Low Blood CD8+ T-Lymphocytes and High Circulating Monocytes Are Predictors of HIV-1-Associated Progressive Encephalopathy in Children

Silvia Sánchez-Ramón, MD, PhD*; José Mª Bellón, MsC*; Salvador Resino, PhD*;
Carmen Cantó-Nogués, PhD*; Dolores Gurbindo, MD‡; José-Tomás Ramos, MD§; and
MªAngeles Muñoz-Fernández, PhD*

ABSTRACT. *Objective.* Human immunodeficiency virus type 1 (HIV-1)-associated progressive encephalopathy (PE) is a common and devastating complication of HIV-1 infection in children, whose risk factors have not yet been clearly defined. Regardless of the age of presentation, PE shortens life expectancy. Paradoxically, as survival of patients has been prolonged as a result of the use of antiretroviral therapy, the prevalence of PE has increased. Therefore, a predictive marker of PE emergence is critical. The objective of this study was to determine in an observational study whether any immunologic (CD4+ and CD8+ T-lymphocyte counts, monocyte counts) or virologic (viral load [VL], biological characteristics of viral isolates) marker might be predictive of PE and whether any particular marker may be involved in the timing of clinical onset of PE.

Methods. A total of 189 children who were vertically infected with HIV-1 were studied retrospectively, 58 of whom fulfilled criteria of the American Academy of Neurology for PE. T-lymphocyte subsets and monocytes in peripheral blood were quantified by flow cytometry. HIV-1 RNA was measured in plasma using a quantitative reverse transcriptase polymerase chain reaction assay. Demographic, clinical, and viro-immunologic characteristics in infants were compared with control groups using logistic regression. Proportions were compared using the χ^2 test or Fisher exact test. For each child, immunologic and virologic markers were analyzed in parallel closely before clinical onset of PE and closely after PE onset and compared by using the Student t test for paired samples.

Results. Overall, mortality of 58 HIV-1-infected children who developed PE was significantly higher than of children who did not develop this complication. Blood CD8+ T-lymphocytes <25% in the first months of life suggested a relative risk of progressing to PE 4-fold higher than those with CD8+ >25% (95% confidence interval: 1.2–13.9) and remained statistically significant after adjustment for treatment. When we compared the PE-positive group with the acquired immunodeficiency syndrome (AIDS)/PE-negative group (children who developed clinical category C and without neurologic manifestations) in a cross-sectional study within 12 months before PE or AIDS diagnosis, respectively, the %CD8+ T-lymphocytes were significantly lower in the PE-positive group. Normalized absolute counts of CD8+ T-lymphocytes with respect to seroreverting children were significantly lower in the group of children with encephalopathy with respect to the AIDS/PE-negative group (data not shown). It is interesting that a statistically significant increase was observed in circulating monocyte percentages and absolute counts shortly before the first neurologic symptoms compared with values after PE was established and with those from HIV-1-infected controls. With respect to AIDS-related events, PE was strongly associated with anemia and lymphoid interstitial pneumonitis in the PE-positive group with respect to a group of children with AIDS but without PE.

Conclusion. HIV-1 infection of the central nervous system (CNS) remains an important clinical concern. The first step toward PE prevention in HIV-1-infected children should be directed at predicting risk of PE and thus the prompt and reliable identification of infants who are at risk for CNS disease progression. Low blood CD8+ T-lymphocytes is a strong early predictive marker of PE emergence in vertical HIV-1 infection. Indeed, among all of the immunologic and virologic variables assessed in this observational study, the only significant difference during the first months of life are the CD8+ T-lymphocytes. A peak of significantly higher peripheral monocytes before the onset of PE with respect to established PE has not been previously described, and strengthens the growing evidence that an increased traffic of monocytes to the brain may be a key factor in triggering neurologic symptoms. The suppression of HIV-1 replication is dependent on the presence of a relatively small number of HIV-1-specific CD8+ T-lymphocytes, and it is possible that the duration of the neurologically asymptomatic phase for any given child may depend mostly on the magnitude of specific CD8+ T-lymphocyte responses. Thus, a decrease of CD8+ T-lymphocytes would diminish the host capacity to control viral infection, as reported in animal models, enabling infected macrophages to cross the blood-brain barrier. Our results advocate the use of CD8+ T-lymphocyte and monocyte counts to follow-up HIV-1-infected children. We suggest that CD8+ T-lymphocytes may be the nexus for many different aspects of the disease, namely loss of control of HIV-1 replication determining higher VL, increased traffic of activated and/or infected monocytes, spread of infection to immune sanctuaries, and finally clinical neurologic emergence of PE. Moreover, we suggest that CD8+ T-lymphocytes or/and monocytes may be used as putative biological markers of neuropathogenicity. This might suggest their use in decision making of when to start more effective antiretroviral regimens for HIV-1 infection of the CNS and the need of new therapies either to preserve or to augment an adequate CD8+ T-lymphocyte immune response. Early detection of children who are at

