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Herpesvirus Seropositivity in Childhood Associates with Decreased Monocyte-Induced NK Cell IFN-γ Production

Shanie Saghafian-Hedengren,2,3*, Yvonne Sundström,3* Ebba Sohlberg,*, Caroline Nilsson,† Annika Linde,‡ Marita Troye-Blomberg,*, Louise Berg,4§ and Eva Sverremark-Ekström4*

EBV infection is inversely associated with IgE sensitization in children, and this association is further enhanced by CMV coinfection. In mice, herpesvirus latency causes systemic innate activation and protection from bacterial coinfection, implying the importance of herpesviruses in skewing immune responses during latent infection. Early control of viral infections depends on IFN-γ release by NK cells, which generally requires the presence of accessory cells. We investigated IFN-γ production by NK cells in PBMCs from children seropositive (SP) for EBV alone, for both EBV and CMV, or seronegative for both viruses. The ability of classical (CD14++CD16−) and proinflammatory (CD14++CD16+) monocytes to induce autologous NK cell IFN-γ was studied by coculture experiments with enriched CD3−CD56+ cells. Transwell experiments were used to evaluate how monocytes interact with NK cells to induce IFN-γ synthesis. SP children had a significantly reduced proportion of IFN-γ+ NK cells and cognate intracellular IFN-γ levels, which was more pronounced in CMV-coinfected subjects. Also, resting PBMCs of SP children displayed lower proportions of proinflammatory monocytes. IFN-γ production by NK cells was dependent on interactions with monocytes, with the proinflammatory subset inducing the highest IFN-γ. Finally, SP children had markedly lower levels of plasma IFN-γ, concurrent with in vitro findings. Herpesvirus infections could be one contributing factor for maturation toward balanced Th1-Th2 responses. Our data indicate that early infection by herpesviruses may affect NK cell and monocyte interactions and thereby also influence the development of allergies. The Journal of Immunology, 2009, 182: 2511–2517.

The viruses EBV and CMV belong to the herpesvirus family and are widely spread in human populations. Primary infections with EBV and CMV often occur during early childhood and are usually asymptomatic or mild. After primary infection, EBV and CMV establish latency and persist for the lifetime of the host. Seropositivity for these viruses can vary among populations dependent on their socioeconomic background, and young children in industrialized countries seem more protected from early infection (1, 2). Latently infected memory B cells are believed to constitute the main site for EBV persistence (3), whereas the location for carriage of infected memory B cells are believed to constitute the main site for EBV persistence (3), whereas the location for carriage of CMV DNA is less clear but has been shown to include cells of the myeloid lineage (4).

Because Ag-specific immune responses require time to develop and expand, components of innate immunity play an important role in the early phase of infections. NK cells are activated early after a viral infection, and one of the central mechanisms by which they mediate antiviral activity is through IFN-γ. This cytokine is a potent activator of APCs by influencing their maturation and antiviral functions (5, 6). IFN-γ can also act as an immunoregulatory cytokine in that it plays a central role in the shaping of Th1 immune responses (7).

Although NK cells can exert immediate attacks, they generally do not become fully functional unless triggered by cytokines that are released upon recognition of viruses or viral components (6). Among these cytokines are the proinflammatory ILs, IL-12, IL-15, and IL-18, which are produced largely by accessory cells, such as dendritic cells (DCs) and monocytes (5, 6, 8, 9). In addition to soluble factors, NK cells can become activated by a number of contact-dependent mechanisms provided by accessory cells, and numerous studies have investigated such interactions between NK cells and DCs (10–12). NK cell and monocyte interactions have received less interest, although it has been demonstrated that depletion of CD14+ cells markedly reduces IFN-γ production by NK cells (13, 14). In addition, blocking of the costimulatory molecules CD40 (15), CD80, and CD86 (16) on activated monocytes has been shown to decrease NK cell IFN-γ production. However, distinction of monocytes on the basis of CD14 expression alone might not be optimal for examining their interactions with other cells, given that monocytes represent a heterogeneous population with functional differences. Monocytes are roughly divided into two major subsets and are classified based on differential expression degrees of CD14 and CD16 (FcγRIII). The larger subset is CD14++CD16− (classical

* Abbreviations used in this paper: DC, dendritic cell; PGN, peptidoglycan; SN, seronegative; SP, seropositive; GeoMFI, geometrical mean fluorescence intensity.

