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Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Age-related changes in the inflammatory responses to viral infections in the central nervous system during childhood

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BACKGROUND: The developmental stages and function of immune cells in the central nervous system during infancy and childhood are poorly understood. We analyzed whether cytokine and chemokine profiles in children and adolescents with viral central nervous system infections were different depending on age.
METHODS: The acute phase cerebrospinal fluid of 80 children (mean age 98 months, range 1–206 months) were analyzed for protein levels of interleukin-1β (IL-1β), IL-1-RA, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, monocyte chemoattractant protein-1 (MCP-1), interferon (IFN) gamma-induced protein 10 (IP-10), IFN-γ, and macrophage migration inhibitory factor (MIF).
RESULTS: We found an age-dependent increased expression of IL-4, IL-6, IL-13, MIF, IP-10, and IFN-γ and a decreased expression of MCP-1 and IL-15 in response to a viral infection of the central nervous system. In contrast, all other cytokines and chemokine were unaffected by the age of the patient.
CONCLUSION: These findings demonstrate that the immunological response to a viral infection matures during childhood and adolescence. This may in turn be of importance for the outcome of a viral infection and the risk for subsequent sequela. It also demonstrates that age is a factor that needs to be considered when using cytokines and chemokines as biomarkers for infections in the central nervous system.

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IMPACT:

- The immunological response to a viral infection matures during childhood and adolescence.
- This may be of importance for the outcome of a viral infection and the risk for subsequent sequela. It also demonstrates that age is a factor that needs to be considered when using cytokines and chemokines as biomarkers for infections in the central nervous system.

INTRODUCTION

The immune system is far from fully developed at birth, even if most cells and soluble factors can be detected very early in life. Peripheral monocytes are high in numbers at birth but with a narrower mode of action which affects cytokine and chemokine production. Similarly, dendritic cells mature over the first years of life, whereas the lymphocyte populations, both B and T cells, are high at the time of birth, and increase over the first year of life to then decrease to adult levels during childhood. However, T cells are impaired both by lack of function and infectious experience, particularly during the first year of life. This is likely multifactorial but lack of interleukin-2 (IL-2) is considered of central importance, as is the T helper type 2 (Th2) tilt due to the lack of interferon-γ (IFN-γ). Likewise, B cell function is thought to develop as a result of infections and reaches adult function around adolescence (for a review of peripheral immune defense maturation, see ref. [1]).

In the central nervous system (CNS), postnatally protected by the blood–brain barrier (BBB), resident microglia, macroglia, and perivascular myeloid cells constitute the resident immune cells. These cells all have the capacity to produce cytokines and chemokines and play a key role in attracting other immunological cells and induce an immunological response. In contrast, lymphocytes are not resident in the CNS but invade upon challenge. The immunological cell populations and the functional potential thus differ between the periphery and the CNS, but if and how the CNS immune response also matures during childhood is poorly understood.

Cytokines and chemokines are key intercellular mediators in inflammation with specific properties that make them useful as potential biomarkers. They also exhibit differential patterns of expression depending on the etiology of inflammation and may be used as biomarkers of viral CNS infections.[2,3] However, whether an age-specific expression during childhood limits the utility of certain cytokines/chemokines has not been described.

In CNS infections, the host–immune response plays an important role in disease severity and outcome and cytokine/chemokine levels in the cerebrospinal fluid (CSF) have been shown to correlate both to severity and outcome.[4–7] Moreover, the host–immune response during CNS infection, measured by, for example, cytokines and chemokines, appears to differ between pathogens, most likely representing different pathogeneses.[8–10] In addition,

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the cytokine/chemokine profile in the CSF may be used to differentiate between viral or autoimmune encephalitis.[11,12]

In order to increase the understanding of the utility of CSF cytokines and chemokines as biomarkers in childhood CNS infections, we examined whether the expression of these molecules varied by age. Our finding of such an immunological maturation demonstrates that age needs to be considered when using cytokines and chemokines as biomarkers.

