# Differential maturation trajectories of innate antiviral immunity in health and atopy

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The rest of the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.


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# Abstract

Background

The maturation of innate immune responses in health and atopy is still incompletely understood.

Methods

We aimed to evaluate age-related trajectories of the TLR3 and TLR7/8 pathways across the lifespan and whether these differ between healthy and atopic individuals. Peripheral blood mononuclear cells (PBMCs) were isolated from 39 otherwise healthy atopic and 39 non-atopic subjects, aged 0-45 years. Selected cytokines involved in antiviral responses were measured by Luminex in culture supernatants of poly(I:C)- and R848-stimulated PBMCs. The non-parametric correlation between age and cytokine expression and differences in developmental trajectories between healthy and atopic were estimated. Patterns of cytokine development were identified with principal component analysis.

Results

Normal innate immune maturation entails significant and progressive age-related changes in the production of IL-1β, TNF-α, MIP-1β, MCP-3, IP-10, IL-10, IL-12p70 and IFN-γ upon TLR3 and/or TLR7/8 stimulation. Individual cytokines made small contributions to the observed variability; chemokines MCP-3 and IP-10 were key contributors. The development of these pathways deviated in atopic subjects with significant differences observed in the trajectories of IL-1β, MIP-1β and IL-10 synthesis.

Conclusion

TLR3 and TLR7/8 pathways mature during childhood, while atopy is associated with an abnormal maturation pattern. Suboptimal responses in Th1, inflammatory cytokine and chemokine production may be implicated in poor antiviral immunity in atopics, while deficient maturation of IL-10 producing capacity in the breaking of tolerance.

**Keywords:** innate immunity; antiviral response; cytokines; chemokines; innate ontogeny.

# 1. Introduction

The neonatal period and early childhood are characterized by increased susceptibility to infections, attributed to the immaturity of several functions of both innate and adaptive immune systems (1, 2). During recent years several studies have sought to gain insight into immune developmental trajectories (3-15). Despite significant variations among published data, it has become obvious that the developmental pattern of cytokine responses is non-linear but rather age- and TLR-specific (16) and may be modified by genetic factors and environmental exposures (15, 17).

Nonetheless, most of these studies have focused on changes occurring during the first months of life (3, 4, 6-9, 13) and only a few have assessed responses during later childhood years (5, 10) or up until adulthood (11, 12, 18). Most of the latter studies have only addressed the TLR4 pathway (10-12). The maturation of the antiviral i.e. TLR3- and TLR7/8-mediated responses from birth to adulthood has not been sufficiently studied up to now, as only 2 studies have included children older than 2 years of age (5, 15).

As the occurrence of viral infections is particularly frequent in children, it is of great importance to characterize the developmental trajectories of the pathways involved in antiviral immunity in detail. In the present study, we investigated the postnatal development of the TLR3 and TLR7/8 pathways from birth to adulthood and the effect of atopy on these maturational trajectories.

# 2. Methods

## 2.1 Population and sample collection

This cross-sectional study was conducted in two sites ("P. & A. Kyriakou" Children's Hospital and "Attikon" General University Hospital) in Athens, Greece and the recruitment comprised a cohort of Caucasian otherwise healthy atopic/allergic and non-atopic/non-allergic children (0-18 years old) and adults (18-45 years old), visiting the centers mentioned above for a planned clinical evaluation or an elective surgery. The study was approved by the Medical Ethics Review Board of the centers involved (protocol numbers 21826/26-11-2012 and EBΔ 528/10-10-13, respectively) and written informed consent was obtained from all participants or the parents/legal guardians of subjects <18 years of age. Exclusion criteria are listed in supplement (Table S1). All participants were evaluated by a specialist and defined as atopic-allergic if they had a clinical history and/or symptoms compatible with eczema, allergic rhinitis, bronchial asthma or food allergy and relevant sensitizations. Sensitization was assessed by skin prick testing to a panel of 8 prevalent local aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat dander, Alternaria alternata, cockroach, 5-grass mix, Olea europaea, Parietaria officinalis) and egg white. In some cases, according to the patient's history, further testing was conducted. Commercial allergen extracts were used (Alyostal, Stallergenes, France). Histamine (10 mg/ml) and saline were used as positive and negative control respectively. A wheal diameter of 3 mm or greater was considered positive (19). In subjects where skin prick tests could not be performed, sensitization was assessed by sIgE measurement by IMMULITE® 2000 immunoassay system (Siemens, Germany) or ImmunoCap allergen-specific IgE blood test (Phadia AB, Uppsala). Values >0.35 kU/L were considered positive. The groups

were matched for age and sex. Clinical data were documented in a standardized manner.

Blood samples were collected in sterile plastic tubes with lithium heparin (BD Vacutainer® Blood Collection Tubes, BD Diagnostics) and were processed within 4 hours of collection.

**2.2 Blood sample processing - Peripheral blood mononuclear cells isolation and in vitro stimulation**

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Biocoll separating solution (BIOCHROM AG), per standardized procedures. PBMCs were collected from the interphase layer, washed with PBS and resuspended in RPMI-1640 supplement with 25 mM HEPES and L-glutamine (Gibco, Invitrogen) with1% of penicillin-streptomycin, 1% non-essential amino acids, 1% sodium pyruvate, 1% L-glutamine 200mM solution, 1% MEM Vitamin Solution, 50 µM β-mercaptoethanol and 10% heat-inactivated fetal bovine serum (FBS) (all purchased from Sigma-Aldrich). Cell viability estimated by trypan blue exclusion was >95%. Upon resuspension, the concentration was adjusted to $10^6$ viable cells/ml. To study TLR3 and TLR7/8 responses, 500 µL of cell suspension were placed in duplicate in flat-bottom 48-well plates and treated with poly(I:C) (20 µg/ml) and R848 (4 µg/ml) respectively or cultured in the presence of 10 µL of supplemented RPMI (control). After 24h of incubation at 37°C under 5% CO2, the culture supernatants were harvested and frozen at -80°C for storage until multiplex analysis.

## 2.3 Cytokine measurements

Frozen culture supernatants were thawed before use. The cytokines IFN-α2, IL-12p70, TNF-α, IL-1β, IL-10, IP-10, MCP-3, MIP-1β, MDC, IL-33, IL-23, IL-27, IL-28A and IFN-γ were detected using Luminex® 200™ (Luminex Corporation, Austin, TX, USA) according to the manufacturer's protocol (Millipore, Milliplex Map, Merck Laboratories, Switzerland). Readings for each sample were obtained in duplicate and the mean value was used for the statistical analysis.

## 2.4 Statistical analysis

Net cytokine responses were calculated by subtracting the background/baseline cytokine concentrations (unstimulated samples) from those in supernatants derived from samples stimulated with TLR agonists. Outliers exceeding four standard deviations from the sample mean were replaced by adjacent values from the remaining data, while any negative values were replaced by zero. We estimated the non-parametric correlation between age and cytokine expression in both healthy and atopic subjects by utilizing the Spearman's rho correlation test. Moreover, a simple linear regression analysis was performed that considered age as the independent variable and each cytokine response as the dependent variable. Differences in developmental trajectories between healthy and atopic subjects were estimated by comparison of the regression curves for each cytokine (regression slopes), with the use of an analysis of variance. The developmental trajectories of cytokines were also depicted using polynomial regression analysis, as in most of the cases a better fit with the biological phenomenon was obtained, according to the adjusted R-squared criterion (Table S2). Finally,

principal component analysis (PCA) was executed in order to visualize subjects and tag their age groups in a 2-dimensional space constructed by the selected cytokines. All p-values were two-sided and were considered significant when $p<0.05$. Analysis was carried out using the R statistical computing software.

