

Plasma levels of Th1 and Th2 cytokines in Ghanaian children with vaccine-modified measles

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ABSTRACT. To understand the pathogenesis of vaccine-modified measles (VMM), we measured plasma levels of IFN- γ and IL-2 (Th1 cytokines), IL-4 and IL-10 (Th2 cytokines), IL-12, TNF- α and TGF- β 1 in children with uncomplicated measles, who had anti-measles IgG antibodies and with a history of immunization on admission (day 0), day 14 and day 60. We compared these to levels in healthy, age-matched, immunized children. Plasma levels of IFN- γ , IL-2 and IL-12 were significantly higher in VMM patients on day 0 compared to healthy controls ($p = 0.023$; $p = 0.018$; $p = 0.001$) respectively. In contrast, plasma IL-4 was lower in VMM patients on day 0 when compared to the controls ($p = 0.009$). Plasma levels of IL-12 remained consistently high on days 14 and 60 ($p = 0.001$; $p = 0.04$), whilst IL-10 levels fell significantly on the same days ($p = 0.002$; $p = 0.001$) respectively. Kinetically, IFN- γ and IL-10 levels decreased consistently from day 0 to days 14 and 60 in VMM patients. In contrast, IL-4 levels increased from day 0 to day 14 and day 60. Our results therefore suggest that VMM is associated with an early up-regulation of Th1 cytokine production and a down-regulation of Th2 cytokine production. The strong Th1 response may be associated with the induction of IL-12 and memory cells, thus contributing to the early resolution of the infection and lack of complications.

Keywords: Vaccine-modified measles (VMM), interferon-gamma (IFN- γ), interleukin (IL), tumor necrosis factor-alpha (TNF- α), tumor growth factor-beta 1(TGF- β 1), T helper (Th)

INTRODUCTION

Cytokines, which are intracellular signaling molecules produced predominantly by T cells and macrophages, play a significant role in the regulation of immunity and inflammation, and coordinate diverse cellular responses. Helper CD4 + T (Th) cells have been classified as Th1 or Th2 on the basis of their cytokine secretion patterns [1]. While Th1 cells produce IFN- γ , IL-2 and TNF- β , preferentially inducing macrophage activation and resistance to intracellular infection leading to a cellular response, Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13 and are important for macrophage deactivation and humoral immunity. These Th cell populations are functionally distinct and cross-regulatory [1, 2]. Responses assessed in children during and after acute measles infection, or immunization with live attenuated measles vaccines have shown that they exhibit immunological patterns consistent with a predominant Th2 response, characterized by

spontaneous release of IL-4, production of high levels of measles virus antibodies and temporary suppression of cell-mediated immunity (CMI) [3-10]. Development of the Th1 response is primarily due to the production of IL-12, which in turn induces the production of IFN- γ from T cells and natural killer (NK) cells and inhibits IL-4 production [11-14]. However, the diminished Th1 responses in measles occur as a result of the down-regulation of IL-12, resulting from the binding of measles virus to the CD46 receptor. This results in the suppression of CMI and an increased susceptibility of patients to secondary infections associated with acute measles virus infection [15].

Despite the availability and wide immunization coverage of measles vaccine, outbreaks of measles still occur with changes in epidemiological patterns, mostly in developing countries. These outbreaks, described as vaccine-modified measles (VMM) exhibit reduced severity, mild clinical symptoms, and occur in older children who have

a history of measles immunization, and are probably due to vaccine failure and waning immunity with age [16-19]. Consistent with these characteristics, there was an outbreak of measles in Ghanaian children, six years or older, with a previous history of immunization. These children showed mild clinical symptoms with reduced incidence of fever, and the presence of anti-measles IgG antibodies [20]. Although VMM is assuming importance, its pathogenesis is not fully understood, and in particular the Th1/Th2 cytokine balance and association with clinical presentation and outcome have not been fully studied. In order to understand the pathogenesis of VMM, we measured plasma levels of IFN- γ and IL-2 (Th1 cytokines), IL-4 and IL-10 (Th2 cytokines), IL-12 and TNF- α (pro-inflammatory cytokines) and TGF- β 1 (anti-inflammatory cytokine) in Ghanaian children with VMM on admission (day 0), at recovery (day 14) and convalescence (day 60).