METHODS

Subjects

In this study, we included children with viral CNS infections that originated from two different cohorts. The original cohort originated from a prospective study of childhood viral encephalitis in which children aged 28 days to 17 years who were admitted to a primary and tertiary care hospital in Stockholm, between May 2011 and May 2016, due to acute encephalitis were included.[13] The diagnosis of encephalitis was based on the following criteria: (1) signs of cerebral dysfunction either as (i) encephalopathy defined as altered consciousness, personality, or behavioral changes lasting for >24 h, or (ii) abnormal electroencephalography findings compatible with encephalitis, plus at least one of the following: abnormal results of neuroimaging compatible with encephalitis, positive focal neurological findings, or seizures. (2) Signs of infection, defined either as pleocytosis (≥6 white blood cells (WBCs)/μL), fever (>38 °C), or elevated infectious parameters, C-reactive protein (CRP), and WBCs. Catarrhalia was considered not to be sufficient. Children with another verified cause of symptoms such as bacterial meningitis or other underlying neurological or metabolic disease or verified genetic epilepsy that per se could explain the symptoms were excluded. Pure ataxia was not considered sufficient neurology for inclusion. In addition to this cohort, children with tick-borne encephalitis (TBE) during 2004–2010 were retrospectively identified and retrieved from the local Swedish Institute for Infectious Disease Control in Stockholm as described previously.[4] The lumbar puncture was performed in the acute phase of the illness upon presentation to healthcare in all children. The samples from both cohorts were stored at −70 °C until analysis.

Analysis of cytokines and chemokines

A premade multiplex assays (Bio-Plex Pro, Life Science Bio-Rad) was used for detection of IL-1β, IL-1-RA, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, IL-18, IFN-γ, monocyte chemoattractant protein (MCP-1), and macrophage migration inhibitory factor (MIF). The multiplex assay was run according to the manufacturer's protocol using Luminex 200 (Luminex Corporation, TX). CSF samples were assessed as undiluted samples of a volume of 50 μL/sample as previous preliminary experiments showed generally low concentrations of several of the measured cytokines in CSF. Due to limitations in volume, only single samples could be run. Standards were added to provide calibration curves. The calibration curves for each analyte were calculated using the Bio-Plex software. Data points below the detection limit were given the value of half the detection limit. Similarly, values above detection were given a value twice the upper limit.

Ethics

Ethical approval was obtained from the local ethics committee in Stockholm prior to the start of the study.

Statistics

Generalized linear models were applied to analyze the different outcome variables and the independent variable age. All outcome variables were transformed. Variables that met the normality assumptions with a log transformation were analyzed with a linear regression model. For variables that did not meet the normality assumptions, data were transformed to a binary outcome and thereafter analyzed with generalized linear models with a log link. Data analysis was performed using R version 3.4.4 and statistical significance was defined as $p < 0.05$.

Table 1. Etiologies and age of cohort.

Etiology	*n*	Mean age (months)	Age span
Corona	1	22	
EBV	1	153	
Entero	2	39	1–77
Herpes	1	11	
Influenza B	2	37	
Metapneumo	1	51	
Rhino	1	151	
Rota	6	33	23–51
TBE	46	125	57–206
Varicella	4	87	50–168
Unknown	15	68	6–202
All	80	98	1–206

RESULTS

Study cohort

We analyzed the expression of cytokines and chemokines in CSF from 80 children with viral CNS infections. The mean age of included children was 98 months (range 1–206 months). The etiology was dominated by TBE virus, but also included herpes simplex virus, Epstein–Barr virus, influenza B virus, rhinovirus, varicella-zoster virus, rotavirus, coronavirus, metapneumovirus, enterovirus, and several cases with an unknown etiology (Table 1).

