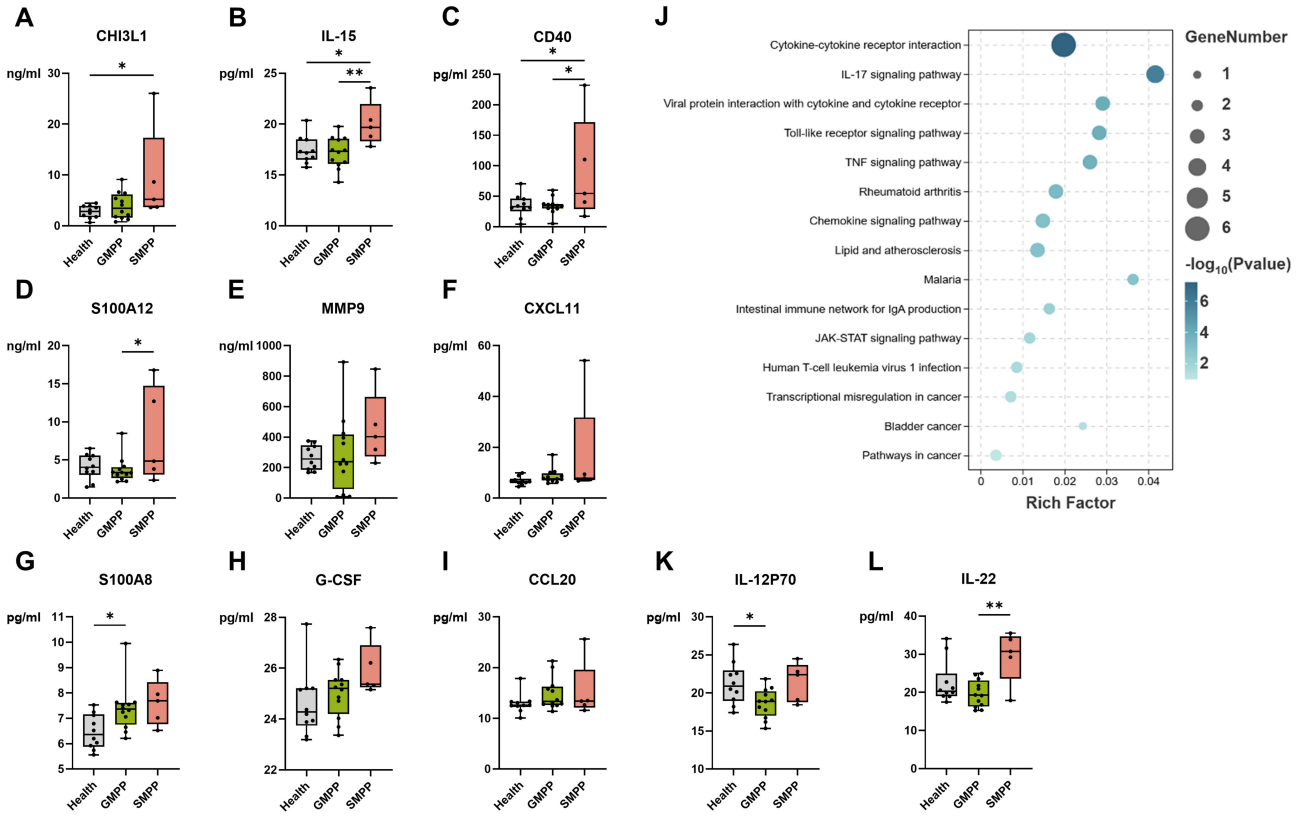

Figure 1 Serum cytokine profiling in 0–2 age group. (**A**) CHI3L1; (**B**) IL-15; (**C**) CD40; (**D**) S100A12; (**E**) MMP9; (**F**) CXCL11; (**G**) S100A8; (**H**) G-CSF; (**I**) CCL20; (**K**) IL-12P70; (**L**) IL-22. The healthy group (Health), GMPP and SMPP are indicated in gray, green, dark respectively. Median and quartiles were displayed using a box plot. Statistical analyses were calculated using a one-way ANOVA or Kruskal–Wallis test with Tukey's posttest. *P < 0.05; **P < 0.01. (**J**) KEGG analysis of cytokines upregulated in MPP groups compared to healthy controls.

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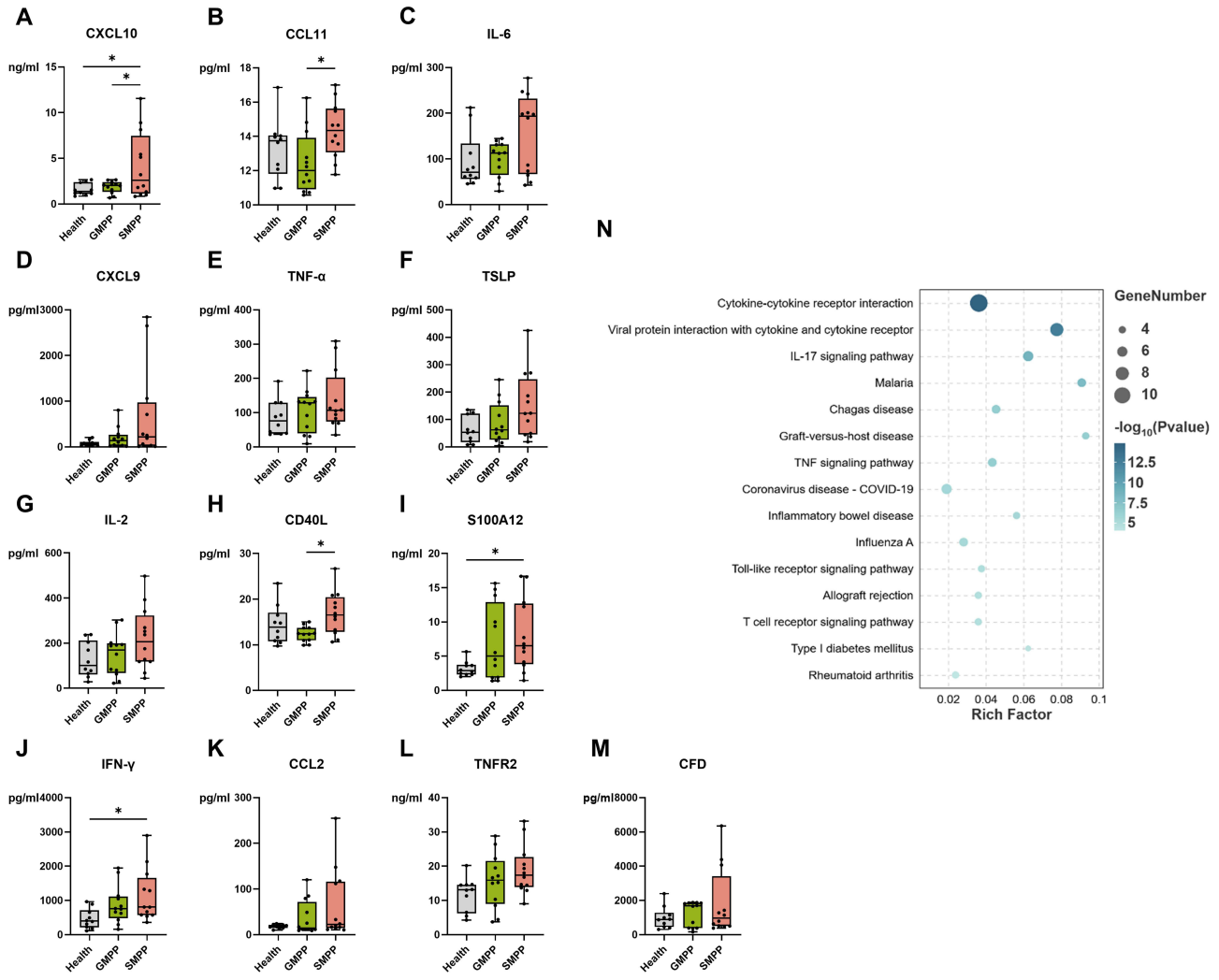