# 3. Results

The clinical characteristics of the subjects are summarized in Table 1. The general characteristics of the groups were similar, with a few exceptions, such as the longer duration of breast feeding in atopics and the more frequent tobacco exposure in house of healthy subjects; parental atopy was, as expected, more prevalent in atopic children. The age distributions of both groups are also shown in supplementary Figure 1 (Figure S1), in form of comparative box-plots. The description of the allergic disease and the sensitization profile in the atopic group are shown in supplement (Figure S2 and Table S3).

## 3.1 Cytokine production in unstimulated cultures

In healthy non-atopic subjects, we observed an age-related increase in the spontaneous secretion of IL-12 ($p=0.046$, $r=0.322$), IP-10 ($p=0.001$, $r=0.511$), IL-10 ($p=0.000$, $r=0.685$) (Figure 1d, 1i, 1k, respectively) and a significant fall in MCP-3 production ($p=0.005$, $r=-0.439$) (Figure 1h). Identical maturation patterns were observed for IP-10 ($p=0.000$, $r=0.635$), IL-10 ($p=0.000$, $r=0.605$) and MCP-3 ($p=0.027$, $r=-0.386$) during childhood (i.e. subanalysis of subjects 0-18 years old). In atopics, a positive age correlation was found only for the secretion of IL-33 ($p=0.012$, $r=0.404$). IL-28

measurements were below detection limits and were excluded. The detailed Rho correlation values (correlation coefficient, r) and their corresponding p-values in healthy and atopic subjects separately, as well as the p-value of the comparison of the simple regression curves for each cytokine (regression slopes) between the two groups are shown on Table 2.

### 3.2 Cytokine production upon TLR3 & TLR7/8 stimulation

Compared to the unstimulated control, there was a statistically significant induction of all cytokines measured upon TLR3 & TLR7/8 stimulation, with the exception of IL-28 and IL-33. Median cytokine values and their interquartile range values in different age groups (0-1, 1-6, 6-12, 12-18, >18 years of age) in healthy and atopic subjects are shown on Table 3**.**

#### 3.2.1 Maturation of TLR3 & TLR7/8 pathways in healthy, non-atopic subjects

As far as the TLR3 pathway is concerned, we observed a significant age-dependent rise in the production of the proinflammatory cytokines IL-1β ($p=0.012$, $r=0.399$) and TNF-α ($p=0.055$, $r=0.310$) from 0-45 years of age. Polynomial analysis curves further showed that the production of these mediators peaks during early adult years and then subsequently slightly falls (Figure 1b, 1c). Moreover, there was a progressive postnatal increase in the C-C chemokine MIP-1β production ($p=0.013$, $r=0.395$) from birth to mid-adulthood (Figure 1g). The maturation of proinflammatory and chemokine innate responses was paralleled by a significant age-related increase in IFN-γ production ($p=0.001$, $r=0.516$), although its induction is indirectly linked to TLR

activation. IFN-γ levels peaked during early adulthood and subsequently stabilized (Figure 1f). In addition, an age-dependent increase was observed in the TLR3-induced anti-inflammatory IL-10 response ($p=0.000$, $r=0.655$), that reached a plateau in mid-adulthood (Figure 1k). Some of the aforementioned correlations were also valid when the childhood age spectrum (i.e. 0-18 years old) was separately assessed, specifically IFN-γ ($p=0.011$, $r=0.438$) and IL-10 ($p=0.004$, $r=0.485$).

Upon R848 stimulation, there was a significant age-related rise in the production of IL-1β ($p=0.007$, $r=0.422$); its production plateaus in mid-adulthood (Figure 1b). Strong age correlations were observed for the chemokines MIP-1β ($p=0.006$, $r=0.430$), MCP-3 ($p=0.020$, $r=0.371$) and IP-10 ($p=0.027$, $r=0.355$). Polynomial regression curves confirmed the progressive rise in MIP-1β and MCP-3 secretion from birth to mid-adulthood (Figure 1g, 1h), whereas for IP-10, a transient peak was observed in early adult years, with a subsequent decrease during adulthood (Figure1i). Postnatal increases from birth to adulthood were also recorded for IL-12p70 ($p=0.009$, $r=0.415$) and IFN-γ ($p=0.000$, $r=0.563$). The stabilization in their production was observed around mid-adulthood (Figure 1d, 1f). Finally, we observed the maturation of IL-10 secretion ($p=0.000$, $r=0.560$). IL-10 production peaks in early adult years and then slightly falls (Figure 1k). The aforementioned correlations were observed during childhood as well. We also discovered an age-related augmentation of IL-23 secretion ($p=0.037$, $r=0.370$); nonetheless, the net values were quite low and therefore data must be interpreted with caution.

Although there was a strong induction of MDC production upon TLR3 and 7/8 stimulation, values showed no specific pattern with age. Finally, net values of IL-27

were low and did not significantly change with advancing age. Correlations of cytokine production with age are visualized in a correlogram (Figure 2).

PCA in healthy subjects shows small progressive changes among distinct age groups during childhood, whereas cytokine responses in teenagers (12-18 years) and adults (18-45 years) were very close (Figure 3A). The variability is driven by the combined expression of many cytokines, among which R848-induced MCP-3, IP-10, IFN-γ, IL-27 and poly(I:C)-induced TNF-α are the principal contributors (Figure 3C).

### 3.2.2 Deviated maturation of TLR3 & TLR7/8 pathways in atopic subjects

In atopic subjects, the observed responses from birth to adulthood differed in many aspects from those described in healthy subjects. In relation to the TLR3 pathway, proinflammatory IL-1β and TNF-α responses did not significantly change over time. The discrepancy in the maturation trajectories between healthy and atopic subjects was also demonstrated by statistically marginal differences between the slopes of the two regression lines for both IL-1β ($p=0.061$) and TNF-α ($p=0.096$). Moreover there were no significant age correlations for IL-12, IFN-γ, MIP-1β, MCP-3 and IP-10. In addition, the comparison of the regression curves for poly(I:C)-induced MIP-1β responses showed a pronounced difference between the slopes of healthy and atopic subjects ($p=0.002$).

A significant age-dependent increase, reaching a plateau in mid-adulthood, was observed in the production of IL-10 in atopics ($p=0.035$, $r=0.339$), as was the case for healthy subjects too (Figure 1k); nonetheless, the comparison of the slopes of the linear

regression curves suggested a steeper postnatal increase in IL-10 production in healthy subjects (p=0.019).

Upon TLR3 stimulation, we did not find any significant age correlations in atopics through the childhood years, with the exception of IL-27 (p=0.049, r=0.351).

