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

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

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

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

1. 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.

2. 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.

3. Factors are created based on the above clusters (6 in each panel):
   - Innate: IL-10 and TNF-a responses to the viral stimuli (PIC, CpG, RSV, RV), IFN-g responses to viral stimuli, IL-12p40 responses to viral stimuli, IL-8 responses to viral stimuli, IFN-a 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.

CpG = C—phosphate—G—
LPS = Lipopolysaccharide
PG = Polyinosinic-polycytidylic acid
PIC = Polyinosinic:polycytidylic acid
RV = Respiratory syncytial virus
RV = Respiratory syncytial virus

Conclusions

1. Cytokine levels appear to be associated with one another within years, but not across years.
2. 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.
3. Cytokines cluster primarily by cytokine in the innate panel and by stimulant in the adaptive panel.
4. 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.