Gut Pathogens

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A triad of gut dysbiosis, dysregulated immunity, and 'leaky' gut characterize HCMV associated neonatal cholestasis

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Abstract

Background Gut microbiome dysbiosis and related immune dysfunction have been associated with the pathogenesis of Human Cytomegalovirus (HCMV) infection in infants with neonatal cholestasis (NC) as previously reported by us. However, the interaction of a perturbed microbiome, HCMV infection, and dysregulated immunity leading to exacerbation of disease severity has not been investigated so far. In this study, we examined the association of gut microbiome, host inflammatory and homeostatic markers that are likely to govern increased pathogenesis of NC in HCMV infected IgM positive infants (N = 15) compared to IgM negative (N = 15) individuals. Stool samples of HCMV infected infants and age-matched healthy controls (N = 10) were assessed for gut bacteria-derived metabolites like short-chain fatty acids (SCFAs), Lipopolysaccharide (LPS), cytokines and markers of gut barrier integrity. Data were correlated with previously determined gut microbiome composition and frequency of immune cell subsets. Finally, validation of clinical potential was undertaken by principal component analysis (PCA) of integrated data to delineate the spectrum of clinical pathology.

Results Significantly lower levels of SCFAs and elevated fecal levels of soluble inflammatory mediators were observed in IgM positive HCMV infected infants. Further, increased plasma LPS levels and markers of gut permeability, suggestive of microbial translocation due to a 'leaky gut' were observed in HCMV infected IgM positive group. PCA of integrated data revealed clearly disparate profiles representative of IgM positive, IgM negative, and uninfected healthy states.

Conclusion Our results suggest the utility of an integrated approach involving dysregulated microbiome-immune axis for gaining a better understanding of pathogenesis associated with HCMV infection in NC.

Keywords SCFA, LPS, Leaky gut, Gut inflammatory markers, Gut-microbiome-immune axis, Cytomegalovirus, Neonatal Cholestasis

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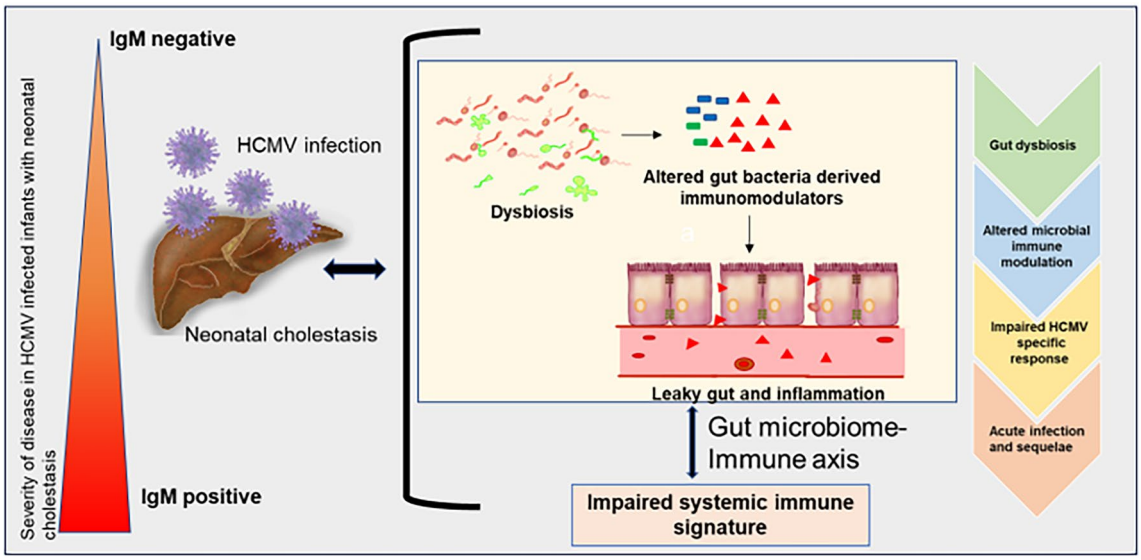
Graphical Abstract



Introduction

The role of gut microbiota in modulation of host immunity and gut barrier function has been well documented. Perturbations or imbalance in the normal composition of gut microbiota, defined as dysbiosis, can lead to immune dysregulation, characterized by elevated pro-inflammatory immune responses, both of which can lead to increased intestinal permeability and subsequently microbial translocation, leading to "Leaky gut syndrome". If not managed, it can further lead to sepsis, systemic inflammation and organ failure [1, 2].

HCMV infection is known to be associated with neonatal cholestasis (NC) [3–7]. Liver pathology is proposed to involve LPS and CD14, where expression of the latter on liver tissues and high levels of plasma endotoxin have been reported in NC [8, 9]. Thus, exposure to systemic LPS would culminate into inflammatory sequelae, including a cascade of pro-inflammatory cytokines, leading to the injury and triggering of cholestasis. Understanding the dysregulated homeostatic mechanisms, by delineating further a perturbed microbiome and loss of gut barrier integrity, would provide potentially actionable targets to improve therapeutic outcomes. Thus, the identification of a broadly applicable therapeutic strategy to ameliorate systemic inflammation associated sequelae may provide a viable adjunct to the current standard of care, which is limited to antiviral therapy and invasive neonatal surgery.

Multiple studies have reported the association between HCMV and neonatal cholestasis [10–13]. In our previous study, we reported a dysbiotic microbiome signature linked with dysregulated immune responses, that was uniquely associated with exacerbated pathology in IgM positive HCMV infected infants with NC [14]. In this study, we attempted to systematically probe the microbiome-immune axis through evaluation of gut microbiota derived metabolites, some of which are known to be potent immunomodulators like SCFAs and LPS. Concurrently, we also evaluated the gut inflammatory milieu (fecal cytokines) together with key regulators of gut epithelial barrier integrity like secretory IgA (sIgA) and zonulin.

