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Original Article

High CD4/CD45RO+ and CD8/CD45RO+ frequencies in children with vaccine-modified measles

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Abstract

Background: Despite availability and wide vaccine coverage, measles infections still occur especially in developing countries. An outbreak of measles occurred among previously immunized older Ghanaian children who had milder clinical symptoms with measles-specific IgG antibodies that could have been attributed to secondary vaccine failure, suggesting that the infection was vaccine-modified measles (VMM).

Methods: Two-color immunophenotyping of the peripheral blood mononuclear cells was performed at acute, recovery and convalescence phases for 19 VMM patients (mean age 6.2 ± 3.5 years) using flow cytometry, and compared with that of 20 healthy, sex- and age-matched controls.

Results: The results showed a significantly higher memory helper ($CD4^+/CD45RO^+$) cell frequency and increased suppressor cell ($CD8^+/CD45R0^+$) frequency in VMM patients compared to healthy controls. There were no complications and all the patients recovered completely.

Conclusions: These findings show that the mild symptoms in patients with VMM may have correlated with the increase of memory T cells, which is in sharp contrast with previous reports on acute measles infection. This may suggest that the intact immunologic memory cells could have been crucial for the resolution of VMM.

Key words immunologic memory, T-cell phenotypes, vaccine modified measles (VMM).

Measles is estimated to kill nearly 900 000 children annually,[1,2] with the burden of mortality being highest among children <2 years old in developing countries where general overcrowding and high population densities are major problems.[3–5] The morbidity and mortality associated with measles are mainly due to secondary infections arising from generalized immunosuppression.[6–9] The immune disturbances associated with measles are low natural killer (NK) cell activity,[10] increased expression of activation markers[11] and downregulation of interleukin (IL)-12 production.[12] In our previous studies we observed immunological unresponsiveness and apoptotic cell death of T cells in Japanese children with uncomplicated measles.[13] We also reported low expression of IL-2R α (CD25) on T cells as well as reduced frequencies of memory suppressor T cells (CD8/CD45RO+) during the acute and convalescent phases of measles in Ghanaian children with uncomplicated measles.[14]

We present for the first time in Ghana, 19 cases of vaccine modified measles (VMM) infection among Ghanaian children. VMM is characterized by a generally mild and frequently significantly afebrile illness that usually follows the regular sequences of events in measles, although with shorter prodromal periods and absence of confluence of the rash.[15] Koplik spots are few and transient; they frequently do not occur at all. VMM occurs under a variety of circumstances, which include secondary vaccine failure and waning immunity with age, but with the presence of anti-measles IgG antibodies in the acute phase of the illness.[16,17] The effects of VMM on cellular immune function in terms of immunosuppression have not yet been fully determined. We report on the increased frequencies of memory helper ($CD4^+/CD45RO^+$) cells as well as memory suppressor ($CD8^+/CD45RO^+$) T cell phenotypes in peripheral circulation of children with VMM during a measles outbreak in Accra, Ghana.

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Materials and methods

Patients

A subset of 19 children, 13 boys and six girls (mean age ± SD, 6.2 ± 3.5 years) who presented at the 37 Military Hospital and the La Polyclinic, both in Accra, Ghana, and who were clinically diagnosed as suffering from uncomplicated measles, were recruited into the study after informed parental consent was obtained. Children who had pneumonia, malaria, gastroenteritis or other complications were excluded. Ethical approval for the study was obtained from the Ethics Committee of the Ministry of Health, Ghana. Case definition of measles was as follows: acute febrile illness with axillary temperature >38°C, morbilliform skin rash and at least one of cough, coryza, koplik spot and conjunctivitis. VMM was characterized by a mild illness with rash, minimal cough, coryza and fever as well as absence of Koplik spot and the presence of anti-measles IgG antibodies in the acute phase of the illness (confirmed serologically).