TLR7/8-induced responses in atopics essentially followed the same pattern with the TLR3 pathway, with an age-related increase in the production of IL-10 from 0-45 years of age (p=0.004, r=0.449). IL-10 production peaked in early adult years and then slightly decreased. Postnatal maturation of IL-10 production was also observed upon TLR7/8 stimulation throughout childhood years. There were no other significant age correlations and moreover, the comparison of the regression curves for R848-induced MIP-1β responses showed a pronounced difference between the slopes of healthy and atopic subjects (p=0.004).

The sharp differences in responses between healthy and atopic subjects are also evident in the correlogram (Figure 2).

PCA in atopic subjects further confirms the deviated maturation in cytokine responses, as the adults' group is close to age groups of infancy and early childhood. (Figure 3B). As shown on Figure 3D, most cytokines studied have small contributions to the variability among age groups in atopics, with R848-induced IP-10, IL-12p70, IL-1β and poly(I:C)-induced IP-10 and MCP-3 being more significant.

# 4. Discussion

In the present study, we describe the normal postnatal development of innate immune responses to virus-mimicking stimuli from birth to 45 years of age and further assess the effect of atopy on this process.

A first, important finding is an age-related increase in Th1 and proinflammatory responses upon TLR3 and 7/8 stimulation. The significant, progressive age-related increase in IL-12, TNF-α and IL-1β production paralleled the maturation of adaptive Th1 (IFN-γ) responses. Interestingly, these associations were only observed in healthy subjects. Our observations are compatible with findings from studies that have assessed the maturation of TLR3 and 7/8 pathways. Indeed, innate IL-12, proinflammatory and IFN responses have been found to increase postnatally, to reach adult levels at an earlier (13) or later time point (7, 9). The only notable exception to the aforementioned trends has been found in a South African cohort (8), where all responses declined with time, possibly due to different genetic and/or environmental factors. In response to viral stimulation, this intrinsic innate immune maturation program, which seems to have a common direction in most populations, presents significant variations in the rate at which changes occur. This may reflect genetic and environmental variability, but it could also be linked to experimental methodology. The effect of atopy on the maturation procedure has been prospectively studied up to the age of 5 years by Tulic et al (15), who also observed a defective postnatal maturation of proinflammatory and Th1-related molecules in atopic subjects. Our data suggest that these deficient responses persist throughout childhood and adult life and may be potentially linked not only to allergic disease onset and persistence, but also to the reported increased occurrence of viral infections in allergic subjects (20-23).

Another important finding is the increase with age in the innate production of chemokines MCP-3, MIP1-β and IP-10 upon viral stimuli. Moreover, according to PCA, R848-induced MCP-3 and IP-10 were among major contributors to the variability observed between distinct age groups during childhood. As the only study assessing postnatal chemokine production with TLR stimulation has been conducted in a South African cohort (8), results were quite the opposite to ours. To our knowledge, this is the first study to address the postnatal development of such molecules across the lifespan, while also assessing differences between healthy and atopic subjects. These chemokines contribute to the ability of the host to orchestrate an inflammatory response principally through chemotactic attraction of innate cells. MCP-3 is mainly implicated in monocyte mobilization, while MIP-1β in macrophage and natural killer (NK) cell migration; IP-10 also promotes NK cell recruitment (24-26). MIP-1β has been shown to promote protease release from monocytes, enhance NK cytolytic activity and DC differentiation. IP-10 also potentiates NK-cell mediated killing and may induce apoptosis, regulate cell growth and proliferation, as well as angiogenesis. Finally, MIP-1β is implicated in DC/ T cell interactions, in the chemoattraction and activation of CD8+ T cells and both MIP-1β and IP-10 play a role in Th1 priming/ polarization of adaptive responses (25, 27-29). There is evidence supporting the contribution of these chemokines to viral clearance. Enhanced production of MIP-1β and IP-10 has been associated with better clinical outcomes in viral infections in humans (30-32).Thus, the deficient maturation of these chemokines observed in atopic allergy may contribute to the perturbed balance of Th1/Th2 adaptive responses in atopic subjects and also to their reported deficiency in the defense against viral infections.

An interesting observation regarding proinflammatory cytokines and chemokines is that, in atopic subjects, their net values exceed those of healthy subjects in early life and this hyperresponsiveness has been also noted by Tulic et al (15). Furthermore, in our cohort, responses in atopics remain rather stable over time, whereas, in healthy subjects, they present positive age correlations.

The anti-inflammatory IL-10 production was found to increase with age both in healthy and atopic subjects, but upon TLR3 stimulation, a steeper increase was observed in non-atopics. Our observation is in line with studies in early life (4, 7, 13) and extends the finding to later time points in childhood and adulthood. Tulic et al also found a significant difference in the trajectories of TLR3- & TLR7/8-induced IL-10 responses over time between healthy and atopic children, with higher secretion in atopics until 2.5 years of age (15). A similar pattern is observed in our study upon TLR3 stimulation, but with a more prolonged hyperresponsiveness in the allergic group, as shown by the time point of section between the 2 regression curves, at around 5 years of age.

Interestingly, there is rising evidence for a functional role of TLR3 in the pathogenesis of asthma in experimental models (33-35) and it has been shown that TLR3 rather than TLR7/8 activation, combined with an inhaled allergen, can lead to the development of allergic airway disease (36); on the other hand, researchers reporting a preventive role of TLR3 on experimental asthma found that it was mediated by IL-12 and IL-10 (37). Speculatively, one might hypothesize that the deficient maturation in the anti-inflammatory TLR3-induced IL-10 responses in atopic subjects becomes obvious approximately at the age when respiratory allergies are mainly initiated, generally and specifically in our cohort as well. The robust maturation of anti-

391 inflammatory IL-10 secretion might be linked to protection from the development of
392 allergies.

393 Beyond single molecules, PCA in healthy subjects showed small progressive
394 changes among age groups during childhood while cytokines made small individual
395 contributions to the observed variability. In atopic subjects, a deviated maturation
396 pattern in TLR3- & TLR7/8-induced cytokine responses was revealed, as the adult
397 response was similar to infancy and early childhood.

398 There are a number of limitations to our study. Firstly, our data are cross-
399 sectional and the observed changes in innate immune parameters may be influenced by
400 inter-individual variability, as well as generational aspects. To minimize impact from
401 factors that are known to affect cytokine responses -such as infections and medication-,
402 strict inclusion criteria were applied. Moreover, we did not identify the cellular source of
403 the cytokines produced after TLR stimulation. Production kinetics differ between
404 cytokines and the selection of one time point, necessary from a logistical perspective, is
405 admittedly suboptimal.

406 Despite its limitations, this is the first report describing age correlations for a
407 multitude of molecules upon virus-mimicking stimuli from birth to adulthood, in both
408 healthy and atopic-allergic subjects. Clear age-related changes of the production of
409 Th1, proinflammatory and anti-inflammatory molecules in healthy subjects complement
410 and extend findings from different populations. On the other hand, the defective
411 maturation of these responses in atopic subjects, links the allergic phenotype with a
412 generalized deviation from normal innate immune system development and may be an

important contributing factor to the increased susceptibility of allergic subjects to viral infections.