risk for developing PE is particularly important because aggressive highly active antiretroviral therapy improves neurologic symptoms, allows possible use of neuroprotective treatment to prevent further development of encephalopathy, and emphasizes the relevance of developing therapies aimed to enhance CD8+ T-lymphocyte function. In conclusion, the surrogate markers routinely used in clinical practice for HIV-1 infection (ie, CD4+ T-lymphocyte counts and VL) seem to be insufficient to evaluate the clinical involvement of the CNS. Other systemic markers, as the recent proposed markers for PE evolution (cerebrospinal fluid VL by lumbar puncture and brain atrophy by cerebral magnetic resonance imaging) are undoubtedly more invasive than measuring CD8+ T-lymphocyte and monocyte counts, when the neurologic manifestations of PE are still preventable. *Pediatrics* 2003;111:e168–e175. URL: http://www.pediatrics.org/cgi/content/full/111/2/e168; *central nervous system, HIV-1-related encephalopathy, development, pediatric AIDS, viral load.*

Human immunodeficiency virus type 1 (HIV-1) displays tropism for both the immune and the nervous systems. HIV-1-associated progressive encephalopathy (PE), among other acquired immunodeficiency syndrome (AIDS)-defining illnesses, has been considered much more common and severe in pediatric patients than in adults.[1,2] It was hypothesized recently that neurologic symptoms that occur within the first year of life may have different pathophysiologic mechanisms and clinical significance than those that occur later, which may in turn reflect the specific timing (in utero vs perinatal) of HIV-1 infection.[1] Regardless of the age of presentation, PE shortens life expectancy; estimates of median survival after the onset of PE have ranged from 6 to 22 months.[3–5]

Antiretroviral therapy (ART) has forever changed the landscape of HIV-1 infection in children,[6–8] reducing opportunistic infections and enabling immune reconstitution.[9–11] However, the impact of new ART regimens on HIV-1 infection of the developing brain is still unknown.[12] Some patients develop PE despite multidrug therapies, and others develop subtle neurobehavioral changes that diminish the quality of their lives, despite the increased survival time.[13] Likewise, as survival of patients has been prolonged, the prevalence of PE has increased.[14]

Therefore, a predictive marker of PE emergence is critical. In this regard, preliminary results in a prospective study showed that peripheral blood CD8+ T-lymphocytes in the first months of life of HIV-infected infants were predictive of emergence of neurologic symptoms related to the PE clinical picture. More important, CD8+ T-lymphocytes were a predictive marker for PE independent of the CD4+ T-lymphocyte and viral load (VL).[15] Here, we sought to

determine in an observational study whether any immunologic (CD4+ and CD8+ T-lymphocyte counts, monocyte counts) or virologic (VL, biological characteristics of viral isolates) marker might be predictive of PE and whether any particular marker may be involved in the timing of clinical onset of PE. To evaluate this hypothesis, we initially examined the occurrence of PE in a cohort of vertically HIV-1-infected children. Next, we tried to identify risk factors both in the mother and in the infant that can predict the risk of developing PE. We subsequently evaluated all of those parameters of children whose encephalopathy had an early onset and compared them with those of children with later-occurring PE.

METHODS

Study Subjects

Between December 1984 and November 2000, 189 children who were born to HIV-1-infected mothers were diagnosed with vertical HIV-1 infection as previously described[16] and were followed from birth at the General University Hospital Gregorio Marañón and Doce de Octubre Hospital in Madrid, Spain. HIV-1-infected infants were classified according to the Centers for Disease Control and Prevention guidelines.[17] Fifty-eight (PE-positive group) of the 189 children met criteria of PE according to the American Academy of Neurology criteria.[18] Encephalopathy was defined as probable when the following criteria were fulfilled: evidence of systemic infection by HIV-1; at least 1 of the following progressive findings: failure to attain or the loss of developmental milestones or of intellectual ability, impaired brain growth or an acquired microcephaly and an acquired symmetric motor deficit verified by clinical examination (eg, slowed rapid movements, abnormal gait, limb incoordination, hyperreflexia, hypertonia, or weakness), neuropsychological test (eg, fine motor speed, manual dexterity, perceptual motor skills), or both; and another cause, including active central nervous system (CNS) opportunistic infection or malignancy, psychiatric disorders (eg, depressive disorder), alcohol or substance use, or acute or chronic substance withdrawal, must be ruled out. Taking into account that cumulative incidence of PE from birth in the 189 HIV-1-infected children was maximal at 24 months of age (19.6%), representing 64% of the 58 children with PE, we studied thereafter separately children who developed PE before and after this age point, as "early-onset PE" and "late-onset PE" groups. PE diagnosis was made by the same attending pediatrician and neuropediatrician at each center. Each of the 58 children of the PE-positive group had an extensive medical history, general physical examination, serial head circumference measurements, and detailed neurologic examination. Two additional groups of 28 children (the PE-negative group) with vertical HIV-infection in all clinical categories—A (n = 14), B (n = 2), and C (n = 12)—and 32 children who developed category C (the AIDS/PE-negative group) but without neurologic manifestations, were randomly selected for comparisons at birth and matched to those in the PE-positive group with respect to age at AIDS presentation, respectively. Control groups had similar demographic characteristics and mothers with similar risk behaviors and were born in the same time points.

