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Original Article

Surface marker patterns of T cells and expression of interleukin-2 receptor in measles infection

MICHAEL MARK ADDAE,[1] YOSHIHIRO KOMADA,[2] KIYOSU TANIGUCHI,[3]* TOSHIYA KAMIYA,[1] MUBARAK OSEI-KWASI,[1] BARTHOLOMEW DICKY AKANMORI[1] AND FRANCIS KWAME NKRUMAH[1]

[1]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana,* [2]*Department of Pediatrics, Mie University School of Medicine and* [3]*Mie National Hospital, Mie, Japan*

Abstract The surface marker patterns of T cells of Ghanaian children during measles infection were studied and an attempt was made to demonstrate T cell activation and viability *in vitro* after activation *in vivo* by measles virus. The frequencies of CD4$^+$ and CD8$^+$ naive T cells in measles patients were high while their memory T cells were remarkably reduced with no sign of proliferation even at the acute phase of the illness. The reduction of memory T cells was prolonged during the convalescent phase (2 months after onset). The anti-CD3 monoclonal antibody-induced expression of interleukin-2 receptor α chain (IL-2R/CD25) was significantly suppressed; however, the addition of phorbol 12-myristate 13-acetate or ionomycin caused a remarkable recovery of CD25 expression. On simple culture, an appreciable proportion of T cells from measles patients died rapidly in contrast with only a few T cells from healthy controls doing so. The suppression of CD25 expression was still demonstrated during the convalescent phase of the disease. Taken together these results suggest unresponsiveness and activation-induced cell death of T cells during severe measles infection in Ghanaian children. Furthermore the prolonged abnormalities of T cells (i.e. decreased memory T cells and inhibition of CD25 expression during the convalescent phase) might be related to post-measles infection immunosuppressive status.

Key words cell death, delayed immunosuppression, Ghana, interleukin-2 receptor, measles infection, memory T cells.

Measles continues to cause significant morbidity and mortality among children worldwide in spite of the availability of effective vaccines. The World Health Organization (WHO) estimates that measles results in the death of about 1.5 million children each year and that 3–5% of the infected children in developing countries either die from the acute disease or the long-term adverse effects after infection.[1–4] Increased mortality for 9 months or longer after measles infection has been reported in some developing countries, a phenomenon that is not recognized in the industrialized countries.

Although the mechanism of measles-induced delayed mortality has not been identified, it is thought to be related to a prolongation of the temporary suppression of the cell-mediated immunity that occurs during measles infection.[2,5] Griffin *et al.* reported low activity of natural killer cells in the peripheral blood of children with measles,[6,7] while Ward *et al.* reported spontaneous proliferation of peripheral blood mononuclear cells (PBMC) in children with measles infection.[8] However, these studies did not cover the convalescent period and it would be worthwhile looking at these and other parameters during the convalescent phase of the illness in order to assess their involvement in the delayed morbidity and mortality observed after measles infection. The need to understand the mechanism of measles-induced delayed morbidity and mortality in developing countries is essential as this could contribute to changes in measles vaccination policies and strategies.

Correspondence: Yoshihiro Komada MD, Department of Pediatrics, Mie University School of Medicine, 2–174 Edobashi, Tsu, Mie 514, Japan. Email: komada@clin.medic.mie-u.ac.jp

*Present address: Kiyosu Taniguchi MD, Section of Immunization program, Infectious Disease Surveillance Center, National Institute of Infectious Disease, Shinjyuku, Tokyo 162, Japan

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We report on a study of the immunophenotyping patterns of PBMC and the expression of interleukin-2 receptor α chain (IL-2R/CD25) during measles infection in Ghanaian children.

Methods

Patients

Forty-six children, 22 males and 24 females, aged 12 years or less (mean age ± SD, 3.5 ± 3.3 years) suffering from acute uncomplicated measles infection were recruited from the Ghana Military Hospital, La Polyclinic and Maamobi Polyclinic in Accra. Patients who developed complications such as rubella, malnutrition or gastroenteritis were not included.

