



Novel Analysis Methods for Longitudinal Cytokine Response Data in a Birth Cohort Study



Cynthia M. Visness,¹ Katy F. Jaffee,¹ Agustin Calatroni,¹ Robert A. Wood,² Peter J. Gergen,³ James E. Gern⁴

¹Rho Federal Systems Division, Inc., Chapel Hill, NC, ²Johns Hopkins University School of Medicine, Baltimore, MD,

³National Institute of Allergy and Infectious Disease, National Institutes of Health, ⁴University of Wisconsin School of Medicine and Public Health, Madison, WI

Background/Objective

Multiplex cytokine ELISA systems represent a major advance in research methods, but the large amount of data generated by repeated sampling in birth cohorts creates new challenges for the analysis of relationships between cytokines, postnatal exposures, and atopy-related outcomes. Improved methods are needed to analyze the evolution of time-related patterns of cytokine production.

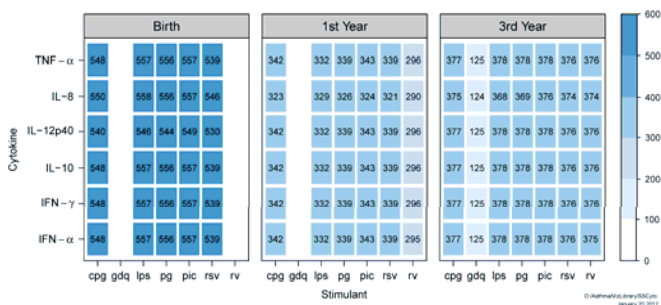
Methods

Participants in the Urban Environment and Childhood Asthma (URECA) longitudinal birth cohort study had mononuclear cell responses of nine cytokines measured in cord blood and at ages 1 and 3 years. Responses were measured to panels of innate and adaptive stimulants, resulting in a large data matrix. Factor, cluster, and principal component analyses were compared as data reduction techniques and to discern patterns of response.

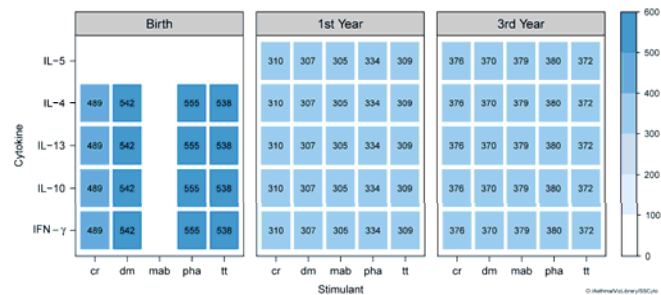
Data Available

Data was collected for the 609 children enrolled in the URECA study, but we focus here on the 560 enrolled in the Atopic cohort. 2 panels of cytokine data were measured. For the innate panel, there are 30 stimulant / cytokine combinations at birth, 36 at year 1, and 42 at year 3. For the adaptive panel, there are 16 stimulant / cytokine combinations at birth and 25 at years 1 and 3.

Innate Panel Available Samples



Adaptive Panel Available Samples



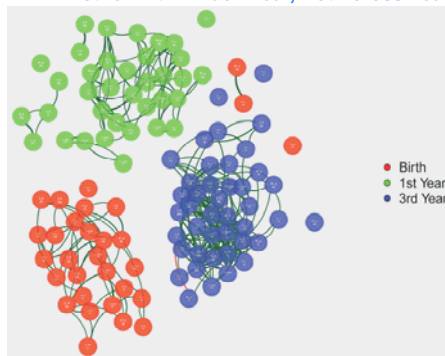
Stimulants Used

CpG = C-phosphate-G
GDQ = Gardiquimod
LPS = Lipopolysaccharide
PG = Peptidoglycan
PIC = Polyinosinic:polycytidylic acid
RSV = Respiratory syncytial virus
RV = Rhinovirus