Figure 2 Serum cytokine profiling in 3–5 age group. (**A**) CXCL10; (**B**) CCL11; (**C**) IL-6; (**D**) CXCL9; (**E**) TNF-α; (**F**) TSLP; (**G**) IL-2; (**H**) CD40L; (**I**) S100A12; (**J**) IFN-γ; (**K**) CCL2; (**L**) TNFR2; (**M**) CFD. The healthy group (Health), GMPP and SMPP are indicated in gray, green, dark respectively. Median and quartiles were displayed using a box plot. Statistical analyses were calculated using a one-way ANOVA or Kruskal–Wallis test with Tukey's posttest. *P < 0.05. (**N**) KEGG analysis of cytokines upregulated in MPP groups compared to healthy controls.

MPP Group C (6–12 Years)

Unexpectedly, 9 cytokines were significantly downregulated in SMPP compared to the other two groups, involving IL-2, PDGF-BB, CCL13, CCL7, IFN-β, IL-12P70, IL-1RA, IL-10, and CD40L (Figure 3A–I). KEGG analysis indicated that these cytokines were principally enriched in the JAK-STAT pathway (Figure 3J). In contrast, 4 cytokines, TNFR2 (P=0.0257), CXCL9 (P=0.2853), CHI3L1 (P=0.6436) and MMP9 (P=0.2306) were elevated in GMPP compared to healthy controls, with further increases observed in SMPP [TNFR2 (P<0.0001), CXCL9 (P=0.0079), CHI3L1 (P=0.2173), MMP9 (P=0.0008)] (Figure 3K–N). The downregulation of the JAK-STAT pathway (IL-10) and upregulation of TNF (TNFR2) and NF-kB (CHI3L1, MMP9) signaling pathways suggest that children with MPP in this age group are in a pro-inflammatory and pro-apoptotic state, which correlates with disease severity.

BALF Cytokine Profiling in MPP Patients is Age-Specific

To gain an in-depth understanding of local pulmonary immune responses, BALF samples were obtained from SMPP of three age groups (0–2, 3–5, and 6–12 years). Cytokine concentrations detected in BALF were adjusted to amount in case of the dilution effect of saline administered during bronchoscopy, as indicated in Methods. This adjustment facilitates comparison among children of different ages and weights.

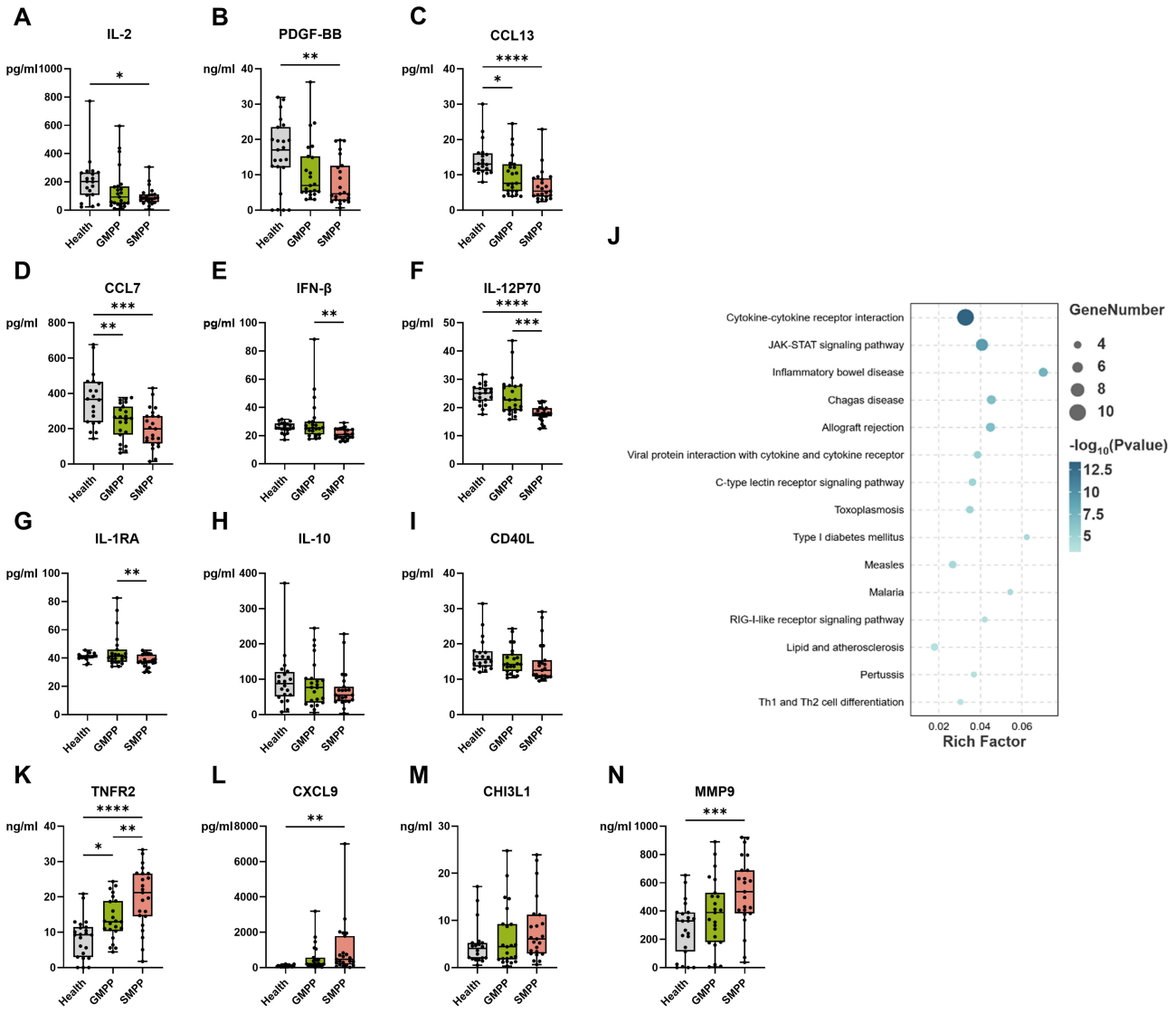

Figure 3 Serum cytokine profiling in 6–12 age group. (**A**) IL-2; (**B**) PDGF-BB; (**C**) CCL13; (**D**) CCL7; (**E**) IFN-β; (**F**) IL-12P70; (**G**) IL-1RA; (**H**) IL-10; (**I**) CD40L; (**K**) TNFR2; (**L**) CXCL9; (**M**) CHI3L1; (**N**) MMP9. The healthy group (Health), GMPP and SMPP are indicated in gray, green, dark respectively. Median and quartiles were displayed using a box plot. Statistical analyses were calculated using a one-way ANOVA or Kruskal–Wallis test with Tukey's posttest. *P < 0.05; **P < 0.01; ***P < 0.001; ****, P < 0.0001. (**J**) KEGG analysis of cytokines downregulated in MPP groups compared to healthy controls.