Gestational age was determined by a combination of prenatal ultrasonography, physical examination of uterine fundal height, and menstrual history. Prenatal drug exposure was defined by maternal prenatal use of illicit drugs (opiates, cocaine, or other injectables) or methadone, as assessed by either a positive interview and/or urine toxicological studies. Patients were considered positive when either criteria was positive. Weight and height *z* scores and head circumferences percentiles were determined by using the Orbegozo's scale for Spanish children as standards.

All medical charts were reviewed retrospectively by the same person on the basis of evocative clinical signs, neuroradiologic examinations, immunologic and virologic markers, treatments, and response to specific treatments. With respect to neuroradiologic examinations (computed tomography [CT] scans, magnetic resonance imaging, or single photon emission CT), we recorded the reports on the results of all of these techniques.

Virus Isolation and Determination of Viral Phenotype

HIV-1 was isolated by a microculture technique, as previously described.[16,19] Briefly, peripheral blood mononuclear cells were isolated from blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Uppsala, Sweden). Both viral culture and polymerase chain reaction assays were performed on the same sample. The criteria for a positive co-culture and determination of the days to first positive were based on p24 HIV results obtained from biweekly culture samples. According to the lag phase before p24 antigen detection and to the level of p24 antigen production by peripheral blood mononuclear cells, HIV-1 isolates from these patients were classified as rapid/high or slow/low.[20] We considered an isolate as syncytium-inducing (SI) when both formation of syncytia in MT-2 cell line under light microscopy and p24 antigen production in the culture supernatants were detected.[16] Moreover, as MT-2 cell line expresses CD4 receptor and CXCR4 (X4) co-receptor but not CCR5 (R5), this cell line may be useful to differentiate X4-using or SI from R5-using or non-SI strains. HIV_{BAL} (R5 or non-SI) and $HIV_{NL4.3}$ (X4 or SI) are routinely used as controls.

Quantitative HIV-1 RNA Assay

Plasma samples were collected into ethylenediaminetetraacetic acid tubes and separated within 4 hours, and the plasma was stored at $-70°C$. HIV-1 RNA was measured in 200 μL of plasma using a quantitative reverse transcriptase polymerase chain reaction assay (Amplicor Monitor, Roche Diagnostic System, Brandersburg, NJ).

Quantification of T-Cell Subsets and Monocytes in Peripheral Blood

T-lymphocyte subsets and monocytes in peripheral blood were quantified by direct immunofluorescence using monoclonal antibodies of the T series and for monocytes (anti-CD14) and flow cytometry (FACScan; Becton-Dickinson, San Jose CA), as previously described.[16,19]

Statistical Methods

In all analyses, HIV-1 RNA levels were transformed to $\log_{10}$ scale to normalize their distribution. Proportions were compared using the χ^2 test or Fisher exact test for expected values below 5. Quantitative variables were expressed as means ± standard error of the mean and compared by using the Student t test or the nonparametric Wilcoxon rank sum test. Kaplan-Meier estimates of the cumulative probability were used for each covariate on the risk of reaching an outcome event and were compared by the log rank test (Mantel-Haenszel). The time to progress to PE and its relative risk were estimated by the proportional hazard Cox regression equation. For ruling out the effect of age on the %CD4$^+$ and %CD8$^+$ T-lymphocytes, their values were adjusted with respect to a cohort of age-matched HIV-1-exposed but uninfected children or seroreverting children (z score).

RESULTS

PE incidence dramatically fell since 1997 from 9.5% to 5.6% in our series, in parallel with the decreased incidence of vertical HIV infection. From 1998, both (vertical HIV infection and PE) incidences are near 0%, as a result of ART during pregnancy and early in life.

Overall, mortality of 58 HIV-1-infected children who developed PE was significantly higher than that of children who did not develop this complication (131 children: 60% vs 44% among non-PE children; $P = .04$).

Characteristics at Birth

Sociodemographic, maternal, and birth characteristics of infants were similar for PE-positive and PE-negative groups (Table 1). No significant differences were noted at birth between early- and late-onset PE-positive and PE-negative groups, respectively (data not shown).

Time to progress to PE was assessed by Kaplan-Meier estimates for the variables studied. Children with low birth weight (<2500 g) progressed to PE faster than those with birth weight >2500 g ($P = .01$; Fig 1A). By Cox regression analysis, children with low birth weight were 2.2-fold more likely to progress to PE than children with birth weight >2500 g (95% confidence interval: 1.1–4.1; $P < .01$). None of the other variables examined was significantly related to risk for progressing to PE.