Materials and methods

Study design

Recruitment of study participants and clinical workup was done as described in our previous study [14]. Briefly, infants with NC (2–5 months, both males and females) were recruited from Bai Jerbai Wadia Hospital for children (BJWHC) in Mumbai and the HCMV infected group was further stratified as IgM positive (N = 21) and IgM negative (N = 25). Age-matched healthy infants (N = 10) were recruited as HCMV negative controls. The clinical data of the recruited participants was reported in our previous study [14] and is reproduced here (Supplementary Table 1, 2). The assays performed in this study were carried out using samples obtained from N = 15 infants each from IgM positive and IgM negative groups, and N = 10 infants from the healthy group as discussed in the earlier report [14].

Gut microbiome profiling

Gut microbiome profiling was carried out in the similar cohort as described earlier. Briefly, DNA was extracted from the fecal samples and paired-end sequencing of V3-V4 amplicons of 16S rRNA was performed with 1 million reads each in the forward and reverse direction with a read length of 2X300 bp on Illumina Miseq. Microbiome analysis was performed as described in the previous study [14]. In this study, only the relative abundance of the bacterial taxa obtained in the previous study were used for correlation and principal component analysis (PCA).

Phenotypic and functional analysis of cellular immune subsets

Immunophenotyping of T cell subsets (CD4 + T cells, CD8 + T cells, CD4 and CD8 naïve / memory and effector memory), Treg cells, NKT-like cells, and NK cells were carried out by surface staining and granzyme B by intracellular staining on whole blood as described in the previous study[14] and reproduced here (Supplementary Fig. 4). In this study, only the relative frequencies of the immune cell subsets reported in the previous study were used for correlation and principal component analysis (PCA).

Estimation of fecal SCFAs

Fecal SCFAs namely- acetate, butyrate, and propionate were estimated in the fecal samples by high-pressure liquid chromatography (HPLC) by following the protocol published by Torii et al. [15]. The absorbance (mAU) obtained for the SCFAs in the fecal samples was used to estimate their concentration using the standard curve.

Determination of fecal and plasma LPS

Fecal and plasma LPS levels were measured by ELISA using the Mybiosource ELISA kit (MBS018465) by following the manufacturer's instructions for sample preparation.

Determination of fecal zonulin, β-defensin2, calprotectin, and sIgA

Estimation of zonulin (BT Lab- E3704hu), β-defensin2 (BT Lab- E1936hu), and calprotectin (BT Lab- 4010hu) in fecal samples was done by ELISA using the commercial kits from Bioassay technology laboratory by following the manual from the respective kits. Fecal sIgA was measured by ELISA with Ridascreen sIgA kit (G09035-96 T) kit and sample preparation was carried out according to the manufacturer's instructions.

Determination of fecal protease activity

Fecal protease activity was quantified in the samples by employing an ELISA-based method as described in Carrol et al. and Tooth et al. [16, 17].

Determination of fecal cytokines and myeloperoxidase (MPO)

The fecal levels of the cytokines IL-6 (ELK1156), IL-8 (ELK1159) TGF-β (ELK1185), TNF-α (ELK1190), IL-23 (ELK1570) IL-1β (ELK1270) and MPO (ELK1062) were estimated using ELISA kits obtained from ELK Biotechnology and instructions in the kit's manual were followed for sample preparation.

PICRUSt2 analysis

Predictive functional analysis of microbiome communities was performed by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) with the q2-picrust2 plugin [18] and data was represented in terms of relative proportion of predicted gene counts obtained through MetaCyc Metabolic Pathway Database.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.4.1 (GraphPad Software, San Diego, California, USA). The levels of fecal metabolites and relative proportions of predicted gene counts obtained in PICRUSt2 analysis were evaluated for normal distribution by the Shapiro–Wilk test. The significance in the fecal metabolites and relative proportions of predicted gene counts was evaluated by the non-parametric Kruskal–Wallis test with Dunn's multiple comparison test. Receiver operating characteristic (ROC) curve analysis was carried out by the Wilson-Brown method with a 95% confidence interval. Spearman-ranked two-tailed correlation was used to correlate fecal metabolite levels with each other and with the relative abundance of microbial taxa and immune cell frequencies obtained in the previous study [14]. P values less than 0.05 were considered significant. Principal component analysis (PCA) was carried out with the fecal metabolites reported in this study, relative abundance of microbial taxa and relative frequencies of immune cell subsets reported in the previous study[14] and reproduced here (Supplementary Fig. 4). The default parameters were employed for PCA as follows; Method-Standardize, Component selection method-Parallel analysis, Percentile level-95%, Random seed-Auto, Number of simulations-1000.

Results

Fecal microbial product levels reflect an altered low Bifidobacterium: Enterobacteriaceae ratio in HCMV infected infants with NC

After assessing the impact of the observed dysbiosis (Supplementary Fig. 1), major gut bacteria-derived short-chain fatty acids namely acetate, butyrate, and propionate were quantified in the fecal samples of healthy and HCMV infected groups. We observed that fecal acetate levels were the highest in the healthy group 175 (54.50–453.2) mmol/L Fig. (1A), which corresponded to the highest Bifidobacterium: Enterobacteriaceae ratio reported for the group above (Supplementary Fig. 1B). The levels of acetate were significantly lower in both HCMV infected groups. There was a strong trend, although not significant ($p=0.321$), towards higher acetate levels in the IgM negative group, 102.3 (8.524–262.8) mmol/L compared to IgM positive group, 47.97 (1.349–173.3) mmol/L which was supported by ROC curve analysis (AUC, 0.7556; Fig. 1A, Supplementary Fig. 2). Butyrate levels were significantly reduced in the IgM positive group, 5.786 (3.474–7.461) mmol/L compared to the healthy, 13.44 (3.409–44.38) mmol/L and IgM negative group, 9.196 (4.325–35.82) mmol/L which was also supported by the ROC curve analysis, with no significant variation in propionate levels. Fecal SCFA analysis was corroborated by PICRUSt2 analysis (Fig. 1B), wherein progressive lower representation of pathways involved in the production of acetate and butyrate from healthy to IgM negative and ultimately IgM positive infants was seen. The high abundance of Enterobacteriaceae observed in the dysbiotic microbiome of HCMV infected infants was further confirmed with a significant and progressively higher level of fecal LPS, which was observed within infected individuals, with the IgM positive group having the highest levels, 14.76 (3.774–36.87) pg/g followed by the IgM negative group, 7.918 (2.590–16.53) pg/g and finally healthy controls, 4.471 (1.702–6.742) pg/g respectively (Fig. 1A). Furthermore, PICRUSt2 analysis, predicted significantly higher biosynthetic potential of gut microbiota for LPS in HCMV infected infants with NC (Fig. 1B).