Clinical diagnosis of acute measles was based on the following criteria: an acute febrile illness (temperature > 38°C), a characteristic generalized maculopapular rash, and at least one of either cough, coryza, conjunctivitis or Koplik spots. Measles hemagglutination inhibition (HI) titers increased in all enrolled patients.

Informed consent of parents or guardians was obtained before blood samples were collected. All samples taken within 7 days after the onset of fever were regarded as the first samples, representing the acute phase of the illness. Second samples, regarded as recovery or convalescent phase samples, were collected 2 months after disease onset at a time when all patients had no clinical signs or symptoms of measles virus infection or other complications. Thirty samples of blood were collected from age-matched healthy children aged 12 years or less and used as controls. They had been free from clinical signs of infection during the preceding 2 months. Measles HI titers obtained from the health controls showed that 6 of the 30 (20%) children studied were seronegative although their vaccination profiles were not available.

An average of 4 mL of blood was taken into K_3EDTA tubes and PBMC were separated from whole blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia Piscataway, NJ, USA), after films had been prepared for the malaria parasites test. Cells were washed twice in RPMI 1640 medium and cell viability was determined by the trypan blue dye exclusion test after resuspension in RPMI 1640 supplemented with 5% heat-inactivated fetal calf serum (culture medium).

Malaria parasites test

Thick blood films of the patients and healthy controls were stained with a 1 in 10 dilution of Giemsa stain (Wako Pure Chemicals Ltd, Tokyo, Japan) and examined for malaria parasites. The test was done to ensure that patients did not have complicated measles and that the healthy controls also were not immunosuppressed as a result of malaria infection.

Immunophenotypic analysis

Two-color immunofluorescence analysis of cell surface markers was performed, as previously described.[9] Anti-CD45 (anti-Hle-1), anti-CD14 (anti-Leu-M3), anti-CD3 (anti-Leu-4), anti-CD19 (anti-Leu-12), anti-CD4 (anti-Leu-3a), anti-CD8 (anti-Leu-2a), anti-HLA-DR, anti-CD16 (anti-Leu-11c), anti-CD56 (anti-Leu-19), anti-CD25 (anti-IL-2R), anti-CD45RA (anti-Leu-18) and anti-CD45RO (anti-Leu-45RO) monoclonal antibodies (mAb) were obtained in fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated forms from Becton Dickinson Immunocytometry System (Mountain View, CA, USA). Peripheral blood mononuclear cells were stained (10 μL of mAb to 100 μL of cells) for 20 min at 4°C. Cells were fixed and resuspended in 0.5 mL FACS buffer. Two-color staining patterns of lymphocytes gated by forward and 90° light scatters were evaluated by using a FACScan flow cytometer (Becton Dickinson Immunocytometry System). A total of 10 000 events per sample tube were collected in list mode and analyzed in a Lysis II Software System (Becton Dickinson Immunocytometry System).

Induction of IL-2R/CD25 expression in vitro

Peripheral blood mononuclear cells (1×10^6/mL) were cultured with anti-CD3 mAb, OKT3 (1 : 5000 diluted immune ascites) and in three other combinations: (i) anti-CD3 mAb and phorbol 12-myristate 13-acetate, (PMA, 10 nmol/L and 50 nmol/L); (ii) anti-CD3 mAb and ionomycin (1 μmol/L); and (iii) ionomycin (1 μmol/L) and PMA (50 nmol/L). A control tube of PBMC in culture medium was always included. Cultures were incubated in humidified atmosphere of 5% CO_2 at 37°C for 3 days. The cells were washed with FACS buffer and stained with anti-CD4, anti-CD8 and anti-CD25 mAb for 20 min at 4°C. Stained PBMC were washed, resuspended in FACS buffer and analyzed on a FACScan flow cytometer. A total of 10 000 events per sample tube were collected in list mode and analyzed in a Lysis II software system (Becton Dickinson Immunocytometry System).

Statistical analysis

All data are shown as mean values ± SD and Student's t-test was used to determine the level of significance of differences in sample means. Differences that failed to achieve a P value of 0.05 or less were considered non-significant.