Cytokine analysis

Analysis revealed age-dependent differences for several cytokines and chemokines. A linear increasing expression with age was seen for IL-4 (Fig. 1a, $p < 0.001$), IL-6 (Fig. 1b, $p < 0.001$), IL-13 (Fig. 1c, $p < 0.001$), IFN-γ (Fig. 1d, $p < 0.001$), and MIF (Fig. 1e, $p < 0{,}001$). Conversely, levels of MCP-1 decreased with increasing age (Fig. 1f, $p = 0{,}02$). The expression of IL-15 and IFN-γ-induced protein 10 (IP-10) also displayed age-dependent differences ($p = 0.005$ and 0.003, respectively), but not with a linear relationship. Rather, the binary statistical analysis revealed an increased chance of obtaining a high value with increasing age for IP-10 and a low value with increasing age for IL-15, but the non-normality of these variables makes interpretation difficult. The levels of IL-1β, IL-1RA, IL-7, IL-8, IL-10, IL-12, IL-17, and IL-18 did not vary with age.

DISCUSSION

The aim of the present study was to study whether the acute CSF cytokine/chemokine levels in response to viral CNS infections varied with age during childhood. As CSF cytokines are considered to correspond to the intrathecal activation of immunoactive cells, a maturation of the immunological response would affect their utility as biomarkers in certain age groups.

The resident immune cells of the CNS are microglia, macroglia, and perivascular myeloid cells. Together with cells invading from the periphery in response to, for example, infection, they will initiate and maintain a complex pattern of inflammatory processes in response to the threat. Although cytokines and chemokines have multiple effects on different types of inflammatory cells, they often have unique properties and patterns of expression which potentially make them useful as biomarkers.