Taken together, these results clearly demonstrate a disparate and dysbiotic gut microbiome in HCMV infected infants with NC that could contribute to a potentially inflammatory milieu in the gut.

Elevated fecal inflammatory markers in HCMV-infected infants with NC

As the dysbiotic signature present in HCMV infected individuals was suggestive of a pro-inflammatory milieu, we quantified the fecal levels of cytokines and markers associated with intestinal inflammation such as calprotectin, β-Defensin 2, and myeloperoxidase (MPO). We observed significantly higher and apparently hierarchical levels of all pro-inflammatory cytokines evaluated in HCMV infected infants with NC compared to healthy controls with IgM positive individuals seeming to have the highest levels (Fig. 2A). This observation also extended to all three inflammation associated markers evaluated. A trend (based on median values), though not significant was observed for the anti-inflammatory cytokine IL-10 with highest levels observed in healthy controls, 652.7 (353–971.6) pg/g and progressively lower levels observed in HCMV infected individuals and no clear trend in TGF-β. ROC curve analysis ascertained the discriminatory potential of some of these significant signatures—IL-1β, IL-6, IL-23 and MPO (Fig. 2B and Supplementary Fig. 2).

Loss of gut integrity and microbial translocation in HCMV infected infants with NC

After elucidating a distinct dysbiotic microbiome and a corresponding pro-inflammatory signature present in HCMV infected individuals, we evaluated fecal levels of sIgA, known to regulate homeostatic composition of the gut microbiota [19] (Fig. 3A), where an incremental and progressively lower level of sIgA was observed from healthy controls, 4546 (3351–5452) μg/g of feces to HCMV infected IgM negative, 2960 (571.7–4355) μg/g of feces and IgM positive individuals, 2378 (910.4–3930) μg/g of feces respectively. Next, we evaluated markers such as fecal protease, indicative of inflammation [28], and zonulin levels, representative of gut permeability [20] (Fig. 3A). Both these markers were significantly and incrementally elevated compared to healthy controls in IgM negative and IgM positive infants respectively, suggestive of impaired gut barrier function within infected individuals. Interestingly, concurrent estimation of plasma LPS revealed detectable levels, which were significantly higher in IgM positive individuals, 31.76 (6.628–68.47) pg/ml. ROC curve analysis (Fig. 3B), depicts the clear discriminatory potential of these markers in IgM positive and IgM negative HCMV infected individuals (AUC-0.72). Taken together, these results clearly demonstrate the occurrence of microbial translocation in conjunction with increased gut permeability within HCMV infected infants with NC.

Microbiome-immune network interactions characterizing HCMV mediated pathology in infants with NC

In an effort to integrate the triad of dysbiotic gut-microbiome, associated microbial products, and observed host immune phenotypes with the pathology and severity of HCMV infection in the context of NC, we obtained an