MATERIALS AND METHODS

Patients and controls

The patients were all recruited during an epidemic of measles in Accra, Ghana from January to March 2000. All children underwent a thorough clinical examination and a detailed clinical history was also obtained. The health cards or immunization records of all the children were also examined. A group of healthy, age and sex-matched children with measles vaccination history were recruited from the nearby Maternal and Child Health Centre, Madina, Ghana as controls [20]. Informed consent was obtained from parents/guardians of the children before being enrolled in the study.

A total of 4.5 ml of venous blood was drawn into K₃ EDTA vacutainer tubes from each patient on the day of admission (day 0), and also from healthy, immunized controls. Subsequently, blood was drawn from these patients on the 14th and 60th day.

The blood was centrifuged at 500g for 10 minutes, and the plasma separated and stored as aliquots, at -20°C.

Thick and methanol-fixed thin blood films from patients and healthy subjects were stained with Giemsa and examined for malaria parasites by microscopy, in order to eliminate malaria.

Ethical approval for the study was obtained from the Ministry of Health, Ghana.

Measurement of cytokine levels in plasma

Plasma levels of IFN- γ , IL-2, IL-12, TNF- α , IL-4, IL-10 and TGF- β 1 were measured using ELISA kits, (Quantikine Immunoassay, R&D Systems Minneapolis, MN, USA), with minimum detectable sensitivity less than 5.0, 7.0, 7.0, 10.0, 3.7 and 7.0 pg/ml respectively. Assays were performed according to the manufacturer's instructions. Briefly, two - fold serial dilutions of the cytokine standards were added in duplicate to the pre-coated microtiter plates. Plasma samples of VMM patients and healthy, immunized children were added to the remaining wells in duplicate and incubated for 2 hours at room temperature. After incubation, the plates were washed to remove unbound proteins and enzyme-conjugated antibody was added. The plates were then incubated at room

temperature for one hour. After washing, the substrate was added for 20 minutes for colour development and the reaction was stopped using a stopping reagent. The colour developed was measured at 450nm. A curve was prepared by plotting the optical densities (OD) measured against the standard concentrations. Thus, the unknown plasma concentrations from the VMM patients and healthy, immunized children were determined using their ODs.

Data presentation and statistical analysis

Statistics are given as means, and 95% confidence interval of the difference. Mean cytokine levels for VMM patients on admission (day 0), at recovery (day 14) and convalescence (day 60) were compared with levels of healthy, immunized children using Student's *t*-test. The levels of the cytokines in plasma for VMM patients on different days (day 0, days 14 and 60) were compared using Student's *t*-test. Values of $p < 0.05$ were considered statistically significant. Where the normality test failed, the Mann-Whitney sum rank test was applied.

RESULTS

Clinical characteristics of the patients

Of the 44 patients clinically diagnosed with uncomplicated measles at the 3 health facilities namely, 37 Military Hospital, La Polyclinic and the Princess Marie Louise Hospital, 32 patients were studied. Their mean age was 7.2 years, (95% confidence interval, 6.3-8.2). An examination of their health cards revealed that whilst four children had previously received measles vaccinations 28 of them could not be confirmed. However, serological tests performed on their plasma samples at onset of the infection using commercial enzyme immunoassay (EIA) kits (Measles IgG (II), Denka Seiken, Tokyo, Japan.), confirmed positive anti-measles IgG antibody titres. Table 1 shows the number of children who presented various combinations of clinical symptoms with positive anti-measles IgG antibodies at the onset of infection. From the clinical symptoms two out of the 32 patients had all the typical symptoms of measles (fever, rash, Koplik spots, cough, coryza and conjunctivitis). There were no complications and all of the patients recovered completely with no mortality [20]. The group of 34 healthy, age and sex-matched, immunized children (controls) had a mean age of 8.1 years (95% C.I., 7.6-8.5).

The study subjects and controls tested negative for malaria parasites.

Levels of plasma cytokines in patients and controls

Mean plasma levels of IFN- γ , IL-2, IL-12, TNF- α , IL-4, IL-10 and TGF- β 1 in VMM patients and healthy, immunized controls are shown in Table 2.

On day 0, plasma levels of IFN- γ , IL-2 (Th1 cytokines) and IL-12 in VMM patients were significantly higher, whilst IL-4 (Th2 cytokine) was lower compared to healthy, immunized controls ($p = 0.023$; ≤ 0.001 ; ≤ 0.001 and 0.009) respectively. Although plasma levels of TNF- α in VMM patients were slightly higher than those in healthy controls, no statistically significant difference was observed ($p = 0.831$). Similarly, plasma

Table 1
Clinical symptoms in children with positive anti-measles virus Ig G antibody at onset of measles infection (where n = number of children with various clinical combinations).