Risk Factors of PE in the First Months of Life

We then analyzed the predictive power of immunologic (%CD4$^+$ and %CD8$^+$ T-lymphocytes) and virologic (VL) markers in the first months of life

TABLE 1. Clinical and Immunologic Characteristics of the PE-Negative and the PE-Positive Groups at Birth and at a Time Point Within the First Months of Life (Range: 2–7 Months)

Characteristic	PE-Negative Group ($n = 28$)	PE-Positive Group ($n = 58$)
At birth		
Gender (M/F)	7/21	23/35
Gestational age at delivery <37 wk	4 (20%)	13 (32%)
Drug exposure during gestation (n [%])	6 (21.4%)	15 (26%)
Birth weight		
Mean ± sem (g)	2.656 ± 132	2.708 ± 82
Low birth weight (<2500 g)	8 (34.8%)	19 (35.8%)
Responsible for the child's care		
Biological mother	11 (58%)	35 (67%)
Other	8 (42%)	17 (33%)
In first months of life		
Age (mo)	5.0 ± 0.3	4.4 ± 0.3
CD4$^+$ T-lymphocytes	36.6 ± 2.8	34.4 ± 3.3
CD8$^+$ T-lymphocytes	26.3 ± 2.5	20.2 ± 2.4*
VL ($\log_{10}$)	4.7 ± 0.3	5.1 ± 0.2
CD4$^+$ <25% T-lymphocytes	24%	21%
CD8$^+$ <25% T-lymphocytes	40.7%	85%†
VL> 5 $\log_{10}$	44%	50%

Values are expressed as mean ± SEM. SEM indicates standard error of the mean.
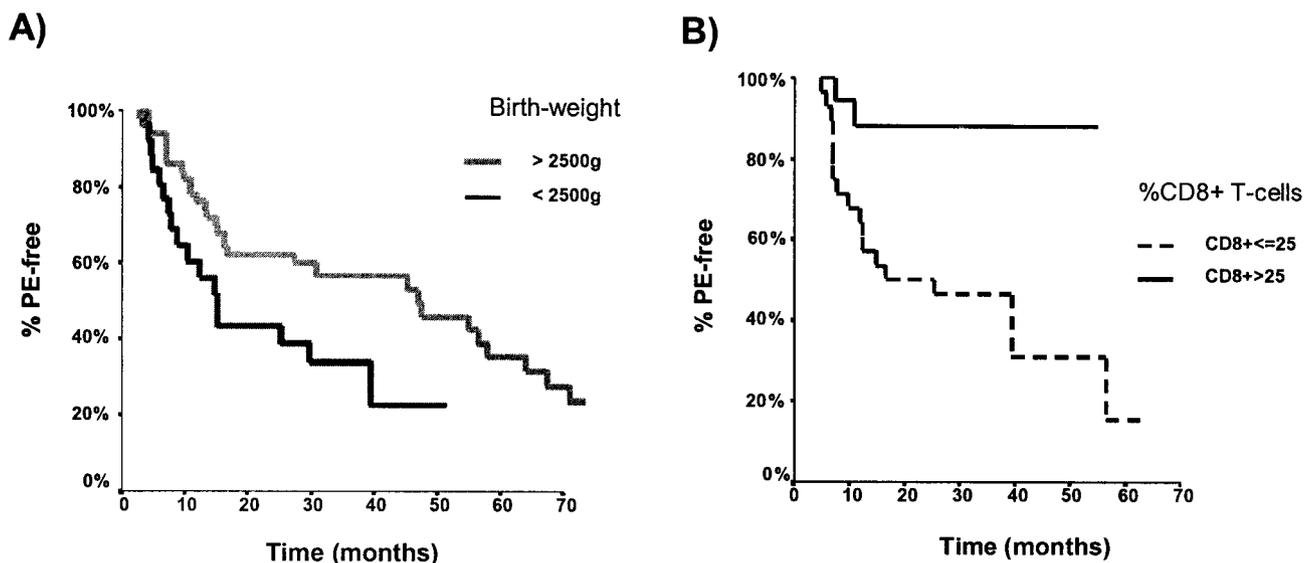* $P < .05$.
† $P < .01$.

Fig 1. Kaplan-Meier curves of progression to encephalopathy, classified according to birth weight of 2500 g (A) and 25% of CD8+ T-lymphocytes (B).

(range: 2–7 months) for PE emergence (Table 1). The %CD8+ T-lymphocytes was significantly lower in the PE-positive group ($P < .05$) and in children with early-onset PE ($P < .05$) than in the PE-negative group. In addition, we studied time of progression to PE by Kaplan-Meier estimates according to a cutoff level for each of the 3 markers, 25% CD4+, 25% CD8+, and 5 log$_{10}$ VL, which have been reported to have prognostic value.[21] Children with CD8+ T-lymphocytes <25% in the first months of life progressed earlier to PE than those with CD8+ T-lymphocytes >25% ($P = .02$), being the median time of progression to PE in the former of 16.7 months versus 55.0 months, respectively (Fig 1B). By Cox regression analysis, CD8+ T-lymphocytes <25% in the first months of life suggested a relative risk of progressing to PE 4-fold higher than those with CD8+ >25% (95% confidence interval: 1.2–13.9; $P = .02$). The results remained significant after adjustment for ART ($P < .05$). No significant correlation between low CD8+ T-lymphocytes and low birth weight was observed in this cross-sectional study.