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## Impact Statement

This study describes the maturation trajectories of proinflammatory, antiviral and regulatory mediators, modelling virus stimulation from birth to adulthood. Normal innate immune maturation entails significant and progressive age-related changes in the production of IL-1β, TNF-α, MIP-1β, MCP-3, IP-10, IL-10, IL-12p70 and IFN-γ, following simulation of the TLR3 &TRL7/8 pathways. The maturation path deviates in atopic subjects with considerable defects observed in the trajectories of IL-1β, MIP-1β and IL-10 synthesis. Suboptimal responses in Th1, inflammatory cytokine and chemokine production may be implicated in poor antiviral immunity in atopics, while deficient maturation of IL-10 producing capacity in the breaking of tolerance. These findings reveal the dynamics of immune imbalance in allergy and set the scene for a time-dependent appreciation of the atopic immune profile.

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# Tables

**Table 1. Clinical characteristics of the study participants.**

Significant differences between the groups were determined by using the Wilcoxon's rank-sum test for continuous data and the z-test for comparisons of proportions.

| | Healthy non-atopic | Atopic | P-value |
|---|---|---|---|
| Group size, n | 39 | 39 | 1 |
| Age, years, median (range) | 10.304 (0-43.25) | 10.752 (0-45) | 0.980 |
| Distribution per age group, n | | | |
| 0-6 months | 6 | 6 | |
| 6-24 months | 6 | 6 | |
| 24-60 months | 7 | 7 | |
| 5-10 years | 7 | 7 | |
| 10-18 years | 7 | 7 | |
| 18-45 years | 6 | 6 | |
| Gender (Female/male, n) | 21/18 | 19/20 | 0.656 |
| Breast feeding (any time) | 79.5% | 87.2% | 0.742 |
| Months, median (range) | 2.82 (0-24) | 5.564 (0-30) | **0.012** |
| Tobacco exposure in house | 61.5% | 33.3% | **0.012** |
| Cigarettes smoked in house, median (range) | 6.897 (0-30) | 5.513 (0-40) | 0.092 |
| Traffic burden (Living in medium & high traffic street) | 28.2% | 43.6% | 0.161 |
| Aerobic exercise | | | |
| >1/week | 75% | 80% | 0.775 |
| >2/week (subjects >5years old, n=20 per group) | 35% | 50% | 0.291 |
| Pets in house | 23.1% | 23.1% | 1 |
| Only dog or cat | 12.8% | 17.9% | |

| | | | |
|---|---|---|---|
| Humidity in house | 35.9% | 35.9% | 1 |
| Older siblings, yes(>1)/total | 64.1(17.9) % | 61.5(15.4) % | 0.818 |
| Attending day care & school (1 month-18 years old, n=30 per group) | 66.7% | 66.7% | 1 |
| Parental atopy | 30.8% | 69.2% | **0.0005** |

**Table 2. Correlation values Rho (correlation coefficient, r) and their corresponding p-values, computed by Spearman's correlation between age and each cytokine, in healthy and atopic subjects separately and p-values of the comparison of the simple regression curves for each cytokine (regression slopes) between the two groups**.

All values are shown for both analyses, the one comprising subjects 0-45 years and the subanalysis referring to childhood, i.e. 0-18 years.

| | **0-45 years** | | | | | **0-18 years** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Healthy** | | **Atopic** | | | **Healthy** | | **Atopic** | | |
| **Feature** | **r** | **p** | **r** | **p** | **p slopes** | **r** | **p** | **r** | **p** | **p slopes** |
| unstimulated.IFN-α2 | 0.207 | 0.205 | 0.307 | 0.057 | 0.642 | 0.318 | 0.071 | 0.303 | 0.086 | 0.797 |
| R848.IFN-α2 | 0.017 | 0.918 | -0.063 | 0.702 | 0.368 | 0.145 | 0.421 | 0.120 | 0.507 | 0.325 |
| pIC.IFN-α2 | 0.2 | 0.222 | -0.027 | 0.87 | 0.104 | 0.099 | 0.584 | 0.039 | 0.828 | 0.689 |
| unstimulated.IFN-γ | 0.043 | 0.793 | 0.246 | 0.131 | 0.893 | -0.337 | 0.055 | -0.002 | 0.990 | 0.412 |
| R848.IFN-γ | 0.563 | **0.000** | 0.215 | 0.188 | 0.368 | 0.545 | **0.001** | 0.283 | 0.110 | 0.660 |
| pIC.IFN-γ | 0.516 | **0.001** | 0.299 | 0.064 | 0.095 | 0.438 | **0.011** | 0.325 | 0.065 | 0.904 |
| unstimulated.IL-1β | -0.149 | 0.365 | -0.028 | 0.868 | 0.936 | -0.177 | 0.323 | -0.033 | 0.857 | 0.320 |
| R848.IL-1β | 0.422 | **0.007** | 0.165 | 0.317 | 0.391 | 0.401 | **0.021** | 0.194 | 0.279 | 0.926 |
| pIC.IL-1β | 0.399 | **0.012** | 0.003 | 0.986 | 0.061 | 0.220 | 0.219 | -0.002 | 0.993 | 0.138 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| unstimulated.MCP-3 | -0.439 | **0.005** | -0.192 | 0.241 | 0.133 | -0.386 | **0.027** | -0.226 | 0.205 | 0.569 |
| R848.MCP-3 | 0.371 | **0.020** | 0.087 | 0.598 | 0.480 | 0.340 | **0.053** | 0.134 | 0.457 | 0.575 |
| pIC.MCP-3 | 0.284 | 0.080 | 0.119 | 0.472 | 0.665 | 0.332 | 0.059 | 0.181 | 0.315 | 0.765 |
| unstimulated.IL-10 | 0.685 | **0.000** | 0.271 | 0.095 | 0.671 | 0.605 | **0.000** | 0.075 | 0.678 | 0.091 |
| R848.IL-10 | 0.560 | **0.000** | 0.449 | **0.004** | 0.859 | 0.496 | **0.003** | 0.488 | **0.009** | 0.844 |
| pIC.IL-10 | 0.655 | **0.000** | 0.339 | **0.035** | **0.019** | 0.485 | **0.004** | 0.267 | 0.133 | 0.606 |
| unstimulated.IL-12p70 | 0.322 | **0.046** | 0.037 | 0.824 | 0.234 | 0.172 | 0.338 | 0.118 | 0.514 | 0.953 |
| R848.IL-12p70 | 0.415 | **0.009** | 0.231 | 0.158 | 0.552 | 0.463 | **0.007** | 0.331 | 0.060 | 0.953 |
| pIC.IL-12p70 | 0.051 | 0.757 | 0.168 | 0.307 | 0.796 | 0.101 | 0.578 | 0.266 | 0.134 | 0.242 |
| unstimulated.IP-10 | 0.511 | **0.001** | 0.256 | 0.115 | 0.153 | 0.635 | **0.000** | 0.182 | 0.310 | 0.114 |
| R848.IP-10 | 0.355 | **0.027** | 0.149 | 0.364 | 0.679 | 0.388 | **0.026** | 0.225 | 0.208 | 0.935 |
| pIC.IP-10 | 0.085 | 0.609 | 0.251 | 0.124 | 0.560 | 0.029 | 0.873 | 0.318 | 0.072 | 0.552 |
| unstimulated.TNF-α | 0.083 | 0.615 | -0.073 | 0.658 | 0.783 | 0.136 | 0.452 | -0.036 | 0.844 | 0.948 |
| R848.TNF-α | 0.277 | 0.088 | -0.136 | 0.407 | 0.087 | 0.267 | 0.134 | -0.047 | 0.795 | 0.374 |
| pIC.TNF-α | 0.310 | 0.055 | 0.055 | 0.740 | 0.096 | 0.193 | 0.282 | 0.098 | 0.586 | 0.319 |
| unstimulated.MDC | -0.016 | 0.924 | -0.062 | 0.707 | 0.758 | 0.215 | 0.229 | 0.093 | 0.607 | 0.628 |
| R848.MDC | -0.222 | 0.174 | -0.113 | 0.494 | 0.757 | -0.173 | 0.335 | -0.07 | 0.699 | 0.497 |
| pIC.MDC | -0.209 | 0.201 | -0.094 | 0.571 | 0.626 | 0.018 | 0.923 | 0.065 | 0.718 | 0.947 |
| unstimulated.MIP-1β | 0.128 | 0.438 | -0.275 | 0.091 | 0.409 | 0.160 | 0.373 | -0.236 | 0.186 | 0.446 |
| R848.MIP-1β | 0.430 | **0.006** | 0.068 | 0.680 | **0.004** | 0.427 | **0.013** | 0.246 | 0.168 | 0.465 |
| pIC.MIP-1β | 0.395 | **0.013** | 0.080 | 0.626 | **0.002** | 0.271 | 0.128 | 0.180 | 0.315 | 0.733 |
| unstimulated.IL-33 | 0.217 | 0.191 | 0.404 | **0.012** | 0.653 | -0.186 | 0.307 | 0.104 | 0.569 | 0.127 |
| R848.IL-33 | -0.009 | 0.955 | 0.137 | 0.413 | 0.735 | 0.219 | 0.227 | 0.176 | 0.334 | 0.861 |
| pIC.IL-33 | -0.025 | 0.881 | -0.108 | 0.517 | 0.599 | 0.172 | 0.346 | 0.037 | 0.840 | 0.373 |
| unstimulated.IL-23 | 0.221 | 0.182 | 0.202 | 0.224 | 0.545 | 0.064 | 0.728 | 0.218 | 0.232 | 0.784 |
| R848.IL-23 | 0.288 | 0.080 | 0.158 | 0.345 | 0.387 | 0.370 | **0.037** | 0.167 | 0.360 | 0.606 |
| pIC.IL-23 | 0.214 | 0.197 | 0.192 | 0.248 | 0.654 | 0.040 | 0.829 | 0.093 | 0.612 | 0.181 |
| unstimulated.IL-27 | -0.025 | 0.883 | -0.045 | 0.791 | 0.822 | 0.039 | 0.832 | 0.009 | 0.962 | 0.814 |
| R848.IL-27 | -0.011 | 0.949 | 0.094 | 0.576 | 0.351 | 0.022 | 0.903 | 0.100 | 0.586 | 0.804 |
| pIC.IL-27 | 0.179 | 0.282 | 0.269 | 0.103 | 0.208 | 0.330 | 0.065 | 0.351 | **0.049** | 0.930 |