Blood samples collected from patients on arrival at the health facilities, regardless of the duration of the fever were taken as the acute phase samples (day 0). The next samples were collected on the 14th and 60th days and regarded as the first and second follow-up or recovery and convalescent phase samples respectively. Twenty samples of blood were collected from age- and sex-matched healthy previously immunized children at the Madina Maternal and Child Health Clinic and used as controls. These children underwent thorough clinical examination by clinicians and were declared free from clinical signs of infection or illness in the period preceding phlebotomy.

Blood samples

A total of 4 mL of venous blood was taken into K_3 ethylenediaminetetraacetic acid vacutainer tubes and delivered to the Noguchi Memorial Institute for Medical Research (NMIMR) within 2–3 h. Peripheral blood mononuclear cells (PBMC) were separated from whole blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Piscataway, NJ, USA) after blood films had been prepared for malaria parasites test. PBMC were frozen in liquid nitrogen using a gradient freezing device[18] and preserved at −180°C until required, while the plasma samples were stored at −40°C until used for antibody testing.

Serology

A sensitive enzyme immunoassay (EIA) for measles antibody was used to quantify measles virus antibodies. Both IgG and IgM antibodies were measured by this method using commercial kits (Measles IgG (II), IgM (II)-EIA, Denka Seiken, Tokyo, Japan). Briefly, sera were thawed, diluted, transferred to microtiter plates pre-coated with antigen according to the manufacturer's instructions. Enzyme-conjugated anti-human IgG or IgM antibodies were added and the plates were incubated for 1 h. After washing the plates, a substrate was added and the color was allowed to develop. Optical density (OD) values were read on the enzyme-linked immunosorbent assay plate reader (MTP-32, Microplate reader, Corona Electric, Ishikawa, Japan). Positive and negative standard sera included in the assay kits were also tested. An antibody index was calculated for each test serum sample, as the ratio of test sample OD to positive control serum OD, and any value >1.2 was considered positive for measles antibodies (Denka Seiken).

Measles virus isolation

Thawed PBMC were co-cultured with B95a cells for the isolation of measles virus as described elsewhere.[19] Briefly, a suspension of 1×10^6 PBMC cells was added to an equal number of B95a cells in RPMI 1640 with 10% fetal bovine serum and incubated for 6 days. Balloon cells and syncytial formation in the cell cultures showed presence of measles virus.

Malaria parasites test

Thick and methanol-fixed thin blood films were made from the venous blood taken from measles patients and controls and stained with a 1:10 dilution of stock Giemsa Stain (Wako Pure Chemicals, Tokyo, Japan) using phosphate buffer pH 7.2 and examined for malaria parasites.

Immunofluorescence assays

Peripheral blood mononuclear cells were stained with fluorescence isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies (Moabs; Becton Dickinson Immunocytometry System, Mountain View, CA, USA). Anti-CD19, anti-CD3, anti-CD4, anti-CD8, anti-HLA-DR, anti-CD45RO antiCD45RA and anti-CD56+16 Moabs were used in the two-color flow cytometric analysis. PBMC were taken out from the liquid nitrogen and thawed at 37°C in a water bath and resuspended in RPMI 1640. Cells were washed twice and viability tests were done using trypan blue exclusion dye test. Cell suspension was made up in RPMI 1640 supplemented with 5% fetal calf serum at 1×10^6/mL. A total of 100 μL of the suspension was stained with 10 μL of the appropriate Moab for 20 min at 4°C. Finally, cells were fixed and resuspended in fluorescence activated cell sorter (FACS) buffer. A two-color staining pattern of lymphocytes gated by forward and 90° light scatter was evaluated using a FACS scan flow cytometer (Becton Dickinson Immunocytometry System, Tokyo, Japan). A total of 10 000 events per sample tube was collected in list mode

and analyzed in Cellquest software system (Becton Dickinson Immunocytometry System).

Data analysis

Data obtained are shown as mean values ± 95% confidence interval. Student's *t*-test was used to compare mean values for the different groups and $P < 0.05$ was regarded as statistically significant.

Results

Patient clinical characteristics

Thirty-eight children were clinically diagnosed with uncomplicated measles. All children in the study had a previous history of immunization against measles based on inspection of immunization records. Measles virus was isolated in the samples of three patients. All study subjects and healthy controls were negative for malaria parasites.