Risk Factors for PE Before Onset of Encephalopathy

For this purpose, we compared the PE-positive group with the AIDS/PE-negative group (32 vertically HIV-1-infected children who developed clinical category C during their lives and without neurologic manifestations) in a cross-sectional study within 12 months before PE diagnosis for the PE-positive group (mean time: 8.2 ± 0.4 months) or to clinical category C or AIDS (including lymphoid interstitial pneumonitis [LIP]) for the AIDS/PE-negative group (mean time, 8.5 ± 1.4 months) (Table 2). Again, the %CD8+ T-lymphocytes were significantly lower in the PE-positive group ($P < .01$) than in the AIDS/ PE-negative group. Higher proportions of children in the PE-positive group ($P < .01$) and in the early-onset PE ($P < .01$) and late-onset PE ($P < .05$) groups had CD8+ <25% T-lymphocytes before PE than the AIDS/PE-negative group before developing AIDS.

Plasma VL levels were higher in the PE-positive group ($P = .05$; Table 2) and in the late-onset PE group ($P < .05$) than in the AIDS/PE-negative group, respectively. Normalized CD8+ T-lymphocytes percentages with respect to seroreverting children were similarly significant (Table 2). Normalized absolute counts of CD8+ T-lymphocytes were significantly lower in the 3 groups of children with encephalopathy with respect to the AIDS/PE-negative group (data not shown). The biological characteristics of viral isolates more frequently observed in peripheral blood before PE onset were SI phenotype (65%) and rapid/high replication kinetics (65%), both corresponding to X4 or lymphocytotropic viral isolates. With respect to AIDS-related events, PE was strongly associated with anemia ($P = .02$) and LIP ($P < .01$) in the PE-positive group (Table 2).

Studies Before and After PE

For each child, immunologic and virologic markers were analyzed in parallel closely before clinical onset of PE (within a 6-month interval before PE; mean time: 4.0 ± 0.6 months) and closely after PE onset (mean time: 3.5 ± 0.6 months) and compared by using the Student t test for paired samples. The percentages of both CD4+ and CD8+ T-lymphocytes were significantly higher before PE onset with respect to the post-PE study ($P < .05$; Fig 2A). When analyzing the influence of ART administered after PE emergence on these parameters by analysis of variance for repeated measures of CD4+ and CD8+ T-lymphocytes and VL adjusting for ART post-PE, only the variation of CD4+ T-lymphocytes was significantly influenced, being the fall-off of CD4+ T-lymphocytes more marked in the children not treated ($P = .01$; Fig 2B).

Peripheral blood monocytes by flow cytometry were similarly analyzed before and after clinical PE onset in the same time points and compared as paired samples. Both percentages and absolute monocyte counts were significantly higher in the

TABLE 2. Clinical and Immunologic Characteristics of the AIDS/PE-Negative and the PE-Positive Groups at a Time Point Before Developing AIDS and PE, Respectively

Characteristic	AIDS/PE-Negative Group ($n = 32$)	PE-Positive Group ($n = 58$)
Age (y)	3.4 ± 0.4	2.7 ± 0.5
Gender (M/F)	15/17	22/36
CDC clinical and immunologic classification		
A	15 (47%)	21 (36%)
B	12 (38%)	32 (55%)
C	5 (15%)	5 (9%)
$CD4^+$ >25%	11 (34%)	25 (38%)
$CD4^+$ 15–25%	8 (25%)	13 (21%)
$CD4^+$ <15%	13 (41%)	18 (41%)
Immunologic and virologic characteristics		
%$CD4^+$ T-lymphocytes	21.4 ± 2.33	19.3 ± 2.7
Z %$CD4^+$ T-lymphocytes	-8.4 ± 0.8	-11.4 ± 1.3
%$CD8^+$ T-lymphocytes	38.8 ± 2.5	23.4 ± 2.7†
Z %$CD8^+$ T-lymphocytes	11.1 ± 1.4	4.8 ± 1.8†
VL (copies/mL)	$82\,425 \pm 25\,039$	$204\,433 \pm 69\,567$*
Log_{10} VL	4.47 ± 0.21	5.10 ± 0.13*
$CD8^+$ <25% T-lymphocytes	18%	58%†
VL> 4.5 log_{10}	56%	76%
VL> 5.5 log_{10}	0%	18%
Non-SI viral phenotype	3 (25%)	7 (35%)
SI viral phenotype	9 (75%)	13 (65%)
Slow/low replication kinetics	3 (25%)	7 (35%)
Rapid/high replication kinetics	9 (75%)	13 (65%)
Clinical characteristics		
Hepatomegaly	8 (50%)	28 (48%)
Lymphadenopathy	5 (31%)	23 (40%)
Candidiasis	3 (19%)	22 (38%)
LIP	1 (6%)	17 (29.3%)†
Anemia	2 (13%)	23 (40%)*
Failure to thrive	3 (19%)	13 (22.4%)
HIV-related myocardiopathy	1 (6%)	12 (20.6%)
Trombocytopenia	2 (12.5%)	9 (15.5%)