Clinical symptoms	n
Rash + Koplik's spot + Cough + Coryza + Conjunctivitis	7
Rash + Koplik's spot + Cough + Conjunctivitis	5
Rash + Cough + Conjunctivitis	4
Rash + Koplik's spot + Cough + Coryza	3
Rash + Fever + Koplik's spot + Cough + Coryza + Conjunctivitis	2
Rash + Cough + Coryza + Conjunctivitis	2
Rash + Koplik's spot + Cough	2
Rash + Cough + Coryza	2
Rash + Fever + Koplik's spot + Cough + Coryza	1
Rash + Fever + Koplik's spot + Cough + Conjunctivitis	1
Rash + Fever + Cough	1
Rash + Coryza + Conjunctivitis	1
Rash + Conjunctivitis	1

Total number of patients = 32

levels of IL-10 (Th2) and TGF-β1 were lower when compared to healthy, immunized controls but again without significance (p = 0.858 and 0.509 respectively).

On day 14, whilst plasma IL-12 levels in VMM patients were significantly higher than in healthy, immunized controls (p = 0.001), IL-10 levels were significantly lower (p = 0.001). Although levels of IFN-γ and IL-2 were higher in VMM patients when compared to healthy, immunized controls (p = 0.254 and 0.05) respectively, TNF-α, IL-4 and TGF-β1 levels were not significantly different between the two groups (p = 0.831; 0.44; 0.705 respectively).

On day 60, plasma levels of IL-2, IFN-γ, TNF-α, TGF-β1 and IL-4 were not significantly different from the levels in the healthy controls. The plasma IL-12 level of VMM patients was however, significantly higher than in the healthy immunized controls (p = 0.04). The plasma levels of IL-10 were lower in VMM patients compared to the healthy controls (p = 0.001).

Plasma IFN-γ and IL-10 levels in VMM patients on day 0 had decreased significantly by day 14 (p = 0.005 and ≤ 0.001 respectively). The levels of the two cytokines had further declined by day 60 (p = 0.005 and ≤ 0.001 respectively). Levels of IL-4 on the other hand, showed a

significant increase by day 60 (p = 0.028). Levels of IL-12, IL-2, TNF-α and TGF-β1 did not show any significant decrease from day 0 to day 60.

DISCUSSION

The results presented here show that plasma levels of IFN-γ and IL-2, both Th1 cytokines, are significantly elevated in VMM patients on admission when compared to healthy immunized children (Table 1). The levels of IFN-γ declined from day 0 to day 60 in VMM patients. This has also been observed in measles virus (MV) infection where levels of IFN-γ were elevated before the onset of rash, rapidly declining thereafter. The production of IFN-γ may have resulted from primed memory Th1 CD4 + and CD8 + T cells, as well as NK cells [21, 22], and its role may have been in the control of VMM infection through elimination of measles virus. This is further supported by a report that has shown that, in mice, IFN-γ production plays a critical role in the protection against MV infection and central nervous system disease [23]. Interleukin-2 is produced by newly stimulated naïve CD4 T cells and primed memory Th1 CD4+ T cells [21, 22], and is known to suppress antibody production by B-cells [24], and in concert with TNF-α, participates in the resolution of a neuron-restricted MV infection in mice [23]. The trend observed in MV infection shows that levels of plasma IL-4, a Th2 cytokine are low during the acute phase but increase rapidly and remain elevated for sometime as a result of the activation of Th2 CD4+ T cells [6, 25]. The pattern of plasma IL-4 in VMM patients in the present study were similar to that reported in MV infection [6, 25]. A recent study has shown that IL-4 promoter gene polymorphism is associated with increased IL-4 synthesis that confers host genetic susceptibility upon subacute sclerosing panencephalitis (SSPE) [26]. Our data therefore show that VMM infection also sequentially activates Th1 and Th2 responses.