When compared to the 0–2 years group, the 3–5 and 6–12 years groups showed significantly lower levels of 13 cytokines, with no significant difference between the latter two groups. These cytokines include GMB, CCL5, IL-1RA, CD40L, CD40, CCL13, IFN-α, IL-2, IL-1α, PDGF-BB, IL-17A, CD274 and IL-4 (Figure 4A–M). KEGG analysis revealed that the activity levels of TLR, RIG-I-like receptor (RLR), and Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathways in local lung lesions were significantly diminished in SMPP of 3–5 and 6–12 years groups compared to the 0–2 years group (Figure 4N). Whereas, IFN-β (P= 0.1971), IL-15 (P= 0.0345) and IL-22 (P= 0.2979) levels were significantly higher in the 3-5-year group compared to the 0–2 year group, with further increase in the 6–12-year group [IFN-β (P= 0.0275), IL-15 (P= 0.0001), IL-22 (P= 0.0209)] (Figure 4O–Q).

Correlation of BALF and Serum Cytokine Profiling

Most cytokines showing significant age-specific changes in BALF had their differences diluted in the serum of corresponding severe patients (Figure 5A–L), except for CCL13, IL-2 and IL-1α, which remained lower in the 6–12 age group compared to the 0–2 [CCL13 (P=0.1044), IL-2 CCL13 (P=0.2705), IL-1α CCL13 (P=0.9373)] and 3–5 age

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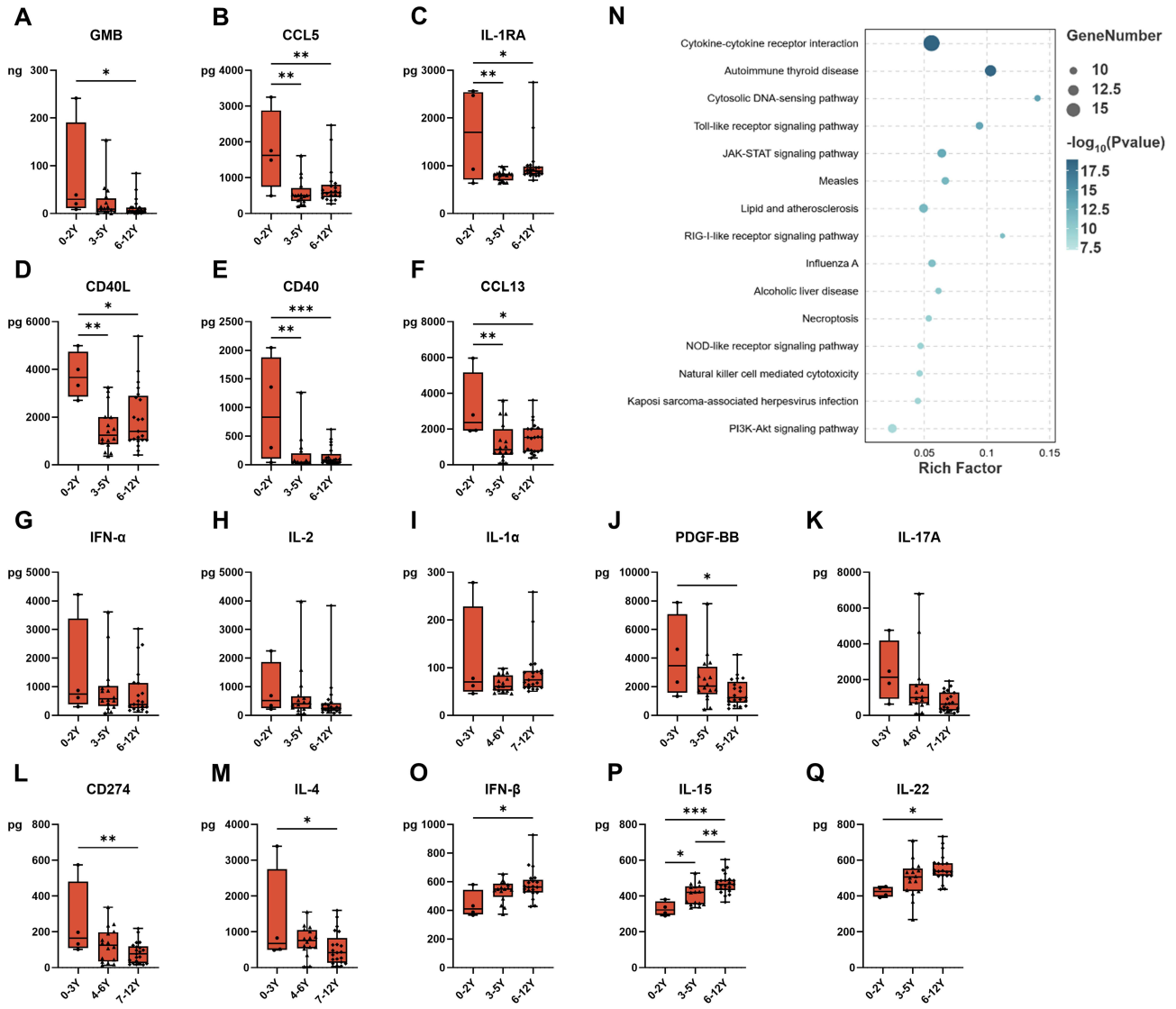

Figure 4 BALF cytokine profiling in SMPP across three age groups. (**A**) GMB; (**B**) CCL5; (**C**) IL-1RA; (**D**) CD40L; (**E**) CD40; (**F**) CCL13; (**G**) IFN-α; (**H**) IL-2; (**I**) IL-1α; (**J**) PDGF-BB; (**K**) IL-17A; (**L**) CD274; (**M**) IL-4; (**O**) IFN-β; (**P**) IL-15; (**Q**) IL-22; (**K**). SMPP of three age groups are marked with different symbols: ● represents 0–2 years, ▲ represents 3–5 years. ♦ represents 6–12 years. Median and quartiles were displayed using a box plot. Statistical analyses were calculated using a one-way ANOVA or Kruskal–Wallis test with Tukey's posttest. *P < 0.05; **P < 0.01; ***P < 0.001. (**N**) KEGG analysis of cytokines downregulated in older age groups compared to 0-2-year group.

groups [CCL13 (P=0.0003), IL-2 CCL13 (P=0.0041), IL-1α CCL13 (P=0.0201)] (Figure 5M–O). Additionally, IFN-β and IL-12P70 also showed a similar declining trend in the serum (Figure 5P and Q). Of the five cytokines, IL-2, IFN-β and IL-12P70 promote the differentiation and activation of T cells and NK cells via the JAK-STAT pathway. IL-1α activates $CD4^+$ T cells and promotes the growth and differentiation of B cells. This collection of cytokines may exhibit a deficiency in Th1/Th2-mediated cellular and humoral immune responses in older patients.