Results

Cell surface marker analysis

The immunophenotyping patterns of B cells and T cell subsets of patients with uncomplicated measles are summarized in Tables 1 and 2. The malaria parasites test showed that none of measles patients or healthy controls enrolled in this study were positive for malaria parasites in peripheral blood. The absolute number of lymphocytes in peripheral blood was significantly reduced during the acute phase of measles virus infection but not at the convalescent phase. The frequency of B and T cells during the acute and convalescent phase of the illness showed no significant changes when compared with healthy controls. Interestingly, however, memory helper T cells ($CD4^+/CD45RO^+$) of all the patients showed very significantly reduced frequency at all stages of the illness as compared with the healthy controls. Memory suppressor T cells ($CD8^+/CD45RO^+$) also showed the same pattern with much lower percentages than the memory helper T cells at all stages of the illness. Activated T cells ($CD3^+/HLA\text{-}DR^+$) remained unchanged, maintaining less than 10% in measles patients. Naive helper ($CD4^+/CD45RA^+$) and suppressor ($CD8^+/CD45RA^+$) T cells also remained almost unchanged during the course of the illness. In addition, almost twice the natural killer (NK) cell population of the healthy controls was obtained for measles patients during the convalescent phase of the disease. However, no significant change in the NK cell frequency between patients and controls was observed during the acute phase.

Induction of IL-2R/CD25 expression

The expression of CD25 on $CD4^+$ and $CD8^+$ T cells at the various stages of the illness was investigated (Figs. 1, 2). In the presence of OKT3 mAb (diluted immune ascites), the expression of CD25 appeared distinctly suppressed during both phases of the illness, compared with the healthy controls. The mean percentages of $CD25^+$ cells in $CD4^+$ and $CD8^+$ T cells of measles patients during the acute phase were significantly lower than those of the healthy controls. In the convalescent phase, the expression of CD25 induced by OKT3 mAb on both $CD4^+$ and $CD8^+$ T cells appeared to have recovered. Nonetheless there is statistically significant difference between patients with uncomplicated measles infection and healthy controls in the expression of CD25 induced by OKT3 mAb stimulation. Values in the patients during the convalescent phase were significantly lower than those in healthy controls. In the

Table 1 Absolute number of leukocytes and lymphocytes of measles patients at different phases of the illness

Cell population (cells/μL)	Healthy controls (mean ± SD) (n = 15)	Measles patients (mean ± SD) Acute (n = 15)	Convalescent (n = 15)
Leukocyte	6834 ± 503	3206 ± 467*	7042 ± 887
Lymphocyte	3133 ± 252	1286 ± 189*	3099 ± 498

*Differences between measles patients and healthy controls were significant, assessed by Student's t-test $P < 0.05$.

Table 2 Surface phenotypes of lymphocytes of measles patients at different phases of the illness

Cell population (percent of positive cells)	Phenotype	Healthy controls (mean ± SD) (n = 30)	Measles patients (mean ± SD) Acute (n = 44)	Convalescent (n = 44)
B cell	CD19	16.5 ± 6.8	17.9 ± 7.6	19.3 ± 4.7
T cell	CD3	50.8 ± 9.3	58.7 ± 11.4	57.7 ± 8.2
Helper T cell	CD4	37.3 ± 6.6	31.4 ± 8.6	34.7 ± 11.2
Suppressor T cell	CD8	20.7 ± 5.1	28.8 ± 9.9	27.4 ± 6.0
CD4/CD8 ratio		1.86 ± 0.49	1.24 ± 0.70	1.38 ± 0.71
Activated T cell	CD3/HLA-DR	9.6 ± 4.3	9.8 ± 7.2	7.3 ± 5.0
Natural killer cell	CD16/CD56	7.2 ± 3.6	7.8 ± 5.9	13.9 ± 6.7*
Naive helper T cell	CD4/CD45RA	28.4 ± 5.4	27.0 ± 7.8	27.7 ± 10.3
Naive suppressor T cell	CD8/CD45RA	22.1 ± 6.4	25.6 ± 7.0	27.4 ± 6.5
Memory helper T cell	CD4/CD45RO	17.6 ± 4.9	4.7 ± 3.3*	6.4 ± 2.9*
Memory suppressor T cell	CD8/CD45RO	3.3 ± 1.4	1.1 ± 0.9*	1.3 ± 1.1*