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monocytes), whereas the remaining subset is CD14+CD16- (non-
classical or proinflammatory monocytes) (17, 18). Proinflamma-
tory monocytes express higher levels of MHC class II and CD86
(17, 18) and are more likely to differentiate into DCs (19). They
also produce larger amounts of proinflammatory cytokines and
(17, 18) and are more likely to differentiate into DCs (19). They
are associated with a bias toward a Th1-type cytokine profile (26).
Twenty-six 2-year-old subjects was observed, whereas CMV infection was
preferentially promote Th2 responses (23, 24).

Our group has previously reported an inverse association be-
tween EBV seropositivity and IgE sensitization in 2-year-old chil-

Table I. Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>CMV− EBV− Children</th>
<th>CMV+ EBV− Children</th>
<th>and/or EBV+ Children</th>
<th>CMV− EBV+ Children</th>
<th>CMV+ EBV+ Children</th>
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<tr>
<td>Group size (n)</td>
<td>26</td>
<td>25</td>
<td>16</td>
<td>9</td>
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<tr>
<td>Sex of child (n; girl/boy)</td>
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<td>9/16</td>
<td>7/9</td>
<td>2/7</td>
<td>NS NS</td>
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<tr>
<td>Delivery mode (n; vaginal/cesarean)</td>
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<td>22/2</td>
<td>14/2</td>
<td>8/1</td>
<td>NS NS</td>
</tr>
<tr>
<td>Maternal age at delivery (yr)</td>
<td>32 (23–42)</td>
<td>28 (21–35)</td>
<td>29 (21–35)</td>
<td>28 (23–35)</td>
<td>0.01 0.03</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.5 (2.7–4.4)</td>
<td>3.7 (3.0–4.8)</td>
<td>3.6 (3.0–4.8)</td>
<td>3.9 (3.4–4.2)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Gestation (wk)</td>
<td>40 (36–43)</td>
<td>40 (38–45)</td>
<td>40 (39–43)</td>
<td>40 (38–42)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Exclusive breast-feeding (mo)</td>
<td>4 (0–10)</td>
<td>4.5 (1–10)</td>
<td>4 (1–10)</td>
<td>4.5 (1–5)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Attending day care (n; yes/no)</td>
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<td>23/2</td>
<td>15/1</td>
<td>8/1</td>
<td>NS NS</td>
</tr>
<tr>
<td>Age at day care start (mo)</td>
<td>19 (14–24)</td>
<td>18 (14–21)</td>
<td>18 (14–21)</td>
<td>17 (14–20)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Housing (n; apartment/house)</td>
<td>14/12</td>
<td>13/12</td>
<td>7/9</td>
<td>6/3</td>
<td>NS NS</td>
</tr>
<tr>
<td>Pets at home (n; dog/cat)</td>
<td>2/5</td>
<td>1/1</td>
<td>1/1</td>
<td>0/0</td>
<td>NS NS</td>
</tr>
<tr>
<td>Siblings (n; 0–5)</td>
<td>16/10</td>
<td>12/13</td>
<td>7/9</td>
<td>5/4</td>
<td>NS NS</td>
</tr>
<tr>
<td>Maternal smoking (n; yes/no)</td>
<td>1/25</td>
<td>3/22</td>
<td>2/14</td>
<td>1/8</td>
<td>NS NS</td>
</tr>
</tbody>
</table>

Statistical comparison between two groups.

Statistical comparison between three groups. Median (range).

EBV and CMV (n = 26) and the other group (n = 25) with subjects SP for either EBV alone (n = 16) or both EBV and CMV (n = 9). Further, buffy coats from healthy adult blood donors were obtained from the blood bank at Karolinska University Hospital (Stockholm, Sweden). This study was approved by the Human Ethics Committee at Karolinska University Hospital (Huddinge, Sweden) and Karolinska Institutet, and the parents provided their informed consent.