To further examine whether systemic and local airway immune responses are coordinated in SMPP, we performed Spearman correlation analyses between paired serum and BALF cytokine levels from the same patients across different age groups. Notably, the majority of cytokines showed no significant correlation between serum and BALF compartments. In the 0–2 years and 3–5 years age groups, none of the 50 cytokines demonstrated significant serum-BALF correlations. In the 6–12 years age group, only CCL13 showed a modest positive correlation between serum concentration and BALF total amount (r=0.4610, P=0.0354, Supplementary Figure 2), while all other cytokines remained

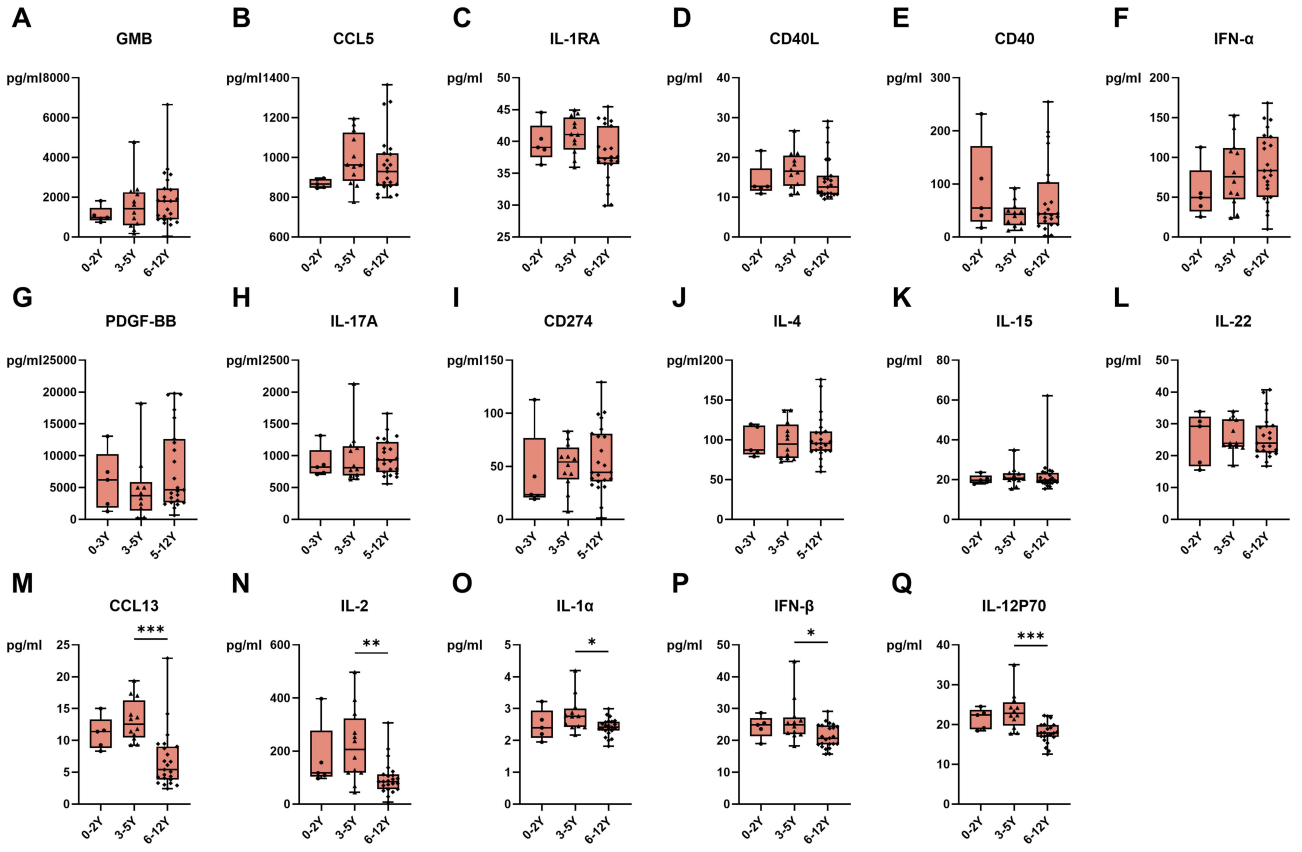

Figure 5 Serum cytokine profiling in SMPP across three age groups. (**A**) GMB; (**B**) CCL5; (**C**) IL-1RA; (**D**) CD40L; (**E**) CD40; (**F**) IFN-α; (**G**) PDGF-BB; (**H**) IL-17A; (**I**) CD274; (**J**) IL-4; (**K**) IL-15; (**L**) IL-22; (**M**) CCL13; (**N**) IL-2; (**O**) IL-1α; (**P**) IFN-β; (**Q**) IL-12P70. SMPP of three age groups are marked with different symbols: ● represents 0–2 years, ▲ represents 3–5 years. ♦ represents 6–12 years. Median and quartiles were displayed using a box plot. Statistical analyses were calculated using a one-way ANOVA or Kruskal–Wallis test with Tukey's posttest. *P < 0.05; **P < 0.01; ***P < 0.001.

uncorrelated. These findings indicate that serum and BALF cytokine profiles largely represent distinct immunological compartments in SMPP, with limited cross-correlation between systemic and local airway immune responses.

On the whole, the age-specific immune patterns observed in the serum of SMPP align with those reflected in BALF, except that serum levels in the 3-5-year group were similar to the 0-2-year group, whereas local lung levels were akin to the 6–12-year group. However, the lack of direct correlation between paired serum and BALF cytokine measurements suggests that systemic and local immune responses are largely compartmentalized rather than simply reflecting each other.

Distribution of SARS-CoV-2 IgG Level in MPP Children

We collected data on all hospitalized children diagnosed with MPP at our hospital from January to February 2024, excluding other infections, resulting in a cohort of 206 cases, together with 126 healthy children as controls. Baseline characteristics of this larger cohort were reported in Supplementary Table 3. Serum samples from these children were tested for SARS-CoV-2 IgG and IgM antibodies. The tests revealed that all children were negative for IgM, indicating the absence of acute SARS-CoV-2 infection. Notably, the incidence and severity of MPP are not influenced by SARS-CoV-2 IgG (Figure 6A and B), although IgG distribution in children is strongly age-related (Figure 6C).