*Differences between measles patients and healthy controls were significant, assessed by Student's t-test $P < 0.05$.

culture without stimulants, the expression of CD25 on both $CD4^+$ and $CD8^+$ T cells was found to be higher in measles patients during the convalescent phase than that in healthy controls.

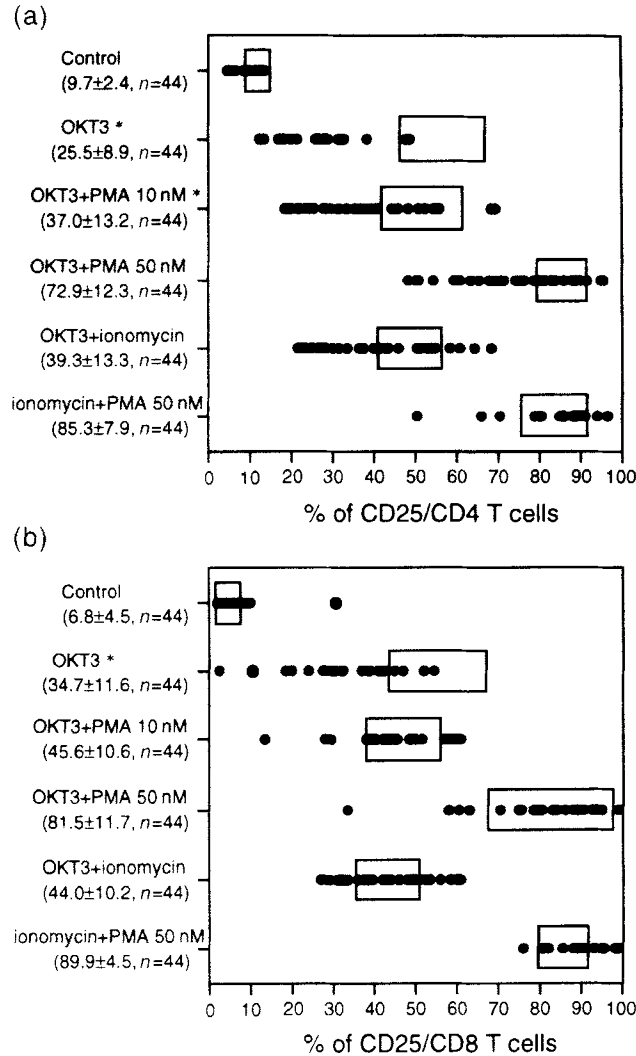

Fig. 1 Expression of IL-2R/CD25 induced with anti-CD3 mAb, PMA, and ionomycin on (a) $CD4^+$ and (b) $CD8^+$ T cells from measles children at the acute phase of the illness. Boxed areas represent mean ± SD of values for $CD4^+$ and $CD8^+$ T cells of age-matched healthy controls ($n = 30$) cultured in control medium (12.1 ± 3.0% and 4.9 ± 2.9% respectively), with OKT3 mAb (56.8 ± 10.2% and 55.3 ± 11.8%, respectively), OKT3 mAb plus 10 nmol/L PMA (51.7 ± 9.7% and 47.1 ± 9.1%, respectively), OKT3 mAb plus 50 nmol/L PMA (85.6 ± 6.2% and 82.5 ± 14.8%, respectively), OKT3 mAb plus 1 µmol/L ionomycin (48.8 ± 7.6% and 43.6 ± 7.5%, respectively), and 1 µmol/L ionomycin plus 50 nmol/L PMA (83.5 ± 8.0% and 85.5 ± 6.0%, respectively). Values in parentheses denote mean ± SD for measles patients and the number of analyzed samples. *Significantly different sample mean from healthy controls, assessed by Student's *t*-test: $P < 0.05$.