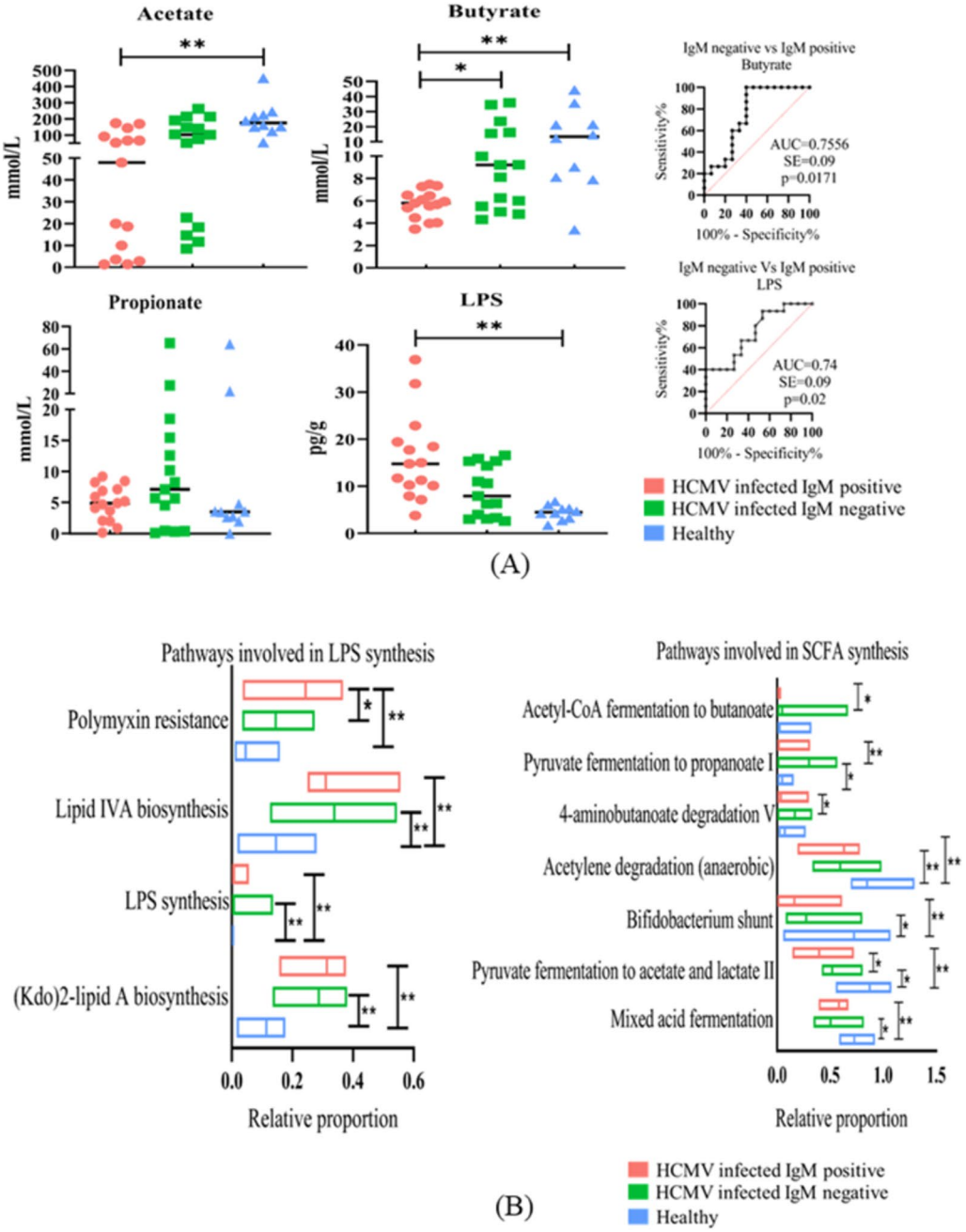


Fig.1 Estimation of fecal microbial products– (**A**) Levels of fecal short-chain fatty acids assessed by HPLC and fecal LPS by ELISA in HCMV infected infants (N = 15 each) with NC compared to healthy controls (N = 10) along with significant ROC curve analysis for butyrate and LPS; (**B**) Relative proportion of predicted significant Metacyc pathways associated with SCFA synthesis obtained by PICRUSt2 analysis. Data represented as Median. Statistical significance calculated by Kruskal –Wallis test with Dunn's multiple comparison test; *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; and ****, $p < 0.001$

extensive correlation network (Supplementary Fig. 3). Figure 4 depicts sub-networks that represent interactions for deciphering potentially actionable pathways for therapeutic interventions in future. Our previously reported associations of Granzyme B expressing NK cells, Gammaproteobacteria and *Finegoldia magna* [14] (reproduced as supplementary Fig. 5) were further extended to include a positive association with fecal LPS (r = 0.5293) and a negative association of this subset with fecal butyrate levels (r = − 0.4686). The latter,