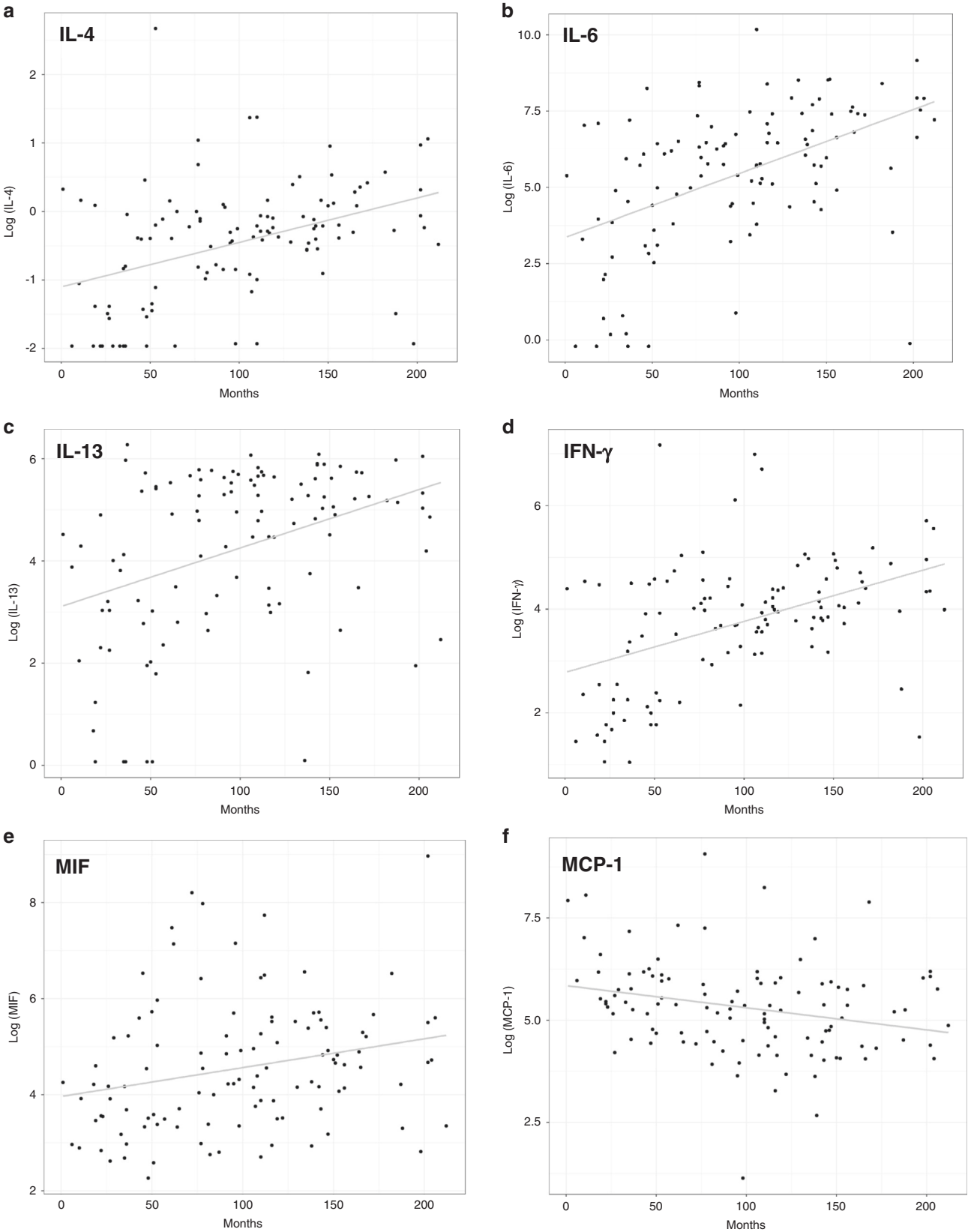

Fig. 1 Cytokine expression during CNS infection. Cytokine expression during CNS infection increased with increasing age for IL-4 (**a**), IL-6 (**b**), IL-13 (**c**), IFN-γ (**d**), and MIF (**e**). Conversely, expression of MCP-1 decreased with increasing age. Values on *y*-axis are log-transformed.

An age-dependent increase was seen in the CSF levels of IL-4. As T cells infiltrate from the periphery upon infection, they most likely follow the maturation observed in the periphery where IL-4 production is age-dependent.[14] IL-4 is of key importance for T cell activation and B cell maturation, and once production is switched on, it is most likely maintained in an autocrine fashion by T cells themselves, also in the CNS. This hypothesis is supported by data showing that the intrathecal production of IL-4 correlates with the progression of Rasmussen encephalitis, a T cell-dominated CNS disease.[15] IL-13 function is closely related to IL-4 along the Th2 axis where the combined effects include altered T cell regulation ultimately favoring an anti-inflammatory response. We found a similar age dependence for IL-13. This combined increase in IL-4 and IL-13 most likely contribute to increased neuroprotection during childhood (reviewed in ref. [16])

The Th1-axis cytokine IFN-γ is also likely to be produced from infiltrating peripheral lymphocytes and the observed age-dependent increase in CSF IFN-γ is in line with the observed maturation of the peripheral IFN-γ response, known to increase with age.[17] Interestingly, mouse strains that produce higher amounts of IFN-γ for a given virus load and T cell level are more susceptible to TBE infections as compared to strains with lower levels.[18] This may be a contributing factor to why clinical TBE disease is more commonly seen in older children with lower levels seen in the younger age groups.

IL-6 is a key player in the acute early pro-inflammatory response following infection. It can be produced by many cells, but macrophages and monocytes are the main cell types. Whereas children reach adult levels of IL-6 production in the periphery ~3 years of age, we here observed a continuous increase in CSF IL-6 during childhood. Although astrocytes and infiltrating lymphocytes may contribute, it is likely that IL-6 in the CNS is produced by resident microglia as well as infiltrating monocytes. While normally quiescent, microglia can become activated by infection through soluble activators such as IFN-γ. Upon activation, microglia will switch on antigen presentation and produce cytokines such as IL-1β, IL-6, TNF, and IP-10.[19] In particular, the slower maturation of the CNS IL-6 response as compared to that seen in the periphery indicates that microglia are of importance. The origin of microglia is complex and not entirely understood. One set of microglia is most likely of mesodermal origin and invade the fetal CNS around the end of the first trimester. These cells can replicate and replenish the microglia pool throughout life. A second set of microglia is thought to invade from the periphery, peri-, and postnatally, and derive from a different lineage than monocytes (reviewed in ref. [20]). The hypothesis of a relationship between microglia and fetal monocytes, which produce less cytokines,[21] may be part of the explanation for the reduced/delayed cytokine response rate intrathecally.