CDC indicates Centers for Disease Control and Prevention.
Results are expressed as mean ± SEM.
* $P < .05$.
† $P < .01$.
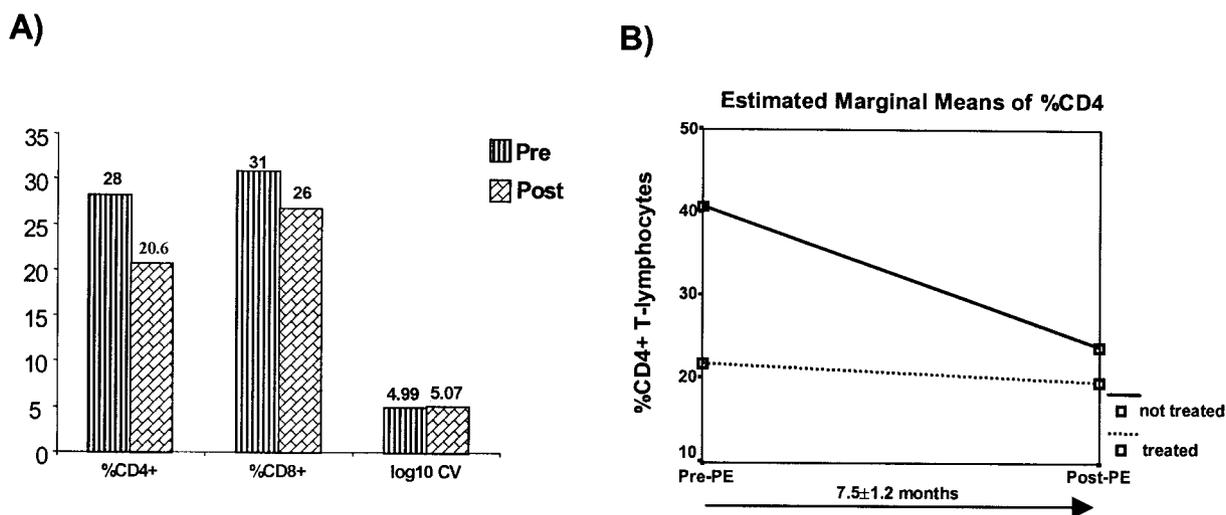
Fig 2. A, Comparison of immunologic (%$CD4^+$ T-lymphocytes and %CD8+ T-lymphocytes) and virologic variables (log_{10} VL) before and after progressive encephalopathy onset. B, Effect of ART given after encephalopathy onset on $CD4^+$ T-lymphocytes percentages.

pre-PE interval than in the post-PE ($P < .05$ and $P < .001$, respectively) and than in a control group of vertically HIV-1-infected children without neurologic manifestations (11 children in clinical category A, 11 in B, and 8 in C; $P < .05$ and $P < .001$, respectively; Table 3). The differences in monocyte absolute counts between the study pre-PE and the control group remain significant after rating the control group in clinical categories A, B, and C ($P < .001$). Besides, absolute counts of monocytes after clinical onset of PE were higher than those of the control group ($P < .001$; Table 3). Of relevance, in 4 children of our cohort who underwent bone marrow biopsies, extensive histiocytic infiltration with in-

TABLE 3. Monocyte Blood Counts by Flow Cytometry in HIV-Infected Children

	% Monocytes			Monocytes/μL		
	Controls	HIV-1	P*	Controls	HIV-1	P*
Pre-PE	7.8 ± 1.5	11.3 ± 1.6	.03	180.1 ± 27.2	794.3 ± 76.1	<.001
Post-PE		7.77 ± 0.8	.25		567.2 ± 85.5	<.001
P		.03			<.001	

Comparison of levels before and after the onset of PE with a control group of children without PE in the 3 CDC clinical categories.
* Significance level between monocyte values of the children with encephalopathy and controls.

creased expression of phagocyte lineage cells together with altered erythropoiesis was found, which would also account for anemia.

Comparison of Early- and Late-Occurring PE in Children

Early-onset PE was found to be more frequently an isolated symptom of AIDS (91%) than late onset PE (24%). Head circumferences of the 37 children with early-onset PE were slightly lower than the 21 with late-onset PE (31.0 vs 32.9 cm, respectively). Similarly, the number of premature children was higher in the early-onset PE group (P = .09), and birth weight was significantly lower in the early-onset PE group than in the late-onset PE group (P = .02). Cerebral atrophy was the main observed finding by CT scan (n = 47), regardless of age of PE onset. Basal ganglia calcifications were found in 46% of children who underwent CT, more predominantly in early-onset PE than in late-onset PE (69% vs 23%).