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| | **0-45 years** | | | | | **0-18 years** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Healthy** | | **Atopic** | | | **Healthy** | | **Atopic** | | |
| **Feature** | **r** | **p** | **r** | **p** | **p slopes** | **r** | **p** | **r** | **p** | **p slopes** |
| unstimulated.IFN-α2 | 0.207 | 0.205 | 0.307 | 0.057 | 0.642 | 0.318 | 0.071 | 0.303 | 0.086 | 0.797 |
| R848.IFN-α2 | 0.017 | 0.918 | -0.063 | 0.702 | 0.368 | 0.145 | 0.421 | 0.120 | 0.507 | 0.325 |
| pIC.IFN-α2 | 0.2 | 0.222 | -0.027 | 0.87 | 0.104 | 0.099 | 0.584 | 0.039 | 0.828 | 0.689 |
| unstimulated.IFN-γ | 0.043 | 0.793 | 0.246 | 0.131 | 0.893 | -0.337 | 0.055 | -0.002 | 0.990 | 0.412 |
| R848.IFN-γ | 0.563 | **0.000** | 0.215 | 0.188 | 0.368 | 0.545 | **0.001** | 0.283 | 0.110 | 0.660 |
| pIC.IFN-γ | 0.516 | **0.001** | 0.299 | 0.064 | 0.095 | 0.438 | **0.011** | 0.325 | 0.065 | 0.904 |
| unstimulated.IL-1β | -0.149 | 0.365 | -0.028 | 0.868 | 0.936 | -0.177 | 0.323 | -0.033 | 0.857 | 0.320 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| R848.IL-1β | 0.422 | **0.007** | 0.165 | 0.317 | 0.391 | 0.401 | **0.021** | 0.194 | 0.279 | 0.926 |
| pIC.IL-1β | 0.399 | **0.012** | 0.003 | 0.986 | 0.061 | 0.220 | 0.219 | -0.002 | 0.993 | 0.138 |
| unstimulated.MCP-3 | -0.439 | **0.005** | -0.192 | 0.241 | 0.133 | -0.386 | **0.027** | -0.226 | 0.205 | 0.569 |
| R848.MCP-3 | 0.371 | **0.020** | 0.087 | 0.598 | 0.480 | 0.340 | **0.053** | 0.134 | 0.457 | 0.575 |
| pIC.MCP-3 | 0.284 | 0.080 | 0.119 | 0.472 | 0.665 | 0.332 | 0.059 | 0.181 | 0.315 | 0.765 |
| unstimulated.IL-10 | 0.685 | **0.000** | 0.271 | 0.095 | 0.671 | 0.605 | **0.000** | 0.075 | 0.678 | 0.091 |
| R848.IL-10 | 0.560 | **0.000** | 0.449 | **0.004** | 0.859 | 0.496 | **0.003** | 0.488 | **0.009** | 0.844 |
| pIC.IL-10 | 0.655 | **0.000** | 0.339 | **0.035** | **0.019** | 0.485 | **0.004** | 0.267 | 0.133 | 0.606 |
| unstimulated.IL-12p70 | 0.322 | **0.046** | 0.037 | 0.824 | 0.234 | 0.172 | 0.338 | 0.118 | 0.514 | 0.953 |
| R848.IL-12p70 | 0.415 | **0.009** | 0.231 | 0.158 | 0.552 | 0.463 | **0.007** | 0.331 | 0.060 | 0.953 |
| pIC.IL-12p70 | 0.051 | 0.757 | 0.168 | 0.307 | 0.796 | 0.101 | 0.578 | 0.266 | 0.134 | 0.242 |
| unstimulated.IP-10 | 0.511 | **0.001** | 0.256 | 0.115 | 0.153 | 0.635 | **0.000** | 0.182 | 0.310 | 0.114 |
| R848.IP-10 | 0.355 | **0.027** | 0.149 | 0.364 | 0.679 | 0.388 | **0.026** | 0.225 | 0.208 | 0.935 |
| pIC.IP-10 | 0.085 | 0.609 | 0.251 | 0.124 | 0.560 | 0.029 | 0.873 | 0.318 | 0.072 | 0.552 |
| unstimulated.TNF-α | 0.083 | 0.615 | -0.073 | 0.658 | 0.783 | 0.136 | 0.452 | -0.036 | 0.844 | 0.948 |
| R848.TNF-α | 0.277 | 0.088 | -0.136 | 0.407 | 0.087 | 0.267 | 0.134 | -0.047 | 0.795 | 0.374 |
| pIC.TNF-α | 0.310 | 0.055 | 0.055 | 0.740 | 0.096 | 0.193 | 0.282 | 0.098 | 0.586 | 0.319 |
| unstimulated.MDC | -0.016 | 0.924 | -0.062 | 0.707 | 0.758 | 0.215 | 0.229 | 0.093 | 0.607 | 0.628 |
| R848.MDC | -0.222 | 0.174 | -0.113 | 0.494 | 0.757 | -0.173 | 0.335 | -0.07 | 0.699 | 0.497 |
| pIC.MDC | -0.209 | 0.201 | -0.094 | 0.571 | 0.626 | 0.018 | 0.923 | 0.065 | 0.718 | 0.947 |
| unstimulated.MIP-1β | 0.128 | 0.438 | -0.275 | 0.091 | 0.409 | 0.160 | 0.373 | -0.236 | 0.186 | 0.446 |
| R848.MIP-1β | 0.430 | **0.006** | 0.068 | 0.680 | **0.004** | 0.427 | **0.013** | 0.246 | 0.168 | 0.465 |
| pIC.MIP-1β | 0.395 | **0.013** | 0.080 | 0.626 | **0.002** | 0.271 | 0.128 | 0.180 | 0.315 | 0.733 |
| unstimulated.IL-33 | 0.217 | 0.191 | 0.404 | **0.012** | 0.653 | -0.186 | 0.307 | 0.104 | 0.569 | 0.127 |
| R848.IL-33 | -0.009 | 0.955 | 0.137 | 0.413 | 0.735 | 0.219 | 0.227 | 0.176 | 0.334 | 0.861 |
| pIC.IL-33 | -0.025 | 0.881 | -0.108 | 0.517 | 0.599 | 0.172 | 0.346 | 0.037 | 0.840 | 0.373 |
| unstimulated.IL-23 | 0.221 | 0.182 | 0.202 | 0.224 | 0.545 | 0.064 | 0.728 | 0.218 | 0.232 | 0.784 |
| R848.IL-23 | 0.288 | 0.080 | 0.158 | 0.345 | 0.387 | 0.370 | **0.037** | 0.167 | 0.360 | 0.606 |
| pIC.IL-23 | 0.214 | 0.197 | 0.192 | 0.248 | 0.654 | 0.040 | 0.829 | 0.093 | 0.612 | 0.181 |
| unstimulated.IL-27 | -0.025 | 0.883 | -0.045 | 0.791 | 0.822 | 0.039 | 0.832 | 0.009 | 0.962 | 0.814 |
| R848.IL-27 | -0.011 | 0.949 | 0.094 | 0.576 | 0.351 | 0.022 | 0.903 | 0.100 | 0.586 | 0.804 |
| pIC.IL-27 | 0.179 | 0.282 | 0.269 | 0.103 | 0.208 | 0.330 | 0.065 | 0.351 | **0.049** | 0.930 |