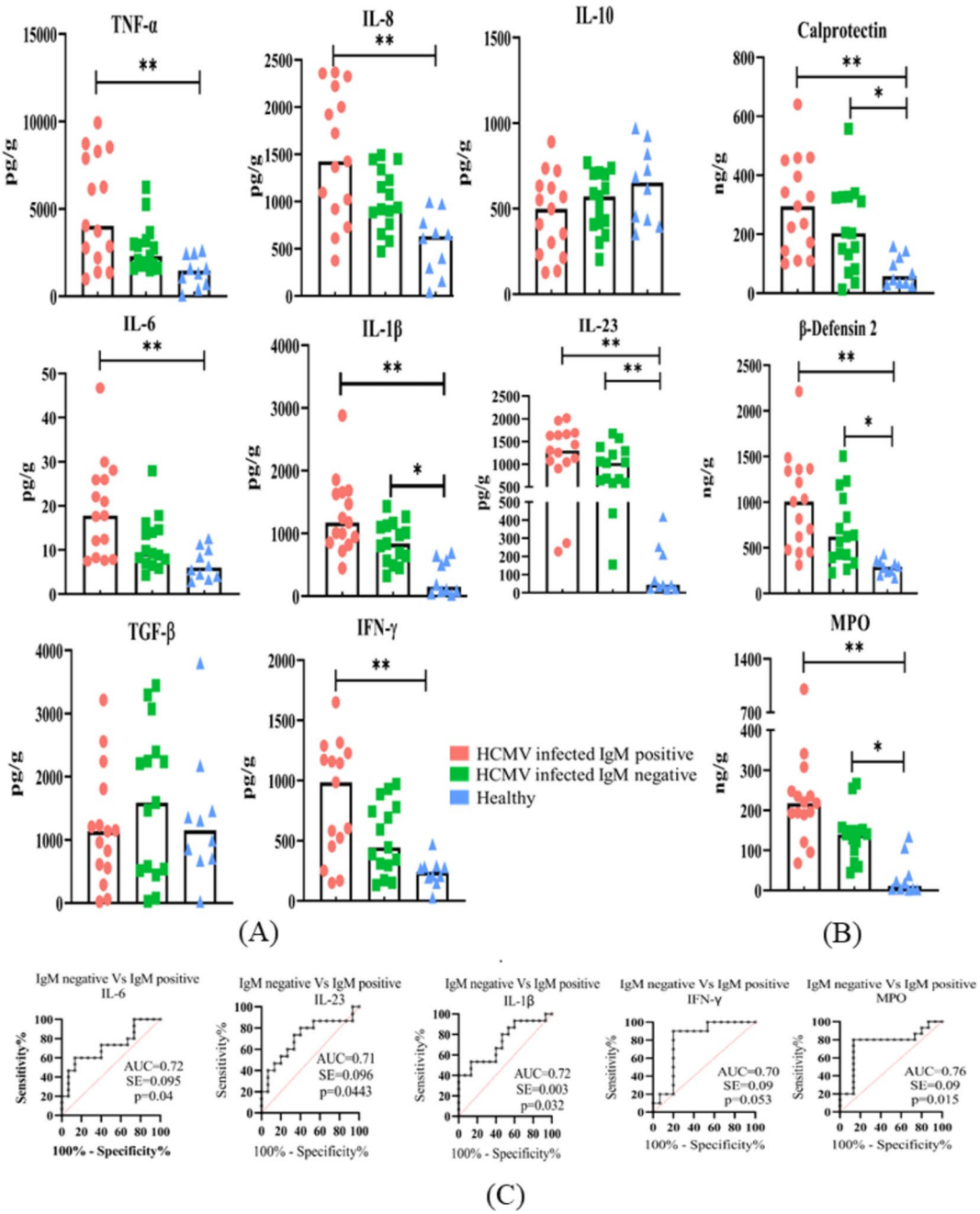


Fig.2 Estimation of fecal inflammatory markers—(**A**) Levels of fecal cytokines and (**B**) gut inflammatory markers assessed by ELISA in HCMV infected infants with NC (N = 15 each) compared to healthy controls (N = 10); (**C**) Significant ROC curve analysis of fecal inflammatory markers between HCMV infected infants with NC. Data represented as Median. Statistical significance was calculated by and Kruskal–Wallis test with Dunn's multiple comparison test; *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; and ****, $p < 0.001$

in turn, were positively correlated with *Bifidobacterium* (r = 0.5492) and negatively associated with fecal LPS (r = − 0.6027) (Fig. 4A). With respect to the T cell compartment (Fig. 4B), we observed a similarly strong negative correlation between CD4 positive Granzyme B expressing T cells and *Finegoldia magna* (r = − 0.5497). Also, a distinct correlation network involving Firmicutes and the CD4/CD8 ratio, a known disease progression marker, was seen, where frequency of this taxa was independently correlated with circulating frequencies of CD4 positive (r = 0.6509) and CD8 positive T cells (r = − 0.6509) and in turn, positively with the CD4/CD8

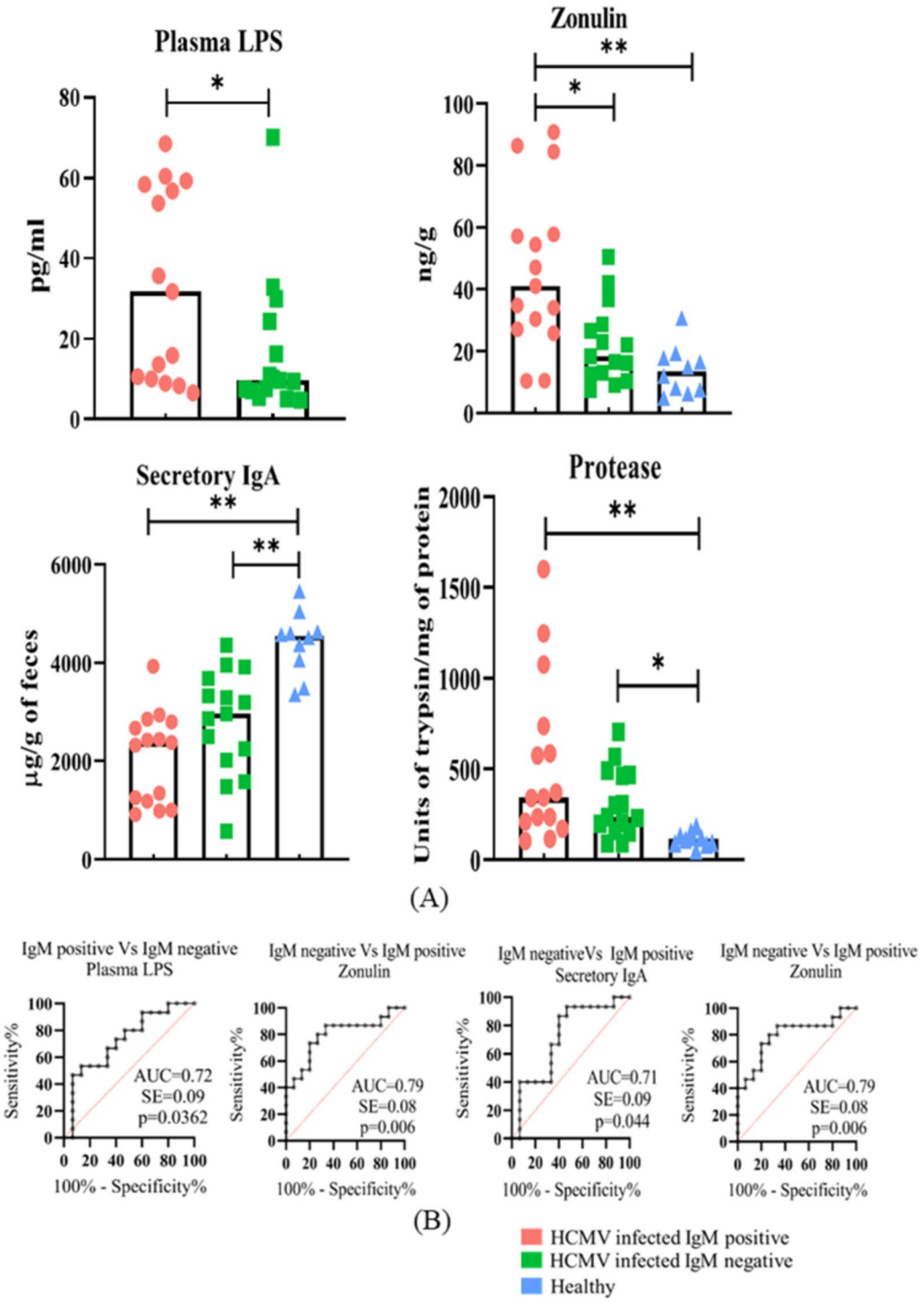


Fig.3 Estimation of fecal gut-integrity and bacterial translocation markers–(**A**) Levels of fecal metabolites assessed by ELISA in HCMV infected infants with NC (N = 15 each) and healthy controls (N = 10); (**B**) Significant ROC curve analysis of fecal gut-integrity markers between HCMV infected infants with NC. Data represented as Median. Statistical significance was calculated by and Kruskal–Wallis test with Dunn's multiple comparison test; *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; and ****, $p < 0.001$