Correlation Between SARS-CoV-2 IgG and Serum Cytokine Profiles is Age-Specific

To give an insight into the potential correlation between SARS-CoV-2 IgG levels and cytokine profiles in children with MPP, the cohort from Table 1—comprising participants whose serum cytokines were detected—was reassigned into IgG-

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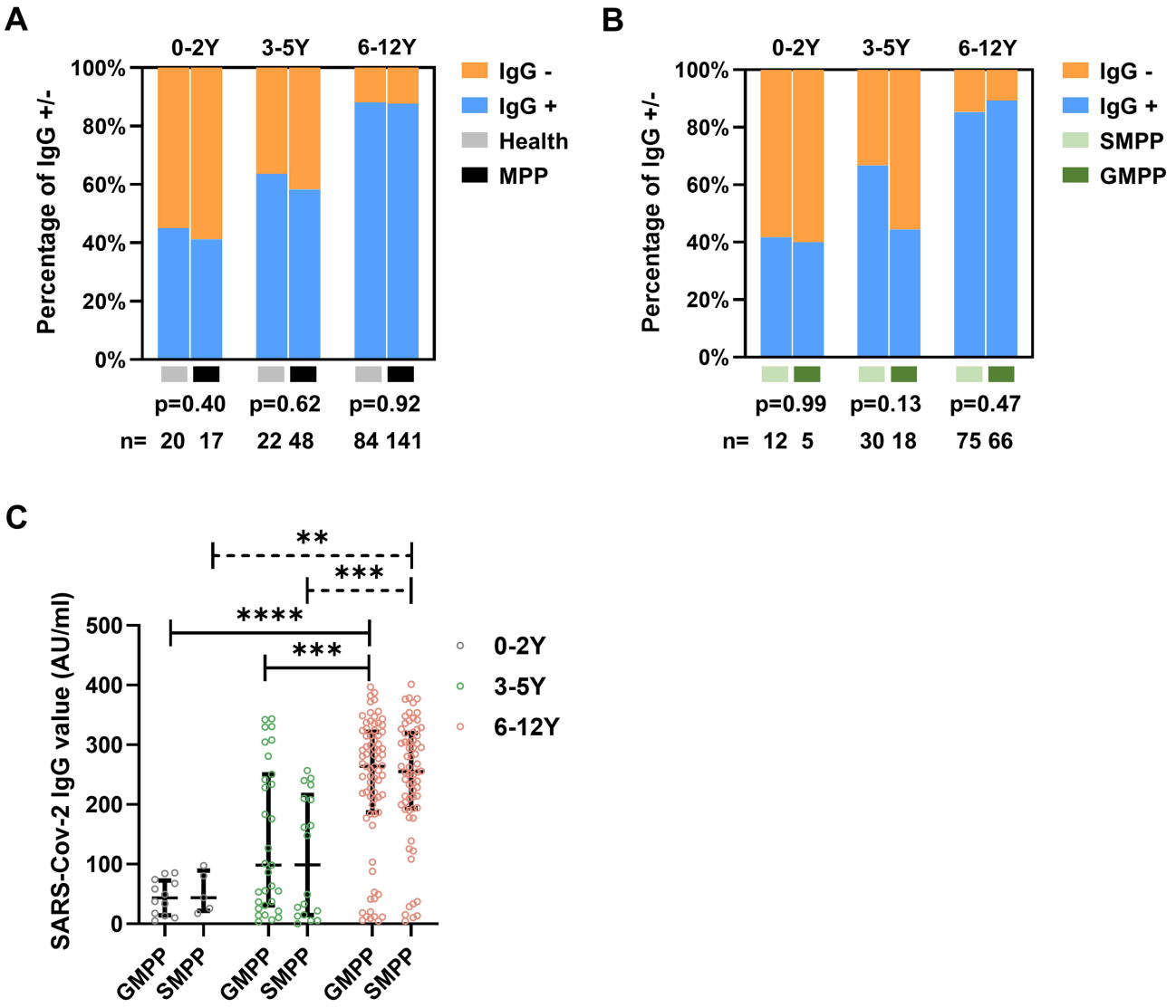

Figure 6 Distribution of SARS-CoV-2 IgG level in MPP Children. Distribution of SARS-CoV-2 IgG positivity between healthy children and MPP patients (**A**), and between GMPP and SMPP (**B**) was compared respectively across different age groups. Categorical variables were analyzed using the Chi-square test or Fisher's exact test. (**C**) Distribution of SARS-CoV-2 IgG level in GMPP and SMPP across three age groups. Statistical analyses were calculated using a two-way ANOVA with Tukey's posttest. Significant differences ($p < 0.05$) were indicated with solid line (to GMPP aged 6–12 years) or dotted line (to SMPP aged 6–12 years), **P < 0.01; ***P < 0.001; ****P < 0.0001.

positive and IgG-negative groups based on their serum SARS-CoV-2 IgG levels. The demographic characteristics of the reassigned cohorts are presented in Supplementary Table 4.

In the 0–2 age group, 8 cytokines [IL-16 (P=0.4877), TWEAK (P=0.2480), IL-12p40 (P=0.4148), CD152 (P=0.0314), PDGF-BB (P=0.8108), S100A12 (P=0.6994), IL-22 (P=0.6746), CCL7 (P=0.0540)] displayed higher levels in the IgG-positive group than in the IgG-negative group (Figure 7A–H), most of which activate inflammatory pathways such as JAK-STAT, NF-κB, and MAPK. Inversely, in the 6–12 age group, IL-7 (P<0.0001) and CCL-13 (P=0.0005) levels were notably lower in the IgG-positive group, suggesting a potential immune suppression related to SARS-CoV-2 IgG (Figure 7I and J). Moreover, CD152, which negatively regulates T cell activity, was elevated in the IgG-positive group also in the 6–12 age group (P=0.1543) (Figure 7D), while CHI3CL1, an apoptosis inhibitor, was slightly higher in IgG-positive children across all age groups (0–2Y: P=0.8307; 3–5Y: P=0.8381; 6–12Y: P=0.4255) (Figure 7K). Nevertheless, no significant cytokine level differences were observed in the 3–5 age group.