To further understand the mechanism of the lack of CD25 expression in the presence of OKT3 mAb alone, PMA and ionomycin were used in various combinations to stimulate both $CD4^+$ and $CD8^+$ T cells. At a PMA concentration of 10 nmol/L, the expression of CD25 on $CD4^+$ T cells of measles patients during the acute phase was significantly lower than that of healthy controls, but not in $CD8^+$ T cells. The percentages of $CD25^+$ cells in $CD4^+$ and $CD8^+$ T cells during the convalescent phase were similar to those of healthy controls. When the PMA concentration was

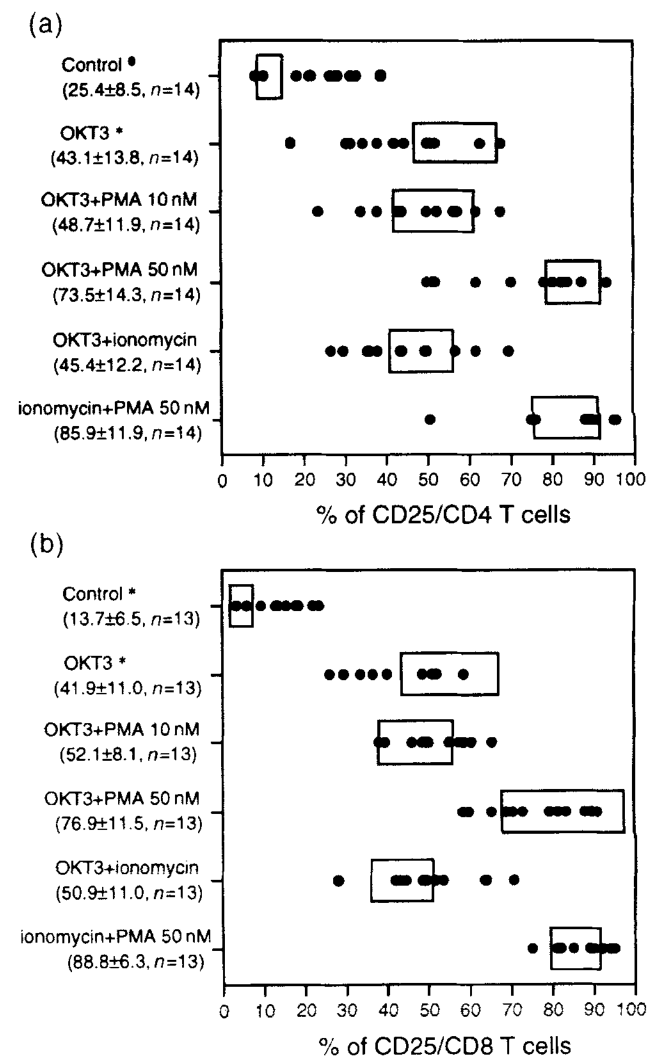

Fig. 2 Expression of IL-2R/CD25 induced with anti-CD3 mAb, PMA, and ionomycin on (a) $CD4^+$ and (b) $CD8^+$ T cells from measles children at the convalescent phase of illness. Boxed areas represent mean ± SD of values for $CD4^+$ and $CD8^+$ T cells of age-matched healthy controls ($n = 30$). Values in parentheses denote mean ± SD for measles patients and the number of analyzed samples. *Significantly different sample mean from healthy controls, assessed by Student's *t*-test: $P < 0.05$.