ratio (r = 0.6509). Interestingly, our previous report on this cohort showed depleted levels of Firmicutes in IgM positive HCMV infected individuals with concurrently lower CD4/CD8 ratios. Propionate and acetate were both positively correlated with this ratio. In addition, propionate was also observed to have a negative correlation with frequency of circulating cytotoxic CD8 positive T cells expressing Granzyme B (r = − 0.5485). Interestingly, we also noted a positive correlation of propionate with fecal LPS (r = 0.3376), suggestive of a potential compensatory response to elevated levels of the former. Next (Fig. 4C), we derived a leaky gut network centred on the microbial translocation marker, LPS, whose levels, as expected, were positively associated with pathogenic bacteria such as *Escherichia* (r = 0.4388) and *Acinetobacter* (r = 0.3478) as well as fecal levels of gut permeability marker zonulin (r = 0.3396). Additionally, Firmicutes and *Bifidobacterium* were positively associated with fecal acetate (r = 0.3624) and butyrate levels (r = 0.5492) respectively, both of which in turn positively correlated with sIgA (r = 0.4117, r = 0.4371) and negatively associated with fecal LPS (r = − 0.3801). Butyrate and sIgA levels also correlated negatively with translocated plasma LPS (r = − 0.4723, r = − 0.4587). Finally, and as expected, butyrate levels were also negatively correlated with fecal zonulin levels (r = − 0.4117) In summation, the observed leaky gut phenotype appeared to be dependent on the interplay of factors governing levels of fecal butyrate, acetate and sIgA. Consequently (Fig. 4D), we report another sub-network where pro-inflammatory mediators were positively correlated with pathogenic bacteria and LPS negatively correlated with taxa known to beneficially influence gut health. Further, IL-10, an anti-inflammatory cytokine was negatively correlated with both Enterobacteriaceae (r = − 0.3482) and LPS (r = − 0.3237).

Correlation analysis was also carried out for the IgM amount expressed in terms of absolute absorbance for the HCMV infected groups with relative abundance of bacterial taxa reported in the previous study [14] and the fecal metabolites assessed in this study (Figs. 1, 2 and 3) (Supplementary Fig. 6). IgM levels (indicative of viral replication) and abundance of inflammation associated bacterial taxa (Gammaproteobacteria) (r = 0.623), microbial translocation markers (Plasma LPS) (r = 0.447) and dysregulated gut integrity markers (Zonulin) (r = 0.554) were positively correlated with each other. On the other hand, IgM levels negatively correlated with butyrate (r = − 0.4) and also with Bacteroides (r = − 0.421), Veillonella (r = -0.492), and also *Bifidobacterium breve* (r = − 0.42).

An integrated signature defining the spectrum of HCMV mediated pathology in NC

In an effort to determine the discriminatory potential of all the above reported data with respect to our study groups, principal component analysis (PCA) was employed. PCA involving all fecal analytes described above may be promising in delineating profiles, distinct from healthy controls and corresponding to varying degrees of pathology in infected individuals (PC1, 40.19%). (Fig. 5A) Cumulative analysis incorporating relative abundance of observed taxa obtained in the previous study [14] and fecal analytes reported in this study also resulted in clearly discriminatory profiles (Fig. 5B, PC1, 20.92%). Finally, we integrated systemic cellular immune subset signatures reported in the previous study[14], where available, into the PCA (Fig. 5C) within a subset of HCMV infected individuals to consolidate the discriminatory gut microbiome—immune axis at play which governs HCMV mediated pathology in the context of NC (PC1, 21.78%).

Discussion

In this study, we attempted to unravel further, gut microbiome-immune axis in the context of exacerbated liver pathology in HCMV infected IgM positive infants with NC, by assessing potent gut microbiome associated immunomodulators and host gut homeostatic factors to support our previously reported observations from this cohort. The significantly reduced Bifidobacterium: Enterobacteriaceae ratio, observed in HCMV infected IgM positive participants, clearly demonstrated a dysbiotic signature that may underlie an increased inflammatory milieu in the context of viral infection. The detection of antiviral IgM is suggestive of an early, immature (non-class-switched to IgG) response that, in the case of infants, would occur during continued viral replication.

(See figure on next page.)

Fig.4 Microbiome-Immune interactions in the pathogenesis of infection- The correlation network represents the pairwise Spearman correlation analysis of the obtained fecal bacterial products (Fig. 1), inflammatory markers (Fig. 2), gut integrity and bacterial translocation markers (Fig. 3) with each other and with relative abundance of bacterial taxa of all the study participants and relative frequencies of immune subsets in HCMV infected infants with NC reported in the previous study [14]. Red lines indicate a positive correlation and blue indicates a negative correlation. The strength of the correlation is indicated by the intensity of the color of the lines in the network. Only the significant correlations have been plotted. The coloured boxes indicate the following: Orange- Relative abundance of bacterial taxa; Pink- Relative frequencies of immune cell subsets; Green-Fecal microbial products; Yellow- Fecal inflammatory markers; Purple- Fecal Gut integrity markers; (**A**) Granzyme B expressing cells regulatory network; (**B**) T cell compartment regulatory network; (**C**) Leaky gut network; (**D**) Inflammatory cascade network