The pro-inflammatory cytokine, IL-12 plays a central role in the pathogenesis of measles infection. The high levels of plasma IL-12 and its persistence throughout up to 60 days in VMM patients, may suggest that it elicited the production of IFN-γ for the activation of Th1 responses leading to cell-mediated immunity and virus clearance by CD8 + cells and the inhibition of IL-4 production [11-14]. Thus the production of IL-12 in VMM patients may suggest an early activation of cell-mediated immunity or a Th1 response. Higher levels of IFN-γ and/or both the

Table 2
Mean concentrations of plasma cytokines in vaccine-modified measles (VMM) patients on days 0, 14 and 60 and a group of healthy immunized children.

Cytokine levels pg/ml	Healthy Control N = 34	VMM Day 0 N = 32	VMM Day 14 N = 32	VMM Day 60 N = 32
IFN-γ	7.3* (3.75-10.85)	40.50* ^a (13.49-67.51)	14.93 ^a (0.01-29.82)	5.27 ^a (3.79-6.76)
IL-2	6.93* (5.97-7.88)	8.79* (7.54-10.44)	8.32 (7.29-9.35)	7.60 (6.58-8.62)
IL-12	0.57* (0.41-0.73)	1.15* (0.98-1.32)	1.10* (0.92-1.28)	0.91* (0.68-1.13)
TNF-α	8.19 (6.76-9.62)	9.70 (7.13-12.26)	7.63 (6.29-8.96)	7.23 (6.16-8.29)
IL-4	3.85* (3.21-4.49)	2.69* ^a (2.09-3.28)	3.51 (2.86-4.16)	3.77 ^a (2.4-4.4)
IL-10	6.90* (5.32-8.48)	6.57 ^a (5.48-7.66)	4.70* ^a (1.69-7.7)	3.73* ^a (1.05-6.42)
TGF-β1	854.1 ^a (556.3-1151.9)	633.4 ^a (421-845.8)	548.8 ^a (365.8-731.8)	381.4 ^a (202.6-560.3)

* Indicates significant difference at p < 0.05 between control and VMM on day 0, day's14 and 60; ^a Indicates significant difference at p < 0.05 between VMM day 0 and VMM day's14 and 60.

presence of IL-12 and IFN- γ have been reported to favour Th1 responses and inhibit Th2 responses [14]. Our results show that there is a significant increase in IFN- γ (Th1) and IL-12, whilst IL-4 levels (or a Th2 response) were significantly lowered in VMM patients confirming the reports of suppressed humoral responses as well as early activation of cell-mediated immune responses in measles [25].

Susceptibility to secondary infection, which leads to complications during acute childhood measles, has been attributed to the temporary suppression of CMI [15]. Our findings thus suggest that the lack of complications observed in these patients with VMM may have been due to the significant increase in Th1 cytokine levels, which activated CMI and rather suppressed Th2 responses that may have blunted responses to new infectious challenges. However, it is also likely that anti-measles antibodies present in these previously immunized children with VMM may have contributed to the resolution of the infection without any complications. Memory B and T cells are crucial to the establishment and maintenance of long-term immunity against infectious agents and indeed, a strong measles virus-specific IgG antibody response forms a major part of the immunity against measles [27]

Some cytokines and chemokines are associated with fever in many disease states. In cerebral malaria for instance, fever is associated with higher levels of plasma TNF- α [28]. Higher levels of TNF- α also exhibit anti-parasitic effects in *Plasmodium falciparum* infection [29]. Our results showed no significant difference in plasma TNF- α between VMM patients on day 0 when compared to healthy immunized children, which could probably explain the absence of fever in most of the VMM patients.

We found that plasma levels of IL-10 (Th2) and TGF- β 1, measured in VMM patients from day 0 to 60, were also lower than those in healthy immunized children. IL-10 (Th2) and TGF- β 1 are macrophage 'deactivators' inhibiting the production of IFN- γ [30] and thus their suppressed levels observed in VMM patients when compared to immunized controls had no effect on the increased levels of IFN- γ on day 0.

A limitation to this study is the lack of data on the production of plasma cytokines by non-vaccinated measles-infected children. However, various studies have shown the pattern of Th1/Th2 cytokines in acute measles, involving unvaccinated children. Nonetheless, the results presented here could lead us to a better understanding of memory responses in vaccinated children.

Our data therefore suggest that a preferential Th1 cytokine (IFN- γ and IL-2) response was produced, together with IL-12, on day 0, with an accompanying down-regulation of Th2 cytokines (IL-4 and IL-10), resulting in both the quick resolution and the lack of complications seen in vaccine-modified measles infection.

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