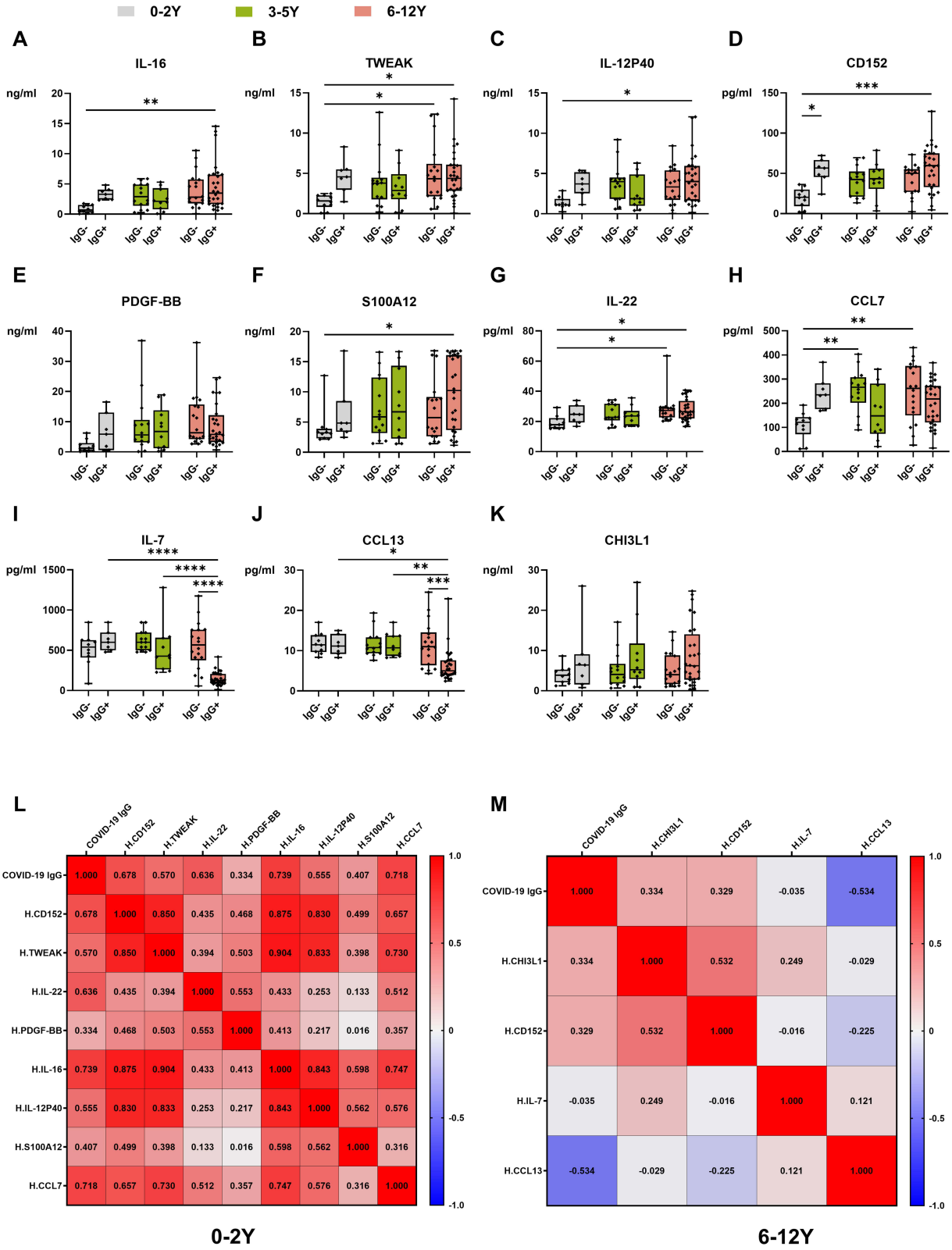

Figure 7 Correlation between SARS-CoV-2 IgG and serum cytokine profiles in MPP patients. The level of serum cytokines in children with MPP: (**A**) IL-16; (**B**) TWEAK; (**C**) IL-12p40; (**D**) CD152; (**E**) PDGF-BB; (**F**) S100A12; (**G**) IL-22; (**H**) CCL7; (**I**) IL-7; (**J**) CCL13; (**K**) CHI3L1. MPP patients were divided into SARS-Cov-2 IgG-negative and IgG-positive groups with different colors (grey: 0–2Y; green: 3–5Y; red: 6–12Y). Median and quartiles are displayed using a box plot. Statistical analyses were calculated using a two-way ANOVA or Kruskal–Wallis test with Tukey's posttest. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. SARS-Cov-2 IgG and serum cytokines correlation heatmap from children with MPP of different age group: (**L**) 0–2Y; (**M**) 6–12Y. The bar represents the range of parameter correlations, ranging from −1.0 (dark blue) to 1.0 (red).

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Spearman correlation analysis showed that in the 0–2 age group, SARS-CoV-2 IgG positively correlated with TWEAK (P=0.017), IL-22 (P=0.006), IL-16 (P=0.001), IL-12p40 (P=0.021), CCL7 (P=0.001), and CD152 (P=0.003). Remarkably, CD152, CCL7, IL-12p40, IL-16, and TWEAK demonstrated strong intercorrelations ($p<0.05$), with each crucial for T cells, monocytes, and macrophages activation (Figure 7L). In the 6–12 age group, SARS-CoV-2 IgG exhibited a pronounced negative correlation with CCL13 ($p<0.001$), while showing slightly positive correlations with CHI3CL1 (P=0.023) and CD152 (P=0.026) (Figure 7M).

Discussion

In this study, our findings uncover that the age-specific immune characteristics against MP in children. Specifically, patients aged 0–2 years primarily involve innate immunity, focusing on the TLR, RLR, and TNF pathways. Abundant studies have highlighted the role of the TLR signaling pathway in MP-induced lung disease, indicating that MP infection can activate TLR2 and TLR4 in host cells, leading to chronic airway inflammation and damage.[17,18] Unlike the broad role of TLR signaling in inflammation and anti-pathogen immunity, RLRs play a critical role in antiviral innate immunity,[19,20] while the TNF pathway is crucial for regulating apoptosis.[21] Concurrently, the IL-17 signaling pathway also plays a significant role in the immune response of infants and toddlers with MPP. Elevated levels of classic neutrophil-recruiting cytokines like G-CSF and IL-17A were detected in both serum and BALF, with strong signals of IL-17 pathway activity and neutrophil activation also observed in the other two age groups.[22] Consistent results were obtained in another study that conducted microbiome and transcriptome analyses using BALF from children aged 2 to 10 with MPP. These findings underscore that the IL-17 signaling pathway and neutrophils represent a fundamental immune response pattern against MP infection across all ages.

By the ages of 3–5 years, the IL-17 signaling pathway-driven cellular immunity as well as humoral immunity further strengthens. Children aged 3–5 are in a phase of rapid immune system development, albeit not yet fully mature. Interestingly, the immune profile of peripheral serum in this age group resembles that of the 0–2 year group, while the local immune characteristics in lung lesions (BALF) are similar to those in the 6–12 year age group. This divergence reflects compartmentalization of immune responses between systemic circulation and the airway in SMPP. Correlation analysis of paired samples revealed that these two compartments are largely independent, with only CCL13 showing modest correlation in the 6–12 years group. This suggests that local airway inflammation and systemic immune responses are regulated through distinct mechanisms rather than one simply reflecting the spillover of the other. Like the skin and digestive tract, the respiratory tract is in direct contact with the external environment and frequently encounters pathogens, necessitating a swifter local immune response mechanism and potentially faster maturation.[23] This compartmentalization underscores the importance of direct airway sampling to accurately assess the pulmonary immune microenvironment, as serum measurements alone cannot capture local immune dysregulation, and emphasizes the need for compartment-specific therapeutic strategies in severe MPP.

For school-aged patients (6–12 years), however, cytokine profiling reveals a highly depressed JAK-STAT signaling pathway and T cell-related immune function, especially in SMPP. Type I interferons (IFN-α/β) and Th1-associated cytokines such as IL-1α, IL-2 and IL-12p70, as well as chemokine CCL13 are markedly suppressed. Surprisingly, IL-1RA, an immunosuppressive factor that exerts anti-inflammatory effects by inhibiting T cell activity,[24] is also significantly reduced. Chen et al collected BALF from children with SMPP during the acute phase and, through T cell sorting and mRNA microarray, found high expression of the apoptosis pathway based on differentially expressed genes (DEGs) from sorted T cells, suggesting that MP infection induces excessive activation, exhaustion, and near-apoptosis of T cells due to the release of pro-inflammatory cytokines and chemokines in the respiratory tract.[25] This finding perfectly aligns with our study results. It is worth pondering why such immune exhaustion appears only in older MPP patients. One hypothesis is that it may be related to a high MP load, as studies have found that levels of pro-inflammatory cytokines like IL-6 and TNF-α, are higher in the high MP load group compared to the low load group in BALF.[26] Furthermore, CCL13 is significantly downregulated in SMPP, which directly reducing the recruitment of monocytes, macrophages, and T cells to the infection site, thereby impairing the body's ability to clear the pathogen.[27]