Separation of plasma and mononuclear cells from blood, cell isolation, and culture.

Following collection of venous blood, plasma was separated after centrifugation and stored at −70°C. PBMCs were isolated by Ficoll-Paque (Phar-
macia-Uipoh) gradient centrifugation and cryopreserved in liquid nitrogen. For the in vitro experiments, PBMCs were thawed, then washed twice in RPMI 1640-HEPES (Life Technologies), and resuspended in RPMI 1640 supplemented with 10% heat-inactivated FCS (HyClone Laborato-
ries), l-glutamine (2 mM/L), penicillin G sodium (100 U/ml), and strep-
tomycin sulfate (100 μg/ml; Merck) to a concentration of 106 cells/ml and cultured for 24 h in 48-well (Corning) or 96-well (Sarstedt) flat-bottom plates. PBMCs were cultured in medium alone or with IL-15 (20 ng/ml; R&D Systems), peptidoglycan (PGN; 1 μg/ml; Staphylococcus aureus; Fluka; Sigma-Aldrich) or IL-15 and PGN in combination. Supernatants were stored at −85°C until analysis. Where indicated, CD3+ CD56− NK cells, CD14+ monocytes, or subpopulations of monocytes (CD14+ CD16+ and CD14+ CD16−) were separated from PBMCs of healthy adult donors, using MACS isolation kits according to the manufacturer’s instruc-
tions (Miltenyi Biotec). Enriched monocytes and autologous NK cells were

Materials and Methods

Characterization and serostatus of subjects

A total of 51 IgE-nonsensitized 2-year-old children (Table I) were selected from an earlier described family cohort (25). The presence of serum IgG against EBV capsid Ag was determined according to previously published immunoassays (30) where a specific fluorescence at a dilution of 1/20 was regarded as a sign of seropositivity. IgG seropositivity to CMV was determined by ELISA using purified nuclear Ags from CMV culti-
vated in human fetal lung fibroblasts (31), where samples with an OD >0.2 at a dilution of 1/100 were considered positive. Subjects were divided into two groups, one group with children that were seronegative (SN) for both

IL-6, IL-10, IL-12p70, IFN-γ, and TGF-β ELISA

To determine IL-6, IL-10, IL-12p70, and IFN-γ concentrations in cell supernatants or in blood plasma; Abs against IL-6, IL-10, IL-12p70, and IFN-γ (Mabtech); human IL-6, IL-10, rIL-12p70, and hIFN-γ (National Biological Standards Board); streptavidin-alanine phosphate-conjugated Abs (Mabtech); and p-nitrophenyl phosphate (Sigma-Aldrich) were used. TGF-β was measured with Quantikine ELISA kits (R&D Systems) ac-
cording to the manufacturer’s instructions. The detection limit was 10 pg/ml for IL-6, IL-10, IL-12p70, and IFN-γ and 4.6 pg/ml for TGF-β (mean value), respectively.
Statistical analysis

The Mann-Whitney U test or Fisher’s exact test was used to evaluate differences between sample groups. Cuzick’s nonparametric test was used to evaluate trends. Outliers are not shown in figures. Data in the text indicate median (numbers in parentheses, range). Statistical analyses were performed with Statistica 7.1 (Statsoft) and Stata 8 Software (Stata) at the Division of Mathematical Statistics (Stockholm University, Stockholm, Sweden).

*p values below 0.05 were considered statistically significant.

Results

Herpesvirus seropositivity is associated with decreased IFN-γ production by NK cells

The IFN-γ production capacity was examined in 2-year-old subjects, either SP for EBV, EBV and CMV or SN for both viruses. PBMCs were stimulated in vitro for 24 h with IL-15 or PGN or with IL-15 and PGN in combination and stained for intracellular IFN-γ. The flow cytometric analysis showed that only a combination of IL-15 and PGN induced effective IFN-γ production. NK cells represented the major IFN-γ⁺ cell population (Fig. 1A), whereas only very low proportions of IFN-γ⁺CD3⁺ cells were detected (Fig. 1B).