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559 **Table 3. Median cytokine values and their interquartile range values in different**
560 **age groups (0-1, 1-6, 6-12, 12-18, >18 years) in healthy and atopic subjects.**

561 The values of most cytokines are in pg/ml, except IL-23, IL-27 & IL-28, which are
562 measured in ng/ml.

*P < 0.05 denotes a significant difference between healthy versus the atopic group at the same age group.

| | Variable | 0-1 years | | 1-6 years | | 6-12 years | | 12-18 years | | 18-45 years | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Healthy | Atopic | Healthy | Atopic | Healthy | Atopic | Healthy | Atopic | Healthy | Atopic |
| UNSTIMULATED | IFN-α2 | 2.2 (1.2 - 3.6) | 3.2 (1.2 - 7) | 5.4 (2.6 - 6.2) | 2.9 (1.2 - 7.9) | 3.9 (1.9 - 8) | 11.9 (9.4 - 12.2) | 24.4 (18.5 - 25.1) | 12.8 (3 - 30.4) | 3.7 (2.8 - 16.9) | 10.5 (2.7 - 25.2) |
| | IFN-γ | 1.6 (1.3 - 2.1) | 0.8 (0.7 - 1.6) | 0.9 (0.4 - 1.2) | 1.1 (0.5 - 1.4) | 1.3 (0.9 - 2) | 1.6 (1.1 - 2.5) | 1 (0.8 - 1.3) | 1.1 (0.7 - 1.9) | 2 (1.3 - 3) | 2.7 (1.6 - 3.9) |
| | IL-1β | 1.8 (0.9 - 2.1) | 2.6 (0.5 - 5) | 1.7 (0.6 - 2.4) | 1.1 (0.5 - 3.1) | 1.1 (0.5 - 3.1) | 1.4 (1.2 - 1.9) | 1 (0.9 - 2.4) | 0.7 (0.6 - 4.4) | 1.4 (0.9 - 1.5) | 1 (0.7 - 1.3) |
| | MCP-3 | 15.6 (15.5 - 15.6) | 15.6 (13.4 - 15.6) | 10.7 (10.7 - 17.8) | 14 (10.7 - 20) | 10.7 (10.7 - 17.3) | 10.7 (4.4 - 11.4) | 10.7 (8.1 - 10.7) | 11.4 (11.1 - 20) | 4.4 (2.9 - 4.4) | 12.8 (6 - 34.2) |
| | IL-10 | 0.9 (0.3 - 1.2) | 1.4 (0.9 - 6.8) | 1.4 (1 - 2.8) | 1.7 (1.1 - 3) | 3.9 (1.4 - 7.9) | 7.6 (6.9 - 8.8) | 4.6 (4 - 7.4) | 2.4 (0.5 - 3.9) | 6.7 (5.8 - 7.2) | 8.4 (4 - 11.2) |
| | IL-12p70 | 1.4 (1.4 - 1.6) | 1.6 (1.6 - 2.1) | 1.6 (1.2 - 1.9) | 1.6 (1.3 - 1.9) | 1.5 (1.5 - 2) | 2.4 (1.8 - 2.4) | 1.6 (1.4 - 2.1) | 2 (1.5 - 2.2) | 2.2 (1.9 - 2.6) | 1.7 (1.2 - 2.8) |
| | IP-10 | 1.1 (0.1 - 2.8) | 3.8 (3 - 17.5)* | 13.1 (4.8 - 35.4) | 12.8 (4.1 - 35) | 29.1 (3.2 - 78.9) | 21.3 (15.5 - 40.2) | 31.7 (20 - 40.9) | 13.3 (5.2 - 35.9) | 13.5 (7.4 - 34.8) | 47.4 (7.8 - 99) |
| | TNF-α | 3 (1.8 - 4.1) | 3.4 (2.4 - 8.2) | 2.9 (1.5 - 4.1) | 4.3 (2.8 - 6.3) | 3.1 (2.2 - 16.6) | 3.1 (2.4 - 6.4) | 4.1 (2.5 - 7.1) | 3.4 (2 - 17.6) | 2.2 (2.1 - 4.4) | 3 (2.2 - 4.2) |
| | MDC | 22.8 (3.2 - 140.6) | 118.4 (60.9 - 218.1) | 151.7 (98.2 - 205.7) | 202.7 (81.9 - 361.5) | 123.1 (54.2 - 535.8) | 146.4 (142.5 - 182.6) | 118 (91.4 - 174.2) | 102.9 (62.9 - 315.5) | 36.6 (22.8 - 56.7) | 56.4 (24 - 210.3) |
| | MIP-1β | 13 (10.2 - 25.9) | 38.5 (30.6 - 52.6)* | 14.3 (11.8 - 30) | 24.5 (15.4 - 34.9) | 26.2 (9.9 - 39.8) | 18.1 (8.5 - 23.1) | 20.7 (16 - 38.5) | 39.4 (12.7 - 60.5) | 30 (17.6 - 30.9) | 19.7 (12 - 35.4) |
| | IL-23 | 0.1 (0 - 0.1) | 0 (0 - 0.1) | 0.1 (0 - 0.1) | 0.1 (0 - 0.1) | 0.1 (0 - 0.1) | 0.1 (0.1 - 0.1) | 0.1 (0 - 0.1) | 0.1 (0.1 - 0.1) | 0.1 (0.1 - 0.1) | 0.1 (0.1 - 0.1) |
| | IL-27 | 0.1 (0 - 0.3) | 0.1 (0 - 0.1) | 0 (0 - 0.1) | 0 (0 - 0.1) | 0.1 (0 - 0.1) | 0 (0 - 0) | 0.1 (0 - 0.2) | 0.1 (0 - 0.1) | 0 (0 - 0) | 0 (0 - 0.1) |
| | | | | | | | | | | | |
| TLR3 | IFN-α2 | 2.2 (0 - 6.5) | 0.9 (0 - 3.3) | 1.8 (0 - 10.5) | 2.7 (1 - 4.2) | 7.5 (5.7 - 11.1) | 1.9 (0 - 7.3) | 2.1 (0 - 4.8) | 2.7 (0 - 13.4) | 4.4 (0.8 - 17) | 1.2 (0 - 3.2) |
| | IFN-γ | 0.5 (0 - 1) | 0.9 (0 - 3.3) | 1.1 (0.5 - 8.2) | 8 (0.4 - 15.8) | 10.3 (4.8 - 15) | 4.8 (2.4 - 11.1) | 17.9 (1.3 - 54.4) | 16.3 (7 - 53.9) | 16.8 (11.5 - 79.1) | 14.8 (5.1 - 24.1) |
| | IL-1β | 182 (53.7 - 421.2) | 277.3 (6.6 - 767.9) | 253.1 (37.7 - 570.1) | 219.5 (134.1 - 312.5) | 436.6 (131.1 - 770.5) | 654.2 (227.5 - 789) | 1416.4 (344.9 - 3044.7) | 148.9 (24.2 - 237.5) | 797.1 (631 - 944.5) | 198.3 (98.9 - 440.9)* |
| | MCP-3 | 95 (10.3 - 210.4) | 261.2 (75.8 - 893.5) | 473.5 (175.1 - 721.4) | 495.7 (63.4 - 1302.5) | 439.1 (233.6 - 1824.4) | 936.8 (906.1 - 1056.6) | 315.7 (139.8 - 682) | 659.1 (318.6 - 1006.8) | 198.9 (149.9 - 642.2) | 549.7 (361.9 - 796.4) |
| | IL-10 | 30.2 (8.3 - 75.9) | 251.9 (2.6 - 453.9) | 152.8 (54 - 317.7) | 109.6 (39.7 - 291.7) | 209.2 (106 - 389.3) | 900.3 (418.5 - 2364.6) | 1170.1 (757.1 - 1732.5) | 366.6 (46.1 - 876.7) | 1826.7 (1385.2 - 3099) | 660.2 (331.2 - 1169.3) |
| | IL-12p70 | 2 (0.2 - 3.5) | 0 (0 - 0) | 5.8 (1.8 - 8.5) | 17 (0.8 - 25.4) | 4.6 (2.7 - 8.2) | 2.3 (0 - 15.2) | 0.5 (0 - 2.3) | 2.6 (0.9 - 21) | 1.5 (0.8 - 3.8) | 1.6 (1 - 4.6) |