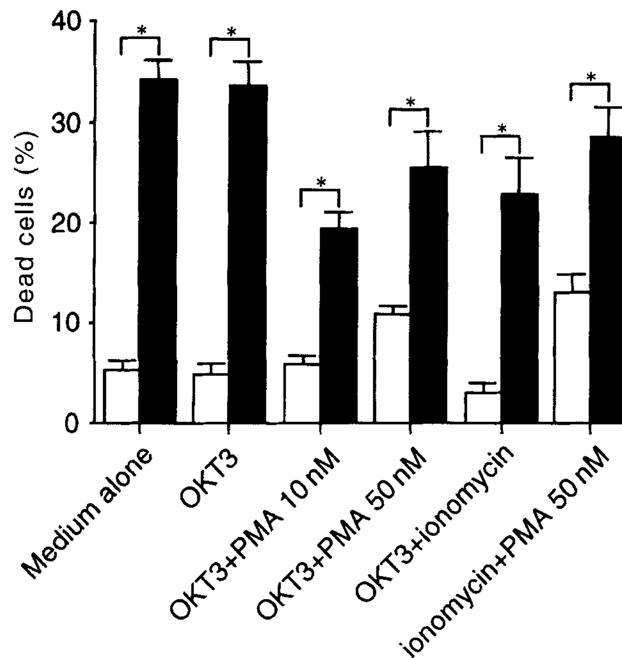

Fig. 3 Cell viability of peripheral blood mononuclear cells from (■) measles children at the convalescent phase of the illness and from (□) age-matched healthy controls. The percentages (mean ± SD) of dead cells taken from patients ($n = 10$) and healthy controls ($n = 10$) were assessed after 5 days' culture in medium alone (34.1 ± 4.0% and 5.2 ± 1.2%, respectively), with OKT3 mAb (33.5 ± 4.4% and 4.8 ± 1.3%, respectively), OKT3 mAb plus 10 nmol/L PMA (19.5 ± 2.5% and 5.8 ± 1.2%, respectively), OKT3 mAb plus 50 nmol/L PMA (25.6 ± 9.6% and 10.9 ± 1.3%, respectively), OKT3 mAb plus 1 μmol/L ionomycin (22.9 ± 8.3% and 3.0 ± 1.0%, respectively), and 1 μmol/L ionomycin plus 50 nmol/L PMA (28.5 ± 6.5% and 13.0 ± 3.7% respectively). *Differences between measles patients and healthy controls were significant, assessed by Student's t-test $P < 0.01$.

increased to 50 nmol/L, significantly higher CD25 expression was obtained from both $CD4^+$ and $CD8^+$ T cells during the acute and convalescent phases. Similarly the use of ionomycin at a concentration of 1 μmol/L in combination with OKT3 mAb appeared to stimulate both $CD4^+$ and $CD8^+$ T cells during the acute and convalescent phases. When PBMC were cultured with PMA (50 nmol/L) and ionomycin (1 μmol/L), CD25 expression on both $CD4^+$ and $CD8^+$ T cells of measles patients during the acute and convalescent phases was similar to that in healthy controls.

Viability of T cells in measles infection

The trypan blue dye exclusion test was used in assessing the viability of PBMC obtained from measles patients during the convalescent phase. Cells were cultured for 5 days in culture medium alone or with anti-CD3 mAb, PMA (10 nmol/L or 50 nmol/L) and ionomycin (1 μmol/L). More dead cells were obtained from the measles samples than from the healthy controls, indicating reduced or decreased viability of PBMC in severe measles patients as compared with healthy controls (Fig. 3). To determine what kind of cells in the measles patients might undergo cell death, the surface phenotypes of the PBMC were compared before and after *in vitro* culture. The percentages of $CD4^+$ and $CD8^+$ T cells before *in vitro* culture (36.8 ± 5.9% and 21.8 ± 4.9%, respectively) were similar to those after *in vitro* culture without stimulants (37.8 ± 7.3% and 22.9 ± 7.4%, respectively), suggesting that both $CD4^+$ and $CD8^+$ T cells in cultures underwent significant cell death.

Discussion

Immune suppression has long been recognized as a consequence of measles infection and a likely contributor to the secondary complications that occur long after the resolution of the disease. We report distinctly reduced memory helper and suppressor T cells in measles patients during both the acute and convalescent phases of the infection. Naive helper and suppressor T cells appear more frequent than memory T cells. It has been reported 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.[10] Therefore, activation and proliferation of memory T cells *in vivo*, especially during the acute phase of the illness, should have occurred in order to demonstrate the competence of the patients' immune system in preventing illness. Our previous report showed that in Japanese patients a high percentage of CD8 T cells during the acute phase of the illness expressed CD45RO antigen in addition to HLA-DR antigens.[9] However, this did not appear to be happening with Ghanaian patients in the present study. Obviously the response to the *in vivo* stimulation or challenge by the measles virus was very poor in the Ghanaian patients. It is possible that the function of T cells in measles patients might be further suppressed due to the complication of an endemic infectious disease, such as malaria, although none of patients studied were positive for malaria parasites. We also found a significant increase of the NK cell population during the convalescent phase of the illness. Although the actual role of these NK cells is unknown, there is a possibility that NK cell function may be activated in compensation for decreased T cell function.

More interestingly, T cells obtained from the measles patients during the convalescent phase underwent significant cell death after 5 days culture. It has been shown that the proliferative response of T cells initially activated by cross-linking the TCR-CD3 complex was severely inhibited when

the activated cells were given an additional signal by religating the TCR-CD3 complex.[11] Moreover the depressed response or reduction of proliferation response would be associated with an induction of programmed cell death (PCD) or apoptosis and T cells undergoing apoptosis resided in the CD45RO^{+} population of T cells.[12] These observations suggest that the majority of the activated CD45RO^{+} T cell population would die through apoptosis after an equivalently strong immune response *in vivo*. We assumed that in the Ghanaian children who acquired measles infection, CD45RO^{+} T cells could be hyperactivated and therefore undergo rapid apoptosis *in vivo* resulting in the prolonged suppression of CD45RO^{+} memory T cells. It is also of interest that CD25 expression without stimulants was significantly higher during the convalescent phase (Fig. 3). These CD25^{+} T cells, probably activated *in vivo*, appeared to be positive for CD45RA antigen and presumably underwent apoptotic cell death *in vitro*. The precise reason why the CD45RA^{+}/CD25^{+} T cells obtained from Ghanaian patients during the convalescent phase are apparently susceptible to the induction of cell death remains to be clarified.

In addition, our results showed that the anti-CD3 mAb-induced expression of IL-2R/CD25 was low or insignificant in the measles patients as compared with the healthy controls. This depressed response observed in Ghanaian patients has been also reported in Japanese patients with measles infection[9] as well as with Epstein–Barr virus-induced infectious mononucleosis.[12] No specific defect in the T cell receptor (TCR)/CD3 pathway could be identified in the present study, but it is of note that the expression of CD25 was restored remarkably when the TCR/CD3 complex was bypassed entirely by using PMA, a potent protein kinase C activator, and ionomycin. These observations suggest that functional and structural alterations of the TCR/CD3 complex might result in a defective activation pathway via the TCR/CD3 complex.

The significant reduction of CD45RO^{+} T cells and suppressed expression of CD25 induction, even during the convalescent phase of the disease, would cause an immunodeficient status in the patients. A similar phenomenon has been suggested to account for the activation-driven cell death of T cells from patients infected with the HIV-1 virus.[13] Hyperactivation-induced cell death may be a mechanism for the removal of abnormally overactivated or hyperactivated cells that may be detrimental to the outcome of an immune response. A recent study has shown that measles virus down-regulates the production of interleukin-12 (IL-12), an important growth factor for Th1-type CD4^{+} T cells that produce IL-2 and interferon-γ (IFN-γ), essential for generation of cell mediated immunity.[14] Therefore if the inhibition of IL-12 production should be prolonged after measles infection, T cells in such patients would not respond or proliferate with TCR/CD3-mediated stimulation. How these events could contribute to delayed mortality in measles children is not immediately clear. The presented data could provide the basis for a more detailed study into the suppression of T cell activation during measles virus infection in developing countries. Children with severe measles infection must necessarily be followed for longer periods before complete recovery of T cell function can be documented. It would be also valuable to extend this investigation to a larger group of subjects in order to fully define the immune response to measles among Ghanaian children.

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