The correlation between SARS-CoV-2 IgG and cytokines is intricate, yet distinct features emerge across different age groups. In the 0–2 age group, macrophage-related (TWEAK, CCL7) and T cell-related (IL-22, IL-16, IL-12p40, CD152)

cytokines exhibit significant positive correlations with SARS-CoV-2 IgG. As children age, these cytokine' levels are consistent with those seen in the IgG-positive in 0–2 age group, suggesting COVID-19 may accelerate their expression and the corresponding immune maturation in early childhood. While the 3–5 age group lacks significant correlations, their highly expressed cytokines are enriched in the Coronavirus disease-COVID-19 pathway. This pathway overlap may account for the marginally higher mild case rate in the IgG-positive group (p=0.13). Contrastingly, in the 6–12 age group, SARS-CoV-2 IgG levels positively with CHI3CL1 and CD152 but significantly negatively correlate with CCL13. Notably, this group also demonstrates the highest IgG positivity rate and SARS-CoV-2 IgG levels across all ages. The previously observed significant reduction of CCL13 in 6–12-year-old SMPP patients further complicates this immunological landscape, hinting that SARS-CoV-2 IgG may impact the immune response in school-aged children with SMPP via the modulation of CCL13 levels.

Our results unveil that in younger children, higher levels of SARS-CoV-2 IgG are associated with a more robust and mature anti-infective immune response. In contrast, for older children, besides providing immune memory against the pathogens, SARS-CoV-2 IgG also appears to plant a landmine of immune exhaustion. The age-specific negative immune impact of COVID-19 may stem from the rapid development of adaptive immunity in older children. As their immune system becoming more active and complex, sometimes may lead to heightened immune responses, excessive inflammation, or immune exhaustion.[28,29] An alternative explanation posits that post-COVID-19 epigenetic reprogramming may account for these observations. Cheong et al, found that changes in the epigenetic landscape of hematopoietic stem and progenitor cells (HSPCs) persisted for months to a year post-severe COVID-19, with elevated cytokine and adhesion molecule gene accessibility.[11] Notably, trained immunity—whereby the innate immune system is conditioned by previous infections or vaccinations to enhance future immune responses[30,31]—works through two main pillars: epigenetic and metabolic reprogramming of cells.[32] A hypothesis suggests that since most vaccinations occur in early childhood, trained immunity mechanisms may help suppress COVID-19 virus replication more effectively in younger children, thereby reducing the immune reprogramming effects of the virus in this group.[32,33] Alternatively, the higher SARS-CoV-2 IgG positivity and concentration in older children might result in exaggerated trained immunity responses,[30] exacerbating the pathogenesis of MPP. Further research is needed to elucidate the precise role of trained immunity in the age-related differences in the clinical spectrum of post-COVID MPP.

This study has several limitations. Primarily, given the retrospective nature our investigation, the sample size is relatively small, particularly in the 0–2 age group (n=5 in SMPP), thereby may impacting the generalizability and statistical power of our findings. Furthermore, a potential selection bias exists in bronchoscopy data. Clinicians are more likely to prescribe this invasive procedure for patients with severe presentations, which led to our focus on comparing cytokine profiles between serum and BALF in SMPP patients. This limited our ability to comprehensively evaluate the diagnostic value of systemic and pulmonary cytokine profiles across the full spectrum of MPP severity, including GMPP cases. Additionally, BALF acquisition during bronchoscopy is subject to considerable procedural variability. Factors such as the volume of saline used or the specific lavage site can significantly influence the detected concentration of cytokine in BALF. To address this and gain deeper molecular insights, future studies could employ single-cell RNA sequencing (scRNA-seq) or assay for transposase-accessible chromatin sequencing (ATAC-seq) on BALF-derived cells. These advanced techniques could provide a more granular and precise understanding of the cellular and molecular dynamics at play in the pulmonary microenvironment of MPP patients.

Our findings carry important implications for optimizing clinical management and guiding future translational research in pediatric MPP. The distinct age-specific immune profiles suggest that therapeutic strategies should be tailored to developmental stage: younger children with IL-17-driven neutrophilic inflammation may benefit from anti-inflammatory interventions, whereas school-aged children demonstrating immune exhaustion and CCL13 suppression may paradoxically require immune-supportive approaches. These cytokine signatures hold promise as prognostic biomarkers enabling early SMPP risk identification. Critically, the age-dependent associations between SARS-CoV-2 IgG and immune dysregulation underscore an emerging post-pandemic paradigm: prior viral exposure, even if subclinical, can fundamentally reshape subsequent immune responses in developmentally specific ways.

To advance this field, several fundamental questions warrant investigation. First, what are the molecular mechanisms underlying CCL13 suppression in school-aged patients with high SARS-CoV-2 IgG levels, and can targeted CCL13

restoration reverse immune exhaustion? Second, what are the underlying mechanisms by which prior SARS-CoV-2 exposure differentially modulates immune responses across distinct developmental stages? Can single-cell multiomics (scRNA-seq, ATAC-seq) identify epigenetic modifications and specific immune cell subpopulations that distinguish trained immunity phenotypes in younger children from exhaustion phenotypes in older children? Third, what mechanisms underlie the extrapulmonary manifestations observed in SMPP patients? As shown in Table 1, circulatory system complications represent a significant proportion of extrapulmonary involvement. Emerging evidence suggests that cytokine storm syndromes share common pathophysiology involving endothelial activation and vascular leak.[34] Identifying endothelial biomarkers (*eg*, angiopoietin-2, soluble thrombomodulin) alongside cytokine profiles may enhance SMPP risk prediction and guide vascular-protective interventions. Addressing these questions will establish a precision medicine framework for pediatric respiratory infections in the post-pandemic era.

Conclusions

Overall, the cytokine profile analysis of serum and BALF indicates distinct immunological response patterns across infants and toddlers (0–2 years), preschoolers (3–5 years), and school-aged children (6–12 years). The balance between innate and adaptive immunity, the hierarchy of signaling pathways, and the activation levels of immune cells exhibit clear age-specific characteristics. Furthermore, despite the COVID-19 pandemic having ended a year ago, children still possess high levels of SARS-CoV-2 IgG antibodies. These antibody levels are correlated with the immune patterns of children with MPP, showing significant age-specific associations. Understanding these differences aids pediatricians in the post-COVID era to select appropriate immunomodulatory treatments, precisely control inflammatory responses, and improve patient outcomes.

Data Sharing Statement

The data sets used and/or analyzed in this study are available from the corresponding author, Zhimin Chen, upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Children's Hospital, Zhejiang University School of Medicine (No. 2024-IRB-0171-P-01) and was conducted in accordance with the Declaration of Helsinki. Given the retrospective nature of this study using residual clinical samples, the Ethics Committee waived the requirement for individual informed consent. All samples were originally collected during routine clinical care and stored according to institutional biobank protocols. The confidentiality of patient information was protected, and individual identifiers were removed and replaced with serial code numbers.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All the authors declare no potential conflicts of interest related to this work.