We also found increasing levels of MIF, another pro-inflammatory cytokine that is most likely also produced by microglia in the CNS and has a role in auto-activating microglia and initiating pro-inflammation.[22] Excessive levels of MIF are associated with poor prognosis in CNS infections[23] and mice deficient for MIF show reduced inflammation and reduced viral loads. This effect has been shown to be dependent on the ability of MIF in regulating the BBB and allowing viral entry into the CNS in the case of murine West Nile encephalitis.[24] The age-dependent increase of MIF is most likely related to the maturation of microglia and may play a role in age-dependent severity observed in human cases of West Nile virus infections.[25]

Astrocytes, the most common cells in the CNS and with a neuroepithelial origin, have the capacity to present antigens, albeit not as well as macrophages, but also to produce significant amounts of cytokines such as MCP-1. This chemokine, also known as chemokine (C–C motif) ligand 2, regulates migration and infiltration of monocytes/macrophages and is believed to play a major role in the recruitment of these cells. MCP-1 was the only cytokine examined that displayed an age-dependent linear decrease in expression following infection. It is also known as a regulator of the integrity of the BBB,[26] raising the possibility that the observed decrease in MCP-1 with age is part of an age-dependent tightening of the BBB.

The findings of increasing expression of IL-15 and IP-10 (also known as C–X–C motif chemokine ligand 10) is somewhat difficult to interpret and will require further confirmation. As data did not meet the normality criteria, a dual analysis was performed where data were transformed to a binary outcome (a high or low value) and thereafter analyzed with generalized linear models with a log link. With this approach, the chance of having a high expression of IP-10 increases with increasing age and, conversely, decreases with increasing age for IL-15. However, alterations are not linear. This may be due to technical detection limits but may also indicate that age is not the only (or even main) factor affecting this increase.

The current study was performed on CSF samples obtained in the acute phase of disease upon presentation to healthcare. The detected cytokine levels, therefore, reflect the acute situation and how they may be used as acute biomarkers. In contrast, they do not give information on the temporal development of responses or the consequent anti-inflammatory responses that may follow. Also, severe symptoms that will lead to seeking healthcare may appear at different times after primary infection for different pathogens.

A limitation of this study is that specific viral etiologies will vary with age with some viral infections predominantly affecting younger children and others being more prevalent among adolescents. This could lead to confounding so that differences are etiology-specific rather than age-specific. However, although controlling for this possible confounder is not possible due to small individual groups and minimal overlap for some etiologies, we do see similar but statistically nonsignificant age-dependent trends also in the larger etiological groups such as the TBE group. Furthermore, it could be argued that a given cytokine/chemokine expression at a certain age reflects the etiologies prevalent for that age group and that findings represent age-specific expression irrespective of etiology. Future studies are needed to address the role of specific pathogens for cytokine induction. We believe that these findings are of importance in evaluating how specific cytokines may be used as biomarkers, as well as offering insight into physiological mechanisms of immunological maturation in the CNS.

Lymphocytes are not resident in the CNS but may invade upon infection or inflammation, this is to some degree also true for peripheral monocytes. The developmental stages during infancy and childhood of immune cells that are resident in the CNS are poorly understood. In this study, we report an age-dependent expression of several cytokines/chemokines in response to a viral infection of the CNS, indicating that the immunological response to a viral infection matures during childhood and adolescence. This may in turn be of importance for the outcome of a viral infection and the risk for subsequent sequela. It also demonstrates that age is a factor that needs to be considered when using cytokines and chemokines as biomarkers for infections in the CNS.

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AUTHOR CONTRIBUTIONS

S.Y., Å.F., and R.W. contributed to conception and design, acquisition, analysis, and interpretation of data; drafting the article; and approve the final version.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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