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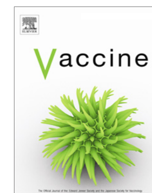

Short communication

Infants with low vaccine antibody responses have altered innate cytokine response

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ABSTRACT

We recently identified a population of 10% of infants who respond with sub-protective antibody levels to most routine primary pediatric vaccinations due to altered innate and adaptive immune responses. We term these infants as low vaccine responders (LVRs). Here we report new data showing that TLR7/8 agonist – R848 stimulation of PBMCs of LVR infants elicit significantly lower IFN-α, IL-12p70 and IL-1β, while inducing higher levels of CCL5 (RANTES) compared to normal vaccine responder (NVR) infants.

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1. Introduction

We have recently identified a population of about 10% of infants who fail to respond or respond with sub-optimal immunity to routine primary pediatric vaccination. We call these infants low vaccine responders (LVRs) [1]. Ten percent translates to 400,000–500,000 children/year in the United States alone. In the initial immune cell characterization of LVR infants we found lower percentages of antigen specific memory B cells, lower levels of T cell activation upon polyclonal stimulation, and lower basal MHC II expression by professional antigen presenting cells (APCs: monocytes, conventional DCs; cDCs, and plasmacytoid DCs; pDCs) [1]. Innate immunity instructs adaptive immunity to subsequently generate antibodies and memory response against infectious diseases. Poor innate immunity in LVR infants may be contributing to their poor adaptive immune response. Further, the suboptimal T and B cell responses in LVR infants [1] makes them rely on a strong innate immune response to protect against viral and bacterial infections. Therefore, we sought to determine the innate cytokine response from LVR infants compared to normal vaccine responder (NVR) infants.

Human conventional DCs (cDCs) and plasmacytoid DCs (pDCs) are the major producers of IL-12p70 and IFN-α/β respectively [2]. IL-12 plays a major role in co-stimulating Th1 immunity and IFN-α/β plays a crucial role in anti-viral immunity [3,4]. IL-12 also mediates DC directed T cell differentiation into T follicular helper cells (Tfh) which in turn facilitates generation of long term B cell memory responses [5]. Since the yield of PBMCs from infants is limited, we used a single TLR agonist which can simultaneously activate all 3 APC subsets. Thus we used a synthetic imidazoquinoline TLR7/8 agonist, resiquimod (R848) because human pDCs express TLR7 whereas monocytes and cDCs express TLR8 [6] and enabled us to study APC specific cytokine responses in peripheral blood. R848 is known to induce IL-12 and IFN-α from monocytes/cDCs and pDCs, respectively [2]. By 1 year of age infants are capable of producing adult level IFN-α and moderate levels of IL-12 [7]. Therefore, we stimulated the infant peripheral blood APCs with R848 to study the innate cytokine responses in LVR

Abbreviations: APC, antigen presenting cell; cDC, conventional DC; DT, diphtheria toxoid; FBS, fetal bovine serum; FHA, filamentous haemagglutinin; IRF, IFN response factors; LVR, low vaccine responders; MHC, major histocompatibility complex; MyD88, myeloid differentiation factor 88; NVR, normal vaccine responders; PBMC, peripheral blood mononuclear cells; PRN, pertactin; PT, pertussis toxoid; PBS, phosphate-buffered saline; pDC, plasmacytoid DC; PRP, polyribosylribitol-phosphate; RANTES, regulated on activation, normal T expressed and secreted; TT, tetanus toxoid; TLR, toll-like receptor; TRIF, also TICAM 1, toll/IL-1 receptor domain-containing adaptor inducing IFN-β.

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infants to determine if they were altered/suboptimal and potentially contributory to the previously observed defective T cell function, B cell memory and reduced vaccine response [1].

2. Methods

2.1. Subjects

Healthy, full term birth infants were enrolled from a middle class, suburban socio-demographic population in Rochester NY in a prospective, longitudinal study as previously described [1]. All vaccines were administered according to the recommended schedule of the CDC ACIP. Children were enrolled at age 6 months upon completion of their primary series of vaccinations from a private pediatric practice (Legacy Pediatrics, Rochester, NY). The blood samples analyzed in the current study for cytokines were from 6 to 9 months old age-matched LVR-NVR infants during healthy routine visits. Written informed consent was obtained from parents of the children in association with a protocol approved by the Rochester General Hospital Institutional Review Board.

2.2. LVR group definition

LVR children as a group has been defined previously [1]. Briefly, we performed a preliminary survey of antibody levels in 107 children against 13 vaccine antigens: DT, TT, PT, FHA, PRN, PRP, Hepatitis B, *Streptococcus pneumoniae* (*Spn*) capsular polysaccharides 6B, 14 and 23F and polio serotypes 1, 2 and 3 at 9–12 months of age. We defined Low Vaccine Responders (LVRs) as those infants who had sub-protective antibody responses to >50% of tested vaccine antigens; all others we defined as Normal Vaccine Responders (NVRs). Statistical analysis of these responses showed that a sub-protective response to 3 out of 6 antigens (among DT, TT, PT, FHA, PRN and PRP) could be used to distinguish LVRs from NVRs in a manner that was not significantly different from analysis of all 13 antigens. Therefore, antibody responses to these 6 antigens were measured in a cohort of 499 children.