Serology showed the presence of anti-measles IgG antibodies at the acute phase in 32/38 (84.2%) of the patients. Table 1 shows the clinical symptoms and the presence of anti-measles antibodies in the acute phase of measles infection. Rash was present in all patients. Most of the patients with anti-measles antibodies IgG–/IgM+ had febrile illness. Whereas five out of 22 patients with measles antibodies (IgM+/IgG+) developed a febrile illness, none in 10 patients with measles antibodies (IgM–/IgG+) did. Considering the mild clinical symptoms with the presence of anti-measles IgG antibodies in the acute phase of illness, it was concluded that the infections in these 32 cases were VMM. A subset of 19 was further selected for lymphocyte surface antigen analysis based on availability of samples at all phases of the illness.

Lymphocyte surface antigen analysis

The results of surface antigen staining are shown in Table 2. Cell surface phenotype patterns did not differ significantly between boys and girls with VMM. Frequency of $CD3^+$ T cells at the recovery and convalescence phase were higher in VMM than in healthy controls. The frequency increased to 69.1% on day 14 and 68.9% on day 60 compared to 60.3% in healthy controls, ($P < 0.05$). $CD4^+$ T cells in VMM patients increased significantly at the recovery and convalescence stage in comparison with the healthy control group ($P < 0.05$). $CD8^+$ T-cell frequency in the VMM patients was also higher at the recovery stage than in the control subjects ($P < 0.05$). No statistically significant differences were observed in $CD3^+$, $CD4^+$, and $CD8^+$ T-cell frequencies between VMM patients at the acute stage of the disease and the control group. More interestingly, there was a significantly higher memory helper ($CD4^+$/$CD45RO^+$) cell frequency ($P < 0.05$) and increased suppressor cell ($CD8^+$/$CD45RO^+$) frequency ($P < 0.05$) in VMM patients compared to healthy controls. There were no significant differences in frequencies of NK cells and naive helper ($CD4^+$/$CD45RA^+$) and naive suppressor ($CD8^+$/$CD45RA^+$) cells between patients and controls. $CD19^+$ B cells were significantly suppressed ($P < 0.05$) at all the various phases of the disease in comparison with the control group. All the patients recruited were without any complications and recovery was complete with no mortality.

Discussion

Despite the high coverage with measles vaccine in most parts of Africa, epidemics of measles still occur with reduced severity in an increasing proportion of older children previously immunized.[17,20–22] In general, the prodromal period in VMM is shorter. Cough, coryza and fever are minimal in VMM, rendering it difficult to diagnose based on clinical symptoms. The present study showed that fever was uncommon among the children with VMM, as would have been expected. In addition to clinical diagnosis, we measured anti-measles antibodies and found that the acute phase samples contained positive IgG antibodies, suggesting that the patients had been immunized previously. Taken together with the general absence of complex signs and symptoms typical for measles in a significant number of the present patients, and the presence of anti-measles IgG

Table 1 Measles antibody levels and clinical symptoms in the acute phase of measles infection in Ghanaian children

Antibody test on day 0	Fever BT > 37.5°C *n* (%)	Symptoms				
		Rash *n* (%)	Koplik's spot *n* (%)	Cough *n* (%)	Coryza *n* (%)	Conjunctivitis *n* (%)
IgM+/IgG– ($n = 6$)	5/6 (83)	6/6 (100)	2/6 (33)	6/6 (100)	3/6 (50)	4/6 (67)
IgM–/IgG+ ($n = 10$)	0/10 (0)*	10/10 (100)	7/10 (70)	7/10 (70)	7/10 (70)	7/10 (70)
IgM+/IgG+ ($n = 22$)	5/22 (23)*	22/22 (100)	14/22 (64)	21/22 (95)	13/22 (59)	16/22 (73)

BT, body temperature.
*$P < 0.05$.