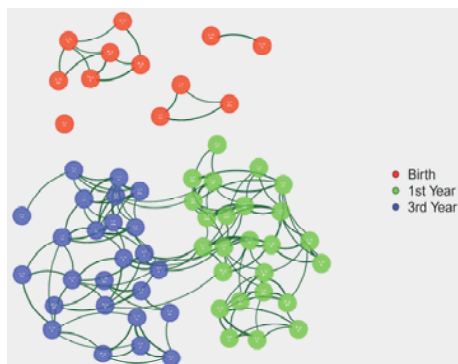
CR = Cockroach
DM = Dust mite
MAB = Anti CD3-Anti CD28 monoclonal antibodies
PHA = Phytohemagglutinin
TT = Tetanus toxoid

Results

Cytokines from the Innate Panel are Primarily Associated with one Another within Each Year, Not Across Years

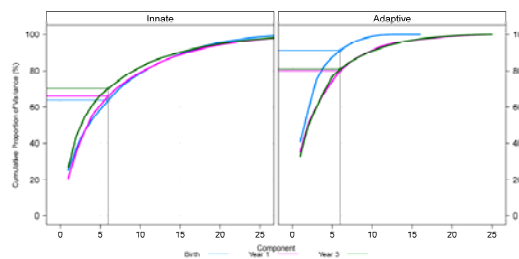


Cytokines from the Adaptive Panel are Primarily Associated with one Another within Each Year, but Some Association Across Years 1 & 3

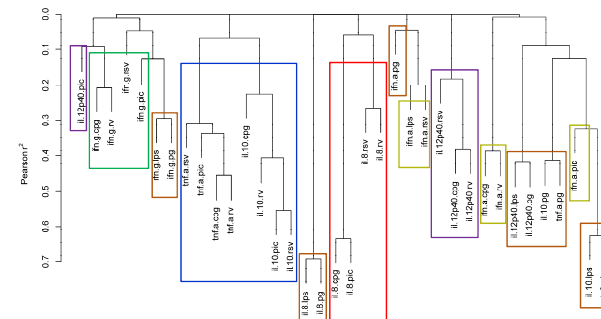


Only tetanus-stimulated cytokine responses are correlated between Years 1 and 3 in the adaptive panel.

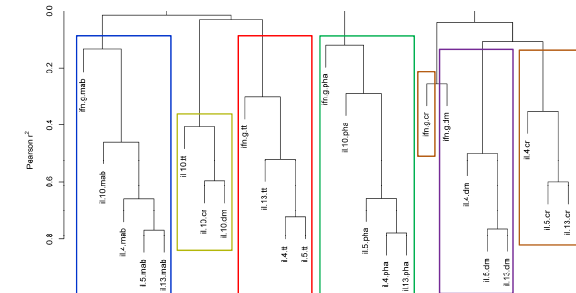
Principal Component Analysis Confirms 6 Factors for Each Panel Accounts for Much of the Variance



The Innate Panel Clusters by Cytokine, Not Stimulant



The Adaptive Panel Clusters by Stimulant, Not Cytokine



- Cytokine response values (pg/ml) were ranked across the 3 time points, separately by stimulant, into 4 groups. Tied values fall into the same group, so they are not necessarily of equal size.
- After standardizing the cytokine values using ranking method above, a score was created for each factor by calculating the mean of the ranks for each stimulant-cytokine response that falls into that factor.
- Factors are created based on the above clusters (6 in each panel):
 - Innate: IL-10 and TNF- α responses to the viral stimuli (PIC, CpG, RSV, RV), IFN- γ responses to viral stimuli, IL-12p40 responses to viral stimuli, IL-8 responses to viral stimuli, IFN- α responses to viral stimuli, and all cytokine responses stimulated by LPS and PG.
 - Adaptive: IL-10 responses to adaptive stimuli DM and CR, responses to DM (no IL-10), responses to CR (no IL-10), all cytokine responses to PHA, all cytokine responses to MAB, all cytokine responses to TT.

Conclusions

- Cytokine levels appear to be associated with one another within years, but not across years.
 - Adaptive panel shows some interaction between years 1 and 3, but further investigation shows that it is only a moderate correlation for the tetanus stimulated cytokines.
- Cytokines cluster primarily by cytokine in the innate panel and by stimulant in the adaptive panel.
 - Note that we clustered independent of the main outcomes – this could produce different factors.
- Principal component analysis shows that 80% of the variance can be explained by 10 components or less – 6 factors should be sufficient.
- No outcomes or clinical findings were taken into account when clustering or creating the factors. We plan to do this analysis in the future, which may give us different results.