The SP group of children had a significantly lower proportion of IFN-γ⁺ NK cells among total NK cells (\(p = 0.019\); Fig. 1C) accompanied by decreased levels of intracellular IFN-γ measured as GeoMFI (\(p = 0.035\); Fig. 1D) after stimulation with IL-15 and PGN than did SN children. When SP subjects were further divided into EBV⁻/⁻CMV⁻ (n = 25) and EBV⁺/⁻CMV⁺ (n = 9). MFI, mean fluorescence intensity.
Influence of monocytes and monocyte subsets on NK cell IFN-γ production in culture with medium alone (□) or with IL-15+PGN (■). A, IFN-γ release from enriched CD3+CD56− NK cells or total CD14+ population alone, or in cocultures (NK:CD14+) from PBMCs after 24 h. B, IFN-γ release from cocultures of enriched CD3+CD56− NK cells with classical (NK:CD14+CD16+) or proinflammatory (NK:CD14+CD16−) monocytes after 44 h. Median ± SD of four to six (A) or three (B) independent experiments from healthy adult donors. Vertical line through bars, assay detection limit.

Herpesvirus seropositivity associates with phenotypically altered CD14+CD16+ monocytes

Next, we wanted to examine if herpesvirus seropositivity also affected monocyte characteristics. There was a clear tendency toward a decreased ratio of CD14+CD16+ monocytes after 24 h in medium alone neither in the proportion of IFN-γ+ NK cells (1% (0–1) and 1% (0–1), respectively) nor in intracellular levels of IFN-γ, measured as GeoMFI, (8 (6–18) and 8 (6–31), respectively). IFN-γ+CD3+ cell proportions did not differ between SP and SN children (medium alone: 0% (0–1) and 0% (0–1), respectively; IL-15 and PGN: 2% (0–27) and 3% (0–67), respectively), nor did the CD3+ IFN-γ levels measured as GeoMFI (medium alone: 8 (6–12) and 8 (6–11), respectively; IL-15 and PGN: 9 (7–19) and 9 (7–21), respectively). There were no significant differences between SP and SN children regarding total NK cells of all lymphocytes (5% (2–21) and 5% (2–12), respectively), CD56bright cells of all NK cells (4% (2–13) and 4% (1–11), respectively), or total CD3+ cells of all lymphocytes (78% (39–86) and 77% (50–88), respectively) after 24 h in medium.

Blood plasma levels of IFN-γ. Children were categorized in A as SN (n = 26) or SP for EBV or EBV and CMV (n = 25) and the SP group is further subdivided in B as EBV+/CMV− (n = 16) and EBV+/CMV+ (n = 9).

NK cell IFN-γ production is more potently induced by CD14+CD16+ monocytes

Given that the proportion of CD14+CD16+ monocytes was reduced in SP children, we wanted to address their potential contribution to the observed NK cell IFN-γ responses. Consistent with previous findings (13, 14), substantial IFN-γ levels in the culture supernatants were detected only in stimulated cocultures of CD14+ cells and NK cells, whereas stimulation of either cell type alone resulted in barely detectable levels of IFN-γ (Fig. 4A). In addition, IFN-γ levels detected in monocyte and NK cell cocultures were comparable with those detected in PBMC cultures (Fig. 4A), indicating that the monocyte is the main NK cell-activating cell type among PBMCs. We observed markedly higher levels of IFN-γ in cocultures of NK cells and CD14+CD16+ monocytes, as compared with NK cell cocultures with CD14+CD16− cells (Fig. 4B). In agreement with other studies (13–16, 32), when enriched monocytes and NK cells were cocultured but separated by transwell inserts, the levels of IFN-γ were decreased, indicating that cell-to-cell contact was required for optimal IFN-γ
production by NK cells (inhibition range, 40–55% from three separate experiments).