| | IP-10 | 3.1 (2.5 - 7.4) | 3.1 (0 - 36.9) | 8.8 (0 - 40.8) | 18.3 (3.8 - 74.4) | 0 (0 - 418.5) | 3.4 (0 - 60.4) | 17.6 (11.9 - 21.6) | 139.9 (71.8 - 225.9)* | 3.5 (1.5 - 32) | 17.1 (6.5 - 147.9) |
| | TNF-α | 203.9 (118.1 - 507.6) | 258.6 (34.2 - 711.6) | 295.2 (132.8 - 713.1) | 302.5 (203 - 607) | 591.7 (289.7 - 879.9) | 502.2 (324.5 - 970.9) | 679.4 (421.2 - 1082.3) | 422.3 (72.3 - 602.2) | 669.6 (648.5 - 1311.3) | 315.3 (141.5 - 654.4) |
| | MDC | 174.9 (143.9 - 486.9) | 414.6 (234.7 - 589.5) | 1465.2 (435.2 - 2092.4) | 1053.6 (620.6 - 1996.6) | 189.9 (146.7 - 1058.7) | 693.7 (580.6 - 1130) | 482.6 (148.1 - 832.7) | 551.8 (307.5 - 690) | 140.9 (100 - 389.6) | 356.3 (246.1 - 438.2) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **MIP-1β** | 827.4 (559.5 - 1562.1) | 1480.1 (707.2 - 5535.3) | 1774.7 (1220.7 - 3498) | 1667.2 (1083 - 2312.8) | 1157.9 (867.1 - 3091.6) | 3829.4 (2755.5 - 6044.5) | 2982 (1989.2 - 4064.8) | 2455.6 (983.3 - 3095.7) | 4133.2 (3440.4 - 9018.9) | 1901.8 (1378.2 - 2070.2)* |
| | **IL-23** | 0 (0 - 0.3) | 0 (0 - 0.3) | 0.2 (0.1 - 0.2) | 0 (0 - 0.4) | 0.1 (0.1 - 0.1) | 0.3 (0 - 0.7) | 0 (0 - 0.1) | 0.1 (0 - 0.5) | 0.4 (0.1 - 0.6) | 0.1 (0.1 - 0.8) |
| | **IL-27** | 0 (0 - 0) | 0 (0 - 0.1) | 0 (0 - 0.1) | 0 (0 - 0.1) | 0 (0 - 0.3) | 0.1 (0 - 0.2) | 0.1 (0 - 0.1) | 0.2 (0.1 - 0.2) | 0 (0 - 0.1) | 0 (0 - 0.3) |
| | | | | | | | | | | | |
| **T L R 7/8** | **IFN-α2** | 123.2 (45.1 - 353.4) | 104.9 (16.4 - 197) | 264.8 (174.9 - 355.8) | 243.7 (61.9 - 471) | 92.4 (62.1 - 330.5) | 202.6 (69.3 - 1002.6) | 179.8 (22.2 - 442) | 178.3 (147 - 309.6) | 106.6 (43.2 - 417.2) | 44 (29.1 - 141.4) |
| | **IFN-γ** | 29.6 (1.5 - 70.5) | 79.6 (26.8 - 266) | 252.8 (49.6 - 426.1) | 138.6 (26.2 - 485.4) | 131.7 (67.1 - 367.5) | 94.2 (82.3 - 1005.9) | 696.1 (317.2 - 1168) | 490.7 (223 - 1748.3) | 840.5 (464.7 - 1612.1) | 273.1 (20.8 - 994.4) |
| | **IL-1β** | 892.8 (430.2 - 1480.5) | 2196.3 (1346 - 8889.9) | 3315 (2572.4 - 9117.9) | 4398.9 (1260.7 - 9795.1) | 2011.6 (1552.9 - 3443.9) | 12712.4 (2154.5 - 15004.4) | 6637.4 (3775.5 - 11247.1) | 9541.7 (4113.2 - 12882.1) | 12224.5 (5878.7 - 12784.4) | 6543.6 (1149.7 - 12760.9) |
| | **MCP-3** | 84.8 (24.8 - 803.5) | 668.8 (411.9 - 1181.5) | 484.8 (336.8 - 839.5) | 1033.9 (59 - 2363.8) | 644.1 (113.5 - 1290.1) | 1386.3 (1181.8 - 2043.6) | 1240.8 (988.5 - 1531.8) | 1458.3 (385.6 - 1639.3) | 1096 (590.9 - 1587.5) | 1138 (744.7 - 1513.1) |
| | **IL-10** | 33 (21.3 - 596.5) | 331.8 (214.4 - 654.2) | 888.4 (347.2 - 1932.4) | 821.3 (428.7 - 1513.8) | 597.1 (299.2 - 1653.1) | 1807.7 (1400 - 2519.2) | 1263 (888.5 - 3280.9) | 1732.3 (1127.9 - 5030.9) | 3160.2 (1225.7 - 3532.4) | 2122.1 (1334.1 - 3018.3) |
| | **IL-12p70** | 5.5 (1.6 - 8.7) | 3.8 (0.9 - 10.1) | 13.6 (8.6 - 20.7) | 13.6 (5.6 - 38.9) | 17.3 (11.5 - 51.6) | 11.7 (6.5 - 13.3) | 32 (23.6 - 34.1) | 49.1 (16.7 - 79.7) | 21.4 (9.8 - 39.5) | 9.8 (6.1 - 18.8) |
| | **IP-10** | 296.9 (51.9 - 716.4) | 561.2 (323.1 - 1264.6) | 715 (542.7 - 1129.6) | 832.1 (402.1 - 1919.5) | 365.1 (248.8 - 1052.8) | 554.4 (455.9 - 1170.7) | 1450.8 (1142.7 - 1620.7) | 2310.3 (639.8 - 4022.1) | 1267.6 (594.6 - 1903.4) | 898.1 (148.5 - 2245) |
| | **TNF-α** | 1586.6 (837.6 - 3269.1) | 5251.3 (2357.3 - 8742.9) | 3708.6 (3218.5 - 5508.9) | 3168.2 (1867.9 - 4249.8) | 2665.6 (1432 - 3550.6) | 3394.2 (3162.4 - 5615.7) | 6579.2 (3974 - 12670.9) | 5551.1 (1941.7 - 17027.3) | 3725.8 (2961.3 - 21613.8) | 2728.8 (1619.2 - 3295.3) |
| | **MDC** | 48.1 (10.1 - 207.2) | 144.8 (8.5 - 260.2) | 37.6 (0 - 68.8) | 70.2 (0 - 242.4) | 137.6 (0 - 214.6) | 2 (1.9 - 250.8) | 50.4 (0 - 116.1) | 82.9 (0 - 161.6) | 1.5 (0 - 35.8) | 24.3 (2 - 123.1) |
| | **MIP-1β** | 898.9 (763.8 - 3273.3) | 4564.3 (1650.6 - 6580.4) | 5438.4 (2597.7 - 6369.9) | 4318.8 (2259.5 - 8196.3) | 4069.9 (2754.5 - 5028.6) | 4012.5 (3248.2 - 10256.7) | 7306.8 (3558.1 - 11573.9) | 5454 (4174.1 - 7634.9) | 6029.4 (4151.2 - 38502.1) | 3823.7 (2054.3 - 3872.9) |
| | **IL-23** | 0 (0 - 0) | 0 (0 - 0.1) | 0 (0 - 0.1) | 0 (0 - 0.1) | 0.1 (0 - 0.3) | 0.2 (0 - 0.3) | 0 (0 - 0.1) | 0.3 (0 - 0.4) | 0 (0 - 0.1) | 0.1 (0 - 0.5) |
| | **IL-27** | 0 (0 - 0.3) | 0.1 (0.1 - 0.1) | 0.1 (0 - 0.1) | 0.1 (0 - 0.3) | 0.2 (0 - 0.2) | 0.1 (0.1 - 0.3) | 0.1 (0 - 0.2) | 0.2 (0 - 0.3) | 0.1 (0 - 0.1) | 0.2 (0.1 - 0.4) |