2.3. PBMC sample processing and in vitro stimulation

PBMC processing was accomplished as previously described [1]. For *in vitro* stimulations, after thawing and washing, PBMCs were counted and rested overnight (200 μl/well at 2×10^6 cells/ml) in 96 well round bottom plates at 37 °C in 5% CO_2 incubator. After resting, total PBMCs were incubated for an additional 24 h at 37 °C in 5% CO_2 incubator with or without R848 (1 μg/ml). After 24 h culture, cells were spun and supernatants were collected and stored at −80 °C until Luminex analysis.

2.4. Cytokine measurement

Supernatants stored at −80 °C were thawed at room temperature and cytokine and chemokine levels of IL-1β, IL-6, IL-8, IL-10, IL12p70, IFN-γ, CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES) and TNF-α were measured from undiluted samples using Bio-Plex Pro Human Cytokine Group I 12-plex assay kit (Bio-Rad Laboratories, Hercules, CA) according to manufacturer's instructions. IFN-α levels were analyzed separately by human IFN-α multi-subtype ELISA kit (Life Technologies). Prepared samples were run on a Bio-Plex 200 system with Luminex xMAP technology (Bio-Rad).

2.5. Statistics

All data were analyzed using Graph Pad Prism Software version 6.04 (GraphPad Software, La Jolla, CA). Luminex and ELISA results were analyzed by nonparametric Mann-Whitney *U* test using background subtracted R848 stimulated individual cytokine values. $p < 0.05$ was considered significant.

3. Results and discussion

3.1. LVR infants induce higher baseline RANTES

We analyzed innate-associated cytokines/chemokines from PBMCs (number of infants per cohort included in figure legends) with and without R848 stimulation. Basal levels from unstimulated PBMCs of most cytokines/chemokines tested were similar between LVR and NVR infants. However, LVR infants had significantly ($p = 0.03$) higher basal RANTES (CCL5) levels compared to NVR infants (Fig. 1). R848 induced RANTES levels from LVR PBMCs were also high ($p = 0.06$) compared to NVRs (Fig. 1). Multiple studies [8,9] have shown that viral URI especially with influenza, rhinovirus and RSV result in elevated serum and nasopharyngeal levels of RANTES (CCL5), IP-10 (CXCL10) and IL-8. RANTES has been shown to induce T cell migration and its expression can be induced by respiratory viral infections [8]. Prospectively collected findings from children in our study cohort and our prior publications shows that LVR children are significantly more prone to influenza and respiratory bacterial infections compared to NVR children ([1] and unpublished observations). Further, RANTES is a 'late' T cell expressed cytokine. Therefore, we speculate that enhanced infection proneness in LVR children might be causing enhanced RANTES levels as a compensatory T cell recruitment mechanism. Fortunately due to herd immunity, LVR children have not shown an identifiable increase in vaccine preventable infections, except for influenza (unpublished observations). The PBMCs used for cytokine measurements in our studies were from LVR infants during "healthy" visits; however, LVRs have a significantly increased frequency of viral upper respiratory infections (URIs) so it is probable that we more often took their samples just prior to or in recovery from viral URIs thereby allowing us to capture high levels of RANTES. In our study, we also saw higher average values of IP-10 and IL-8 in LVR infants emphasizing the higher proinflammatory status of LVR compared to NVR infants (data not shown).

3.2. LVR infants induce low IFN-α, IL-12 and IL-1β

In contrast to higher levels of RANTES, LVR PBMCs stimulated with R848 secreted significantly ($p < 0.05$) less IFN-α and IL-12p70 compared to NVR infants (Fig. 2A and B) suggesting a reduced capacity to respond to viral and bacterial infections. In addition, LVR infant PBMCs secreted significantly ($p < 0.05$) lower pro-inflammatory IL-1β (Fig. 2C) with R848 stimulation compared to NVR infants. IFN-α plays a critical role in anti-viral immunity and IL-12p70 co-stimulates an effective Th1 response to infections [3,4].

We previously reported low APC-MHC II and IRF7 expression levels indicating altered innate immune response in LVRs [1]. pDCs are one of the main cellular targets of R848 and the substantial IFN-α/β production by pDC is mediated by constitutive expression of IRF7 [10]. Recently, Love et al. [11] demonstrated that in an *ex vivo* human PBMC experimental model, production of IFN-α occurs through a TLR7 (and TLR9) – IRF7 dependent pathway. Hence, the low IFN-α level in R848 induced PBMCs from LVR infants may be a direct result of suboptimal IRF7 transcription.

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