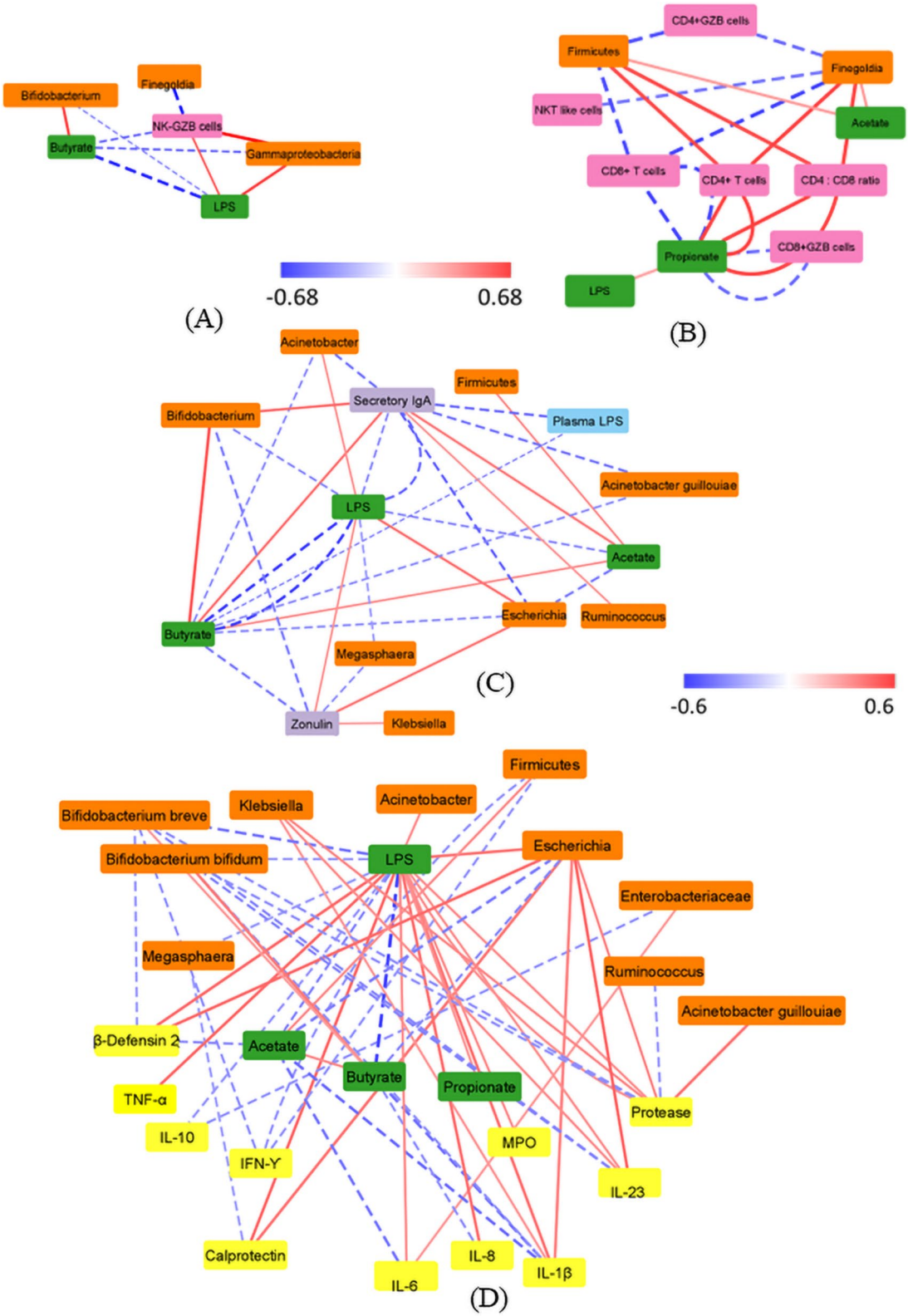


Fig.4 (See legend on previous page.)

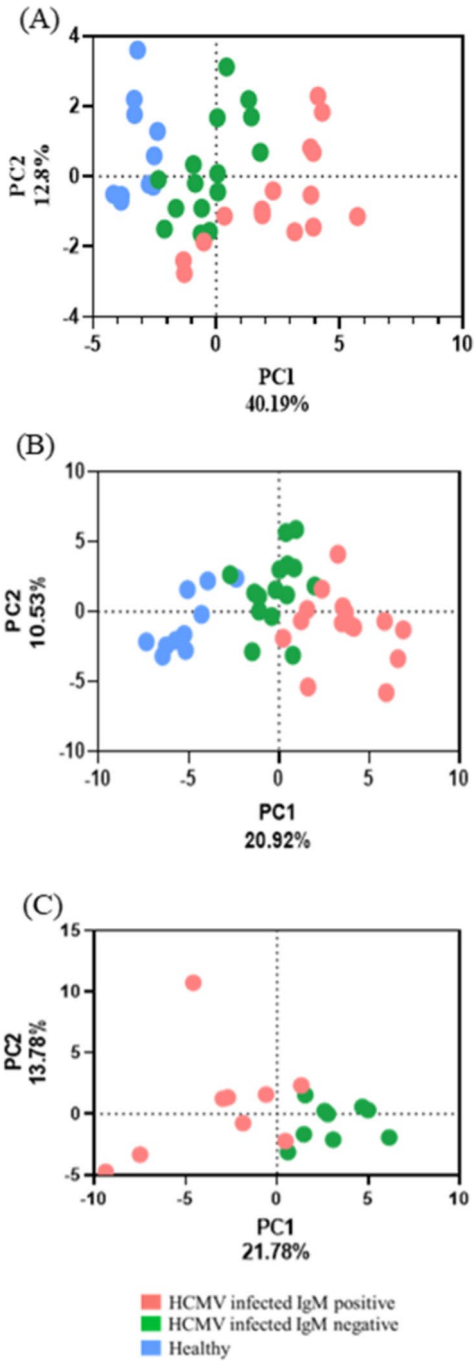


Fig.5 Principal component analysis based on; (**A**) Estimated fecal bacterial products, inflammatory and gut integrity markers of HCMV infected infants (N = 15 each) with NC and healthy controls (N = 10); (**B**) Estimated fecal bacterial products, inflammatory and gut integrity markers and relative abundance of bacterial taxa of HCMV infected infants with NC (N = 15 each) and healthy controls (N = 10); (**C**) Estimated fecal bacterial products, inflammatory and gut integrity markers, relative abundance of bacterial taxa and relative frequencies of immune cell subsets of HCMV infected infants with NC (N = 9 each)

Also, as reported by us earlier [14], HCMV IgM positive infants, compared to IgM negative infants showed higher immune activation, increased CD4:CD8 ratio, elevated cytotoxic potential of immune cells like CD4, CD8, NK and NKT-like cells expressing granzyme-B.