References

1. Sun Y, Li P, Jin R, et al. Characterizing the epidemiology of mycoplasma pneumoniae infections in China in 2022-2024: a nationwide cross-sectional study of over 1.6 million cases. *Emerg Microbes Infect*. 2025;14(1):2482703. doi:10.1080/22221751.2025.2482703
2. Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. *N Engl J Med*. 2015;372(9):835–845. doi:10.1056/NEJMoa1405870
3. Narita M. Pathogenesis of extrapulmonary manifestations of mycoplasma pneumoniae infection with special reference to pneumonia. *J Infect Chemother*. 2010;16(3):162–169. doi:10.1007/s10156-010-0044-x
4. Zhang L, Lai M, Ai T, et al. Analysis of mycoplasma pneumoniae infection among children with respiratory tract infections in hospital in Chengdu from 2014 to 2020. *Transl Pediatr*. 2021;10(4):990–997. doi:10.21037/tp-21-139
5. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. *Arch Dis Child*. 2021;106(5):429–439. doi:10.1136/archdischild-2020-320338
6. Gadsby NJ, Reynolds AJ, McMenamin J, et al. Increased reports of mycoplasma pneumoniae from laboratories in Scotland in 2010 and 2011 - impact of the epidemic in infants. *Euro Surveill*. 2012;17(10). doi:10.2807/ese.17.10.20110-en
7. Lu Y, Wang Y, Hao C, et al. Clinical characteristics of pneumonia caused by mycoplasma pneumoniae in children of different ages. *Int J Clin Exp Pathol*. 2018;11(2):855–861.
8. Li H, Li S, Yang H, Chen Z, Zhou Z. Resurgence of mycoplasma pneumonia by macrolide-resistant epidemic clones in China. *Lancet Microbe*. 2024;5(6):e515. doi:10.1016/s2666-5247(23)00405-6
9. Meyer Sauteur PM, Beeton ML, Pereyre S, et al. Mycoplasma pneumoniae: delayed re-emergence after COVID-19 pandemic restrictions. *Lancet Microbe*. 2024;5(2):e100–e1. doi:10.1016/s2666-5247(23)00344-0
10. Edens C, Clopper BR, DeVies J, et al. Notes from the field: reemergence of mycoplasma pneumoniae infections in children and adolescents after the COVID-19 Pandemic, United States, 2018-2024. *MMWR Morb Mortal Wkly Rep*. 2024;73(7):149–151. doi:10.15585/mmwr.mm7307a3
11. Cheong J-G, Ravishankar A, Sharma S, et al. Epigenetic memory of coronavirus infection in innate immune cells and their progenitors. *Cell*. 2023;186(18):3882–902.e24. doi:10.1016/j.cell.2023.07.019
12. Al-Aly Z, Xie Y, Bowe B. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature*. 2021;594(7862):259–264. doi:10.1038/s41586-021-03553-9
13. Phetsouphanh C, Darley DR, Wilson DB, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. *Nat Immunol*. 2022;23(2):210–216. doi:10.1038/s41590-021-01113-x
14. Schultheiß C, Willscher E, Paschold L, et al. The IL-1β, IL-6, and TNF cytokine triad is associated with post-acute sequelae of COVID-19. *Cell Rep Med*. 2022;3(6):100663. doi:10.1016/j.xcrm.2022.100663
15. Zhong H, Yin R, Zhao R, Jiang K, Sun C, Dong X. Analysis of clinical characteristics and risk factors of plastic bronchitis in children with mycoplasma pneumoniae pneumonia. *Front Pediatr*. 2021;9. doi:10.3389/fped.2021.735093
16. Mu H, Chen J, Huang W, et al. OmicShare tools: a zero-code interactive online platform for biological data analysis and visualization. 2024;3:e228. doi:10.1002/imt2.228
17. Arora S, Ahmad S, Irshad R, et al. TLRs in pulmonary diseases. *Life Sci*. 2019;233. doi:10.1016/j.lfs.2019.116671
18. Chen M, Deng H, Zhao Y, et al. Toll-like receptor 2 modulates pulmonary inflammation and TNF-α release mediated by mycoplasma pneumoniae. *Front Cell Infect Microbiol*. 2022:12. doi:10.3389/fcimb.2022.824027
19. Kato H, Takeuchi O, Sato S, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature*. 2006;441(7089):101–105. doi:10.1038/nature04734
20. Wang BX, Fish EN. Global virus outbreaks: interferons as 1st responders. *Semin Immunol*. 2019;43. doi:10.1016/j.smim.2019.101300
21. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell*. 2003;114(2):181–190. doi:10.1016/s0092-8674(03)00521-x
22. Zhu Y, Luo Y, Li L, et al. Immune response plays a role in mycoplasma pneumoniae pneumonia. *Front Immunol*. 2023:14. doi:10.3389/fimmu.2023.1189647
23. Dzidic M, Boix-Amorós A, Selma-Royo M, Mira A, Collado M. Gut microbiota and mucosal immunity in the neonate. *Medical Sciences*. 2018;6(3):56. doi:10.3390/medsci6030056
24. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2017;281(1):8–27. doi:10.1111/imr.12621
25. Chen X, Liu F, Zheng B, et al. Exhausted and apoptotic BALF T Cells in proinflammatory airway milieu at acute phase of severe mycoplasma pneumoniae pneumonia in children. *Front Immunol*. 2022:12. doi:10.3389/fimmu.2021.760488
26. Deng F, Cao H, Liang X, et al. Analysis of cytokine levels, cytological findings, and MP-DNA level in bronchoalveolar lavage fluid of children with mycoplasma pneumoniae pneumonia. *Immun Inflamm Dis*. 2023;11(5). doi:10.1002/iid3.849
27. Li L, Dai F, Wang L, et al. CCL13 and human diseases. *Front Immunol*. 2023:14. doi:10.3389/fimmu.2023.1176639
28. Morhart P, Kehl S, Schuh W, et al. Age-related DIFFERENCES IN IMMUNE REACtions to SARS-CoV-2 spike and nucleocapsid antigens. *Vivo*. 2023;37(1):70–78. doi:10.21873/invivo.13055
29. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc R Soc B*. 2015;282(1821). doi:10.1098/rspb.2014.3085
30. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol*. 2020;20(6):375–388. doi:10.1038/s41577-020-0285-6
31. Kerboua KE. The perplexing question of trained immunity vs adaptive memory in COVID-19. *J Med Virol*. 2020;92(10):1858–1863. doi:10.1002/jmv.26083
32. Fanucchi S, Domínguez-Andrés J, Joosten LAB, Netea MG, Mhlanga MM. The intersection of epigenetics and metabolism in trained immunity. *Immunity*. 2021;54(1):32–43. doi:10.1016/j.immuni.2020.10.011

33. Filippatos F, Tatsi EB, Michos A. Immune response to SARS-CoV-2 in children: a review of the current knowledge. *Pediatr Investig*. 2021;5 (3):217–228. doi:10.1002/ped4.12283
34. Demosthenous C, Evangelidis P, Gatsis A, et al. Endothelial injury following CAR-T cell immunotherapy for hematological malignancies. *Cancers*. 2025;17(17):2876. doi:10.3390/cancers17172876