**Herpesvirus seropositivity in children is associated with reduced levels of plasma IFN-γ**

We further investigated whether the observed in vitro differences in IFN-γ production between the groups of children were evident also in vivo and determined plasma IFN-γ levels. In accordance with our in vitro results, we found significantly lower levels of circulating IFN-γ in the plasma of the SP children ($p = 0.048$, Fig. 5A). Again, the same pattern was found when the SP subjects were subgrouped in which CMV coinfection was associated with the lowest IFN-γ levels ($p = 0.015$; Fig. 5B). We also quantified IL-6 in plasma but found no significant differences between the groups. Similarly to culture supernatants, there was a strong correlation between plasma IFN-γ and IL-6 ($r_s = 0.90$, $p < 0.001$).

**Discussion**

Throughout the process of virus-host coevolution, herpesviruses have developed an array of immunomodulatory mechanisms to avoid detection and destruction by the host’s immune system. Although loss of immune control can lead to herpesvirus reactivation from latency and result in serious disease (2–4), delayed primary infection with herpesviruses in affluent societies (1, 2) coincides with higher incidence of allergic disorders (32), which in contrast indicates a beneficial role for these viruses.

In connection with this, our group has previously reported an inverse association between EBV seropositivity and IgE sensitization in 2-year-old children (25). Recent observations from our laboratory did not provide support for a Th1-biased cytokine profile in EBV SP children (26), which could have explained the protection against the allergic phenotype (32, 33). However, latent herpesvirus infections in mice result in potentiated innate activity and increased systemic IFN-γ levels (29). To elucidate the mechanism behind protection against IgE sensitization in SP infants, we focused on monocyte and NK cell interplay that gave their interaction has been suggested to play a role in the early control of viral infections and might be important for the initiation and shaping of subsequent adaptive responses (10, 12).

The cooperation between accessory cells and NK cells has recently been investigated in relation to diseases such as malaria (13, 14), rheumatoid arthritis (34), atopic dermatitis (35), and respiratory allergic disorders (36). To our knowledge, we are the first to examine monocyte-induced IFN-γ synthesis in NK cells in relation to early life viral infections. We demonstrate that EBV seropositivity in infants is associated with reduced monocyte-induced NK cell IFN-γ production, with regard to both the proportion of IFN-γ⁺ NK cells and to their cognate intracellular IFN-γ levels. This reduction was even more pronounced in children who were SP for both EBV and CMV. Data on intracellular NK cell and cell culture IFN-γ paralleled, which strongly argues against different kinetics in the synthesis of this cytokine between the groups. The activation degree of CD14⁺ cells after PGN challenge has been shown to correlate with released IL-6 (37). Interestingly, a strong correlation was found between IL-6 and IFN-γ levels in culture supernatants after stimulation although no differences in IL-6 levels were seen between the groups.

PBMCs from SP children showed a strong tendency toward a lower proportion of proinflammatory monocytes. When assessing their functional properties, we found that they triggered IFN-γ release by NK cells more potently than classical monocytes. This effect could potentially be due to an increased capacity to secrete IL-12 (38), which is a key inducer of IFN-γ responses (39), but levels of IL-12p70 in cell culture supernatants were below the detection limit of our assay. The lower CD14⁺CD16⁺ ratio in SP children could not be explained by decreased production of TGF-β or IL-10, cytokines that have been shown to induce CD16 expression on monocytes (40, 41). However, we cannot rule out that the EBV and CMV encoded homologs of human IL-10 could have affected the quantification of IL-10 in plasma (42). To our knowledge, IFN-γ has no clear involvement in regulation of CD16 on monocytes (43, 44). Furthermore, NK cells can be regulatory by killing immature DCs (45) or overactivated macrophages (46), but whether preferential elimination of the CD14⁺CD16⁺ cells could be an explanation behind decreased proportions in SP children remains to be elucidated.

Interestingly, plasma IFN-γ levels in SP children were significantly lower than in their SN counterparts, concurrent with our in vitro data. Again, CMV coinfection was associated with the lowest levels of IFN-γ. The reason behind this finding is unknown but is indicative of in vivo interactions between EBV and CMV with immunomodulatory consequences, something which is supported by a study from Aalto et al. (47) showing that primary CMV infection induced reactivation of latent EBV. Thus, in the double-positive children, CMV coinfection may intensify EBV-induced immune scarring. Furthermore, IL-6 and IFN-γ levels correlated, suggesting a possible in vivo monocyte and NK cell interaction. The fact that the NK cell IFN-γ production in vitro and plasma levels of IFN-γ paralleled could imply that NK cell-derived IFN-γ was the major source of IFN-γ in plasma. Indeed, T cells are also important in controlling viral infections (48), but here their contribution to the plasma IFN-γ is unclear. However, because the SP subjects had lower IFN-γ in their plasma, and we have observed an overall increased cytokine production by PHA-stimulated PBMCs from SP children (26), this strongly argues against T cells as the major contributors to the differences in plasma IFN-γ between the groups.

A modulated accessory cell function due to herpesvirus infections may influence NK cell IFN-γ responses in SP subjects. For instance, infection of monocyte-derived DCs with CMV (49) and HSV 1 (50) inhibits their maturation process as well as their release of inflammatory cytokines. Ex vivo data obtained here from SP infants strengthens these findings and suggests that reduced proportions of CD14⁺CD16⁺ cells might play a part in attenuated NK cell IFN-γ responses after pathogen encounter. In a model of transendothelial trafficking, CD14⁺CD16⁺ cells showed a higher likelihood to differentiate into DCs (19); suggesting that they could represent physiologically relevant blood precursors of migrating DCs, with the potential to interact with NK cells at sites of inflammation or in secondary lymphoid tissues (51). In addition to the decreased ratio, CD14⁺CD16⁺ monocytes from SP subjects who displayed an activated phenotype expressed lower levels of CD11c, implying that a modulation of migratory behavior might occur (52).

Exposure to pathogenic and nonpathogenic agents in childhood strongly influences the progressive shift from the allergy-related Th2 profile toward a balanced Th1-Th2 immunity (33). At first sight, the present data seem difficult to reconcile with our previous data of a high cytokine production in SP children (26). Intriguingly, the two studies indicate that EBV and CMV seropositivity is associated with a lower NK cell but higher T cell cytokine response. A viral-induced modified balance between mononcytic subsets might be one possible explanation behind this observation.

The finding of lower plasma IFN-γ in SP children was unexpected since recent findings in mice with herpesvirus latency suggested the opposite (29). Besides, acute EBV infection in adults causes mononucleosis, which is accompanied by higher in vivo
IFN-γ (53) and increased NK cell activity (54). Whether damped
enized innate responses in infants are a consequence of herpesvirus
infection, or may cause higher susceptibility to infection, remains
an open question. A differential predisposition for acquiring infec-
tions might exist (55, 56), and a very potent innate reaction toward
viruses could hypothetically block active infections and preclude
adaptive immune responses. Because additional data in favor of
this hypothesis is lacking, we rather speculate that herpesvirus in-
fections attenuate innate responses in small children, which could
explain why they typically have asymptomatic primary infections.
Indeed, it would have been interesting to determine at which stage
of viral infection this innate suppression takes place. This is dif-
ficult, however, given that the relation between development of
humoral responses and infection time in asymptomatic infants is
not established.

The fact that a number of the 2-year-old subjects were still SN
could also reflect differential exposure to viruses. However, there
were no differences between the groups in exposure factors such as
duration of breast-feeding, number of siblings, or day-care attend-
ance (Table I).

In summary, we speculate that early life infection by herpesvi-
ruses may attenuate innate responses with particular influence on
monocyte-NK cell interactions, with possible implications in the
development of allergies.

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References
Sci. USA 91: 2384–2389.
4. Sinclair, J. 2008. Human cytomegalovirus: latency and reactivation in the my-
7. Romagnani, S. 2004. Immunologic influences on allergy and the TH1/TH2 bal-
inhibitory dendritic cells in a model tissue setting.
9. Medzhitov, R. 2007. Recognition of microorganisms and activation of the
natural killer cells via CD40-CD40L interaction.
K. Soderstrom. 2007. Natural killer cells trigger differentiation of monocytes into
recognized via the NKp30 receptor by activated NK cells. J. Exp. Med. 195: 343–351.