Gut microbiota derived SCFAs have been described to exert a spectrum of immune-modulatory activities, dominated by anti-inflammatory effects, preservation of the gut epithelial barrier and preventing growth of pathogenic bacteria [21–23]. Indeed, in our correlation network, we observed a strong negative correlation of fecal butyrate levels with *Escherichia*, whose enrichment in the IgM positive HCMV participants may have occurred through impairment of butyrate/PPARγ signaling, favoring the growth of Enterobacteriaceae [22]. The latter, were also conversely correlated with fecal IL-10 and IL-6 levels, where HCMV IgM positive individuals showed highest and lowest levels of IL-6 and IL-10 respectively. Intestinal inflammation by NETosis assessed to an extent by elevated levels of fecal MPO [24] and calprotectin [25] levels, is known to alter the tight junctions, increasing the intestinal permeability and microbial translocation [26]. In our study, MPO was positively correlated with the granzyme containing immune cells, gammaproteobacteria, pro-inflammatory cytokines, and zonulin. Thus, NETosis can further be explored in the pathology of viral infection in mediating tissue injury. Our findings also corroborated with studies that have shown elevated protease levels to be associated with inflammatory diseases like IBD and other gastrointestinal diseases [27, 28]. Similarly, Human β-defensin-2, an inducible antimicrobial peptide that is triggered in the intestinal epithelial cells at the onset of inflammation, assessed as a marker of intestinal inflammation was elevated in the HCMV infected groups and correlated with other pro-inflammatory markers [29–31].

Butyrate promotes epithelial barrier functions by stabilizing HIF (hypoxia-inducible factor) [22]. In our study, we observed a negative correlation of butyrate and sIgA, with zonulin and LPS. This negative correlation suggests a putative cross-talk of dysregulated microbial colonisation and altered gut permeability leading to a 'leaky gut' that would occur in HCMV infected IgM positive infants. We also observed a negative correlation of sIgA with LPS and granzyme expressing NK cells which have been shown to contribute to the pathogenesis of cholestasis [32, 33]. Indeed, Maidji et al. demonstrated the role of HCMV in causing intestinal barrier dysfunction, by impairing the integrity of intestinal cells and IL-6 [34]. Thus, these studies suggest a possible etiology for microbial translocation of bacterial products including LPS that would induce systemic and dysfunctional immune activation including increased cytotoxic potential in

lymphoid subsets as well as a skewed CD4/CD8 T cell ratio observed in the IgM positive HCMV infected individuals from our study. A further contribution to HCMV mediated exacerbated pathology may be contributed by elevated levels of pro-inflammatory cytokines like IL-6, IL-8, TNF-α, IL-1β, and IL-23 in the IgM positive cohort. Our findings in IgM negative infants were interesting as, these individuals, while infected, had relatively lower degree of dysbiosis, immune dysregulation, breach of the gut barrier, and inflammation compared to IgM positive infants. Santos Rocha et al. have reported a modulatory role for butyrate in balancing HCMV viral reactivation and persistence with dampening of inflammatory sequelae where SPF animals when infected with HCMV increased colonization of butyrate producing commensals and thus ameliorated the potential inflammatory effects of viral replication [38]. Thus, the IgM negative HCMV infected group, through preserved butyrate levels may have experienced a less severe HCMV mediated pathology that concurred with the clinical findings in both ours as well as earlier studies reporting on IgM positive HCMV infected infants with NC [6, 14, 35] and can also be seen in studies involving butyrate as an important mediator in ameliorating the severity of cholestasis [36, 37].

Indeed, when our assessed parameters encompassing the triad of gut microbiota profiles, microbial SCFAs, LPS as well as soluble and cellular immune signatures were integrated, we obtained discriminatory patterns that reflected distinct clinical states with IgM positive HCMV infected infants and healthy infants occupying opposite ends of the spectrum.

Cumulatively, through this work, we show that acute HCMV infection, accompanied by IgM production, most likely due to higher levels of active viral replication, mediated severe pathology in infants with NC. The infection in turn may have been a result of gut dysbiosis with a polarization towards an inflammatory milieu in the gut by reduced levels of SCFAs and accompanying impairment in sIgA in IgM positive infants. This would culminate in altered gut permeability along with translocation of LPS causing further systemic immune activation with accompanying sequelae. Our findings highlight the need for conducting mechanistic studies in suitable model systems that might provide evidence for a role of dysregulated gut microbiome-immune axis in determining disease severity in HCMV infected infants with NC. These in turn might open avenues for developing microbial interventions to alleviate the exacerbated pathology in HCMV infected infants with NC. Our study also highlights the utility of an integrated approach towards delineating the role of gut microbiome, through a dysregulated microbiome-immune axis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-024-00663-3.

Supplementary Material 1

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Author contributions
VB and VP conceived the project. VB and VP designed the strategy for microbiome and immune signatures analysis respectively. KK performed all the assays and analysed the data under the supervision of VB. GB performed HPLC for SCFA analysis under the supervision of VB. GB, VP1, and JP performed the PCR and serological diagnostic assays. HP and SV were involved in immunophenotyping assays and analyses. IS was the clinical co-investigator who identified the problem and led the recruitment of the subjects involved in the study. HM and AG were the clinicians who assisted in the recruitment of study subjects. KK, VB, and VP wrote the manuscript. All others read and approved the final version of the manuscript.

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Data availability
Sequence data that support the findings of this study can be accessed at http://www.ncbi.nlm.nih.gov/bioproject/909467. The bioproject ID is PRJNA909467.

Declarations

Ethics approval and consent to participate
The ethics approval was obtained by approval by ICMR-NIRRCH Ethics Committee for Human Studies (No. 277/2015) and IEC-BJWHC (IEC-BJWHC/AP/2015/005-version 02).

Competing interests
The authors declare no competing interests.

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