Table 2 %positive cells of surface marker antigens expressed on lymphocytes

T-cell phenotypes	Control, $n = 20$, mean (95%CI)	Acute VMM day 0, $n = 19$, mean (95%CI)	Recovery VMM day14, $n = 19$, mean (95%CI)	Convalescence VMM day 60, $n = 19$, mean (95%CI)
CD3	60.28 (55.74–64.83)	66.77 (61.21–72.34)	69.13* (64.36–73.90)	68.98* (63.70–74.27)
CD3/HLA-DR	8.66 (7.78–9.54)	11.75 (7.86–15.65)	11.56 (8.84–14.29)	7.88 (6.24–9.51)
CD4	34.25 (30.82–37.68)	37.05 (32.18–41.92)	37.89* (34.87–40.90)	40.40* (37.11–43.69)
CD8	20.56 (18.33–22.78)	25.05 (21.25–28.85)	24.58* (21.80–27.36)	22.48 (20.22–24.74)
CD19	24.08 (20.51–27.65)	15.13* (13.12–17.13)	14.51* (11.94–17.09)	13.76* (10.76–16.75)
CD56+CD16	7.15 (5.47–8.83)	7.11 (4.82–9.40)	6.10 (4.37–7.82)	7.68 (4.76–10.60)
CD45RO/CD4	7.97 (6.60–9.33)	13.47* (9.10–17.84)	12.37* (9.59–15.15)	11.43* (8.71–14.15)
CD45RO/CD8	1.71 (1.15–2.27)	3.53 (2.57–4.50)	3.25* (2.37–4.14)	2.16 (1.52–2.81)
CD45RA/CD4	26.35 (22.84–29.86)	19.43 (15.68–23.19)	21.32 (17.89–24.75)	24.43 (20.48–28.37)
CD45RA/CD8	16.23 (13.61–18.86)	16.95 (14.52–19.38)	16.88 (14.54–19.22)	17.17 (15.39–18.94)

CI, confidence interval; HLA, human leukocyte antigen; VMM, vaccine-modified measles.
*$P < 0.05$.

antibodies in the acute phase of the illness, it is quite likely that the infection was VMM.

It has been established that T cells are important in both B-cell anti-viral antibody responses and effector cells for the clearance of virus-infected cells. In the case of T cells, helper ($CD4^+$) and suppressor ($CD8^+$) cells actively participate in the cellular response. During measles infection, $CD8^+$ T cells eliminate virus-infected cells by major histocompatibility complex class 1 restricted cytotoxic mechanisms.[6] $CD4^+$ T cells respond to measles infection by secreting cytokines, which in turn activate cells required for virus elimination. We previously reported that plasma levels of interferon-γ, IL-2 and IL-12 were significantly higher in VMM patients on day 0 compared to healthy controls. In contrast, plasma IL-4 was lower in VMM patients on day 0 when compared with the controls.[23] IL-12 production has been shown to be impaired in a majority of patients with naturally acquired measles infection, providing a potentially unifying mechanism for the prolonged abnormalities in cellular immune responses observed during and after measles.[12]

Our previous study found low frequencies of $CD45RO^+$ cells in Ghanaian children with uncomplicated measles.[14] However, in the present study, flow cytometric analysis of PBMC from VMM patients showed significantly higher frequencies of $CD4^+/CD45RO^+$ cells ($P < 0.05$). The frequencies of $CD8^+/CD45RO^+$ cells were also higher in VMM than in the healthy controls ($P < 0.05$), at the recovery phase of the disease. These results strongly suggest that an immunologic memory is intact and perhaps crucial in the control as well as the resolution of VMM. It is well known that upon stimulation by specific antigen, naive T cells lose CD45RA antigen, acquire CD45RO antigen and are finally recruited into the peripheral pool of memory T cells.[24–26] Memory is the hallmark of an immune system required for the efficacy of vaccination in preventing infections. Immunologic memory is recognized by the capacity of a previously immunized host to respond more rapidly and with a greater intensity to a secondary antigenic challenge. Activation and proliferation of memory T cells *in vivo,* most importantly during the acute phase of the illness, occurred to demonstrate the competence of the patients' immune system in preventing illness or reducing complications. At the convalescence phase of VMM, this illustrated the preparedness of the host to respond more rapidly and with greater intensity to a secondary antigenic challenge.

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