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# Figure legends

**Figure 1. Maturation trajectories of TLR3 and TLR7/8-induced cytokine production from birth to adulthood (0-45 years).**

Fresh PBMCs from 39 healthy and 39 atopic subjects were stimulated for 24h with poly(I:C) and R848 and selected cytokines were measured by Luminex® 200™ in culture supernatants. Data are expressed in the form of net cytokine values (i.e. difference after the subtraction of baseline cytokine concentrations in TLR-stimulated samples) and plotted as individual points on the graph, both for healthy (blue) and atopic (red) subjects. The y-axes represent cytokine concentrations in pg/ml or ng/ml (IL-23) and x-axes age in years. All data were included to estimate the non-parametric correlation between age and cytokine expression using Spearman's rho test. Moreover, polynomial regression analysis was performed, taking into account age as the independent variable and each cytokine response as the dependent variable.

**Figure 2. Correlogram depicting correlations of selected cytokines in healthy (n=39) and atopic (n=39) subjects with age.**

Spearman's rho correlation between any cytokine and age is symbolized with a circle. Blue circles define positive correlations and red circles define negative correlations, while size is proportional to rho. Healthy and allergic are considered separately. Asterisks correspond to statistically significant p-values (* as for <0.05, ** as for <0.01, *** as for <0.001).

**Figure 3. PCA and age tagging of the healthy (A) and atopic- allergic (B) subjects, as well as the contributions of the selected cytokines for healthy (C) and atopic-allergic (D) subjects to the PCA.**

Two separate PCA procedures were performed: one with the original selected cytokines (colored points), and the other with the selected cytokines aggregated with median per age group (colored tags). Red dashed lines denote average contributions.

Data were centered and scaled prior to PCA.