DISCUSSION

HIV-1 infection of the CNS remains an important clinical concern. One of the primary challenges in the care of infants and children who are infected with HIV-1 remains the early and accurate diagnosis of PE to initiate early, aggressive, specific ART in hopes of preventing devastating effects of HIV on the developing brain.[22] The first step toward PE prevention in HIV-1-infected children should be directed at predicting risk of PE and thus the prompt and reliable identification of infants who are at risk for CNS disease progression. Although PE unquestionably shortens life expectancy,[3] a 4.4-year median survival of our series of 58 HIV-1-infected children with PE must be interpreted in the context of an HIV-1-infected population, so we compared background mortality in a similar group of children without PE. Future progress should be measured not merely in terms of extending the life expectancy of children with PE but by preventing neurologic damage when it is still possible and therefore in the first stages of CNS infection and particularly toward primary prevention of disease.

Whether an HIV-1-infected child develops neurologic disease and how early the clinical signs of infection appear are most likely the net result of both viral virulence and host factors. Important viral factors include cell tropism and strains that determine neurovirulence. The host factors include the cellular expression of viral co-receptors and maintenance of competent immune responses. The conclusions drawn by different cross-sectional and longitudinal studies in HIV-1-infected children to date have failed to estimate the timing of onset of PE with the routine progression markers and other parameters used in the follow-up of HIV-1-infected children in clinical practice. Thus, the longitudinal nature of this study enables some inferences to be drawn concerning the timing of the onset of neurologic abnormalities. In that context, CD8$^+$ T-lymphocyte counts and monocytes may serve to predict the risk of PE and the time of clinical emergence, respectively.[23]

Evidence for higher peripheral monocytes in adult patients with HIV-1-associated dementia than in patients without dementia, with a unique subset of activated monocytes, once encephalopathy is established, has been previously reported.[24,25] More important, a peak of significantly higher peripheral monocytes before the onset of PE with respect to established PE has not been previously described, and strengthens the growing evidence that an increased traffic of monocytes to the brain may be a key factor in triggering neurologic symptoms. Mononuclear phagocytes (brain macrophages and microglia) are the main HIV-1 reservoir in the CNS, and the abundance of macrophages in the brain seems to be the best correlate with severity of PE.[26–28] However, the peak of monocytes may also indicate a reaction to PE in its onset, and not a pathogenic mechanism.

The higher frequency of PE as AIDS-defining illness when occurring before 2 years of age has been previously described,[1,3,4] suggesting that the key pathogenic mechanisms responsible for progression of PE may vary with age of onset. In this regard, it has been suggested that HIV-1-infected infants with rapid progression are reflecting in utero HIV-1 infection.[16,29–31] Likewise, early-onset PE may be the consequence of in utero infection of the brain.[1] Head circumference, in utero exposure to illicit drugs, and birth weight were prognostic markers for progression to PE.[1] However, in both early- and late-onset PE, the entry of the virus into the CNS takes place during the development of the brain and during the process of myelination. HIV-1 encephalopathy in young children could be a very specific consequence of the interaction between HIV-1 and the developing immune and nervous systems and thus might require a specific therapeutic approach.[1]

We previously demonstrated in a prospective study that CD8$^+$ T-lymphocyte counts in the first months of life were significantly associated with the risk for emergence of neurologic signs defining probable PE (cognitive deterioration, cerebral atrophy, progressive motor dysfunction, gait disorder, reflex

disturbances, abnormal muscle tone, and HIV-1 encephalitis.[15] Of note, there were not differences in confounding factors, particularly equal number of patients exposed to drugs in utero. The most striking result is that among all of the immunologic and virologic variables assessed in this observational study, the only significant difference during the first months of life and before the onset of PE are the CD8^{+} T-lymphocytes.

Cytotoxic CD8^{+} T-lymphocytes (CTLs) kill virus-infected cells, and their role in the control of HIV-1 infection in vitro and in vivo is well established, although only a few studies have analyzed their prognostic value in the follow-up of infected adults[32–34] and children.[21,35,36] The suppression of HIV-1 replication is dependent on the presence of a relatively small number of HIV-1-specific CD8^{+} T-lymphocyte clones, and it is possible that the duration of the neurologically asymptomatic phase for any given child may depend mostly on the magnitude of specific CD8^{+} T-lymphocyte responses.[37] Thus, a decrease of CD8^{+} T-lymphocytes would diminish the host capacity to control viral infection, as reported in animal models,[38] enabling infected macrophages to cross the blood-brain barrier. Compatible with this, HIV-1-infected macrophages by HIV-1-specific CTLs have been reported to be less effectively destroyed than HIV-1-infected CD4^{+} T-lymphocytes.[39] Therefore, a drop in number of CTLs will favor escape of circulating infected macrophages from lysis. Recent studies have shown increased numbers of activated CD8^{+} T-lymphocytes in the brain correlating with neurologic dysfunction in animal models[40] or in specimen brain biopsies from HIV-infected children with encephalopathy.[41]

Although some authors have advocated the key significance of high VL for the increased traffic of peripheral monocytes into brain in adults HIV-1-associated dementia,[25] we did not find significant differences between children with and without PE, in accordance with recent results in adult patients.[42,43] In fact, children show extremely high VL levels since early in life,[44–47] even under highly active ART, ruling out its possible role as a triggering factor for the onset of the neurologic picture. There was a trend for SI viral phenotype with rapid/high replication kinetics before PE emergence, as has been described soon before progression to AIDS in children.[48] However, given the compartmentalization of HIV, circulating viral isolates may only be reflecting the progression of the disease but not specifically indicating the course of CNS involvement.[49] Anemia was a good predictor of PE in agreement with other authors[3,5] and may suggest in part hematopoiesis impairment.[25,50] Also, we observed LIP before PE in a significantly higher number of children than in an age-matched group of HIV-positive children before undergoing non-PE AIDS-defining illnesses.

Our results advocate the use of CD8^{+} T-lymphocyte and monocyte counts to follow-up HIV-1-infected children. We suggest that CD8^{+} T-lymphocytes may be the nexus for many different aspects of the disease, namely loss of control of HIV-1 replication determining higher VL, increased traffic of activated and/or infected monocytes, spread of infection to immune sanctuaries, and finally clinical neurologic emergence of PE. Moreover, we suggest that CD8^{+} T-lymphocytes and/or monocytes may be used as putative biological markers of neuropathogenicity. This might suggest their use in decision making of when to start more effective ART regimens for HIV-1 infection of the CNS and the need of new therapies either to preserve or to augment an adequate CTL immune response. Early detection of children who are at risk for developing PE is particularly important because aggressive highly active antiretroviral therapy improves neurologic symptoms, allows possible use of neuroprotective treatment to prevent further development of encephalopathy, and emphasizes the relevance of developing therapies aimed to enhance CD8^{+} T-lymphocyte function.[51]

CONCLUSION

The surrogate markers routinely used in clinical practice for HIV-1 infection (CD4^{+} T-lymphocyte counts and VL) seem to be insufficient to evaluate the clinical involvement of the CNS. Other systemic markers, as the recent proposed markers for PE evolution (CSF VL by lumbar puncture[52] and brain atrophy by cerebral magnetic resonance imaging[38]) are undoubtedly more invasive than measuring CD8^{+} T-lymphocyte and monocyte counts, when the neurologic manifestations of PE are still preventable.

REFERENCES

1. Tardieu M, Le Chenadec J, Persoz A, et al. HIV-1-related encephalopathy in infants compared with children and adults. French Pediatric HIV Infection Study and the SEROCO Group. *Neurology.* 2000;54: 1089–1095
2. Mintz M. Clinical comparison of adult and pediatric NeuroAIDS. *Adv Neuroimmunol.* 1994;4:207–221
3. Cooper ER, Hanson C, Diaz C, et al. Encephalopathy and progression of human immunodeficiency virus disease in a cohort of children with perinatally acquired human immunodeficiency virus infection. Women and Infants Transmission Study Group. *J Pediatr.* 1998;132:808–812
4. Lobato MN, Caldwell MB, Ng P, Oxtoby MJ. Encephalopathy in children with perinatally acquired human immunodeficiency virus infection. Pediatric Spectrum of Disease Clinical Consortium. *J Pediatr.* 1995; 126:710–715
5. McArthur JC, Hoover DR, Bacellar H, et al. Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology.* 1993;43:2245–2252
6. Raskino C, Pearson DA, Baker CJ, et al. Neurologic, neurocognitive, and brain growth outcomes in human immunodeficiency virus-infected children receiving different nucleoside antiretroviral regimens. Pediatric AIDS Clinical Trials Group 152 Study Team. *Pediatrics.* 1999;104(3). Available at: www.pediatrics.org/cgi/content/full/104/3/e32
7. Englund JA, Baker CJ, Raskino C, et al. Zidovudine, didanosine, or both as the initial treatment for symptomatic HIV-infected children. AIDS Clinical Trials Group (ACTG) Study 152 Team. *J Clin Invest.* 1997;99: 2837–2841
8. Melvin AJ, Mohan KM, Arcuino LA, Edelstein RE, Frenkel LM. Clinical, virologic and immunologic responses of children with advanced human immunodeficiency virus type 1 disease treated with protease inhibitors. *Clin Exp Immunol.* 1997;110:15–21

9. Carcelain G, Debre P, Autran B. Reconstitution of CD4+ T lymphocytes in HIV-infected individuals following antiretroviral therapy. *Curr Opin Immunol.* 2001;13:483–488

10. Resino S, Sánchez-Ramón S, Bellón JM, Correa R, Abad ML, Munoz-Fernandez MA: Immunologic recovery after 3 years' antiretroviral therapy in HIV-1-infected children. *AIDS.* 2002;16:483–486

11. Correa R, Munoz-Fernandez MA. Viral phenotype affects the thymic production of new T cells in HIV-1-infected children. *AIDS.* 2001;15:1959–1963

12. Husstedt IW, Frohne L, Bockenholt S, et al. Impact of highly active antiretroviral therapy on cognitive processing in HIV infection: cross-sectional and longitudinal studies of event-related potentials. *AIDS Res Hum Retroviruses.* 2002;18:485–490

13. Price RW, Yiannoutsos CT, Clifford DB, et al. Neurological outcomes in late HIV infection: adverse impact of neurological impairment on survival and protective effect of antiviral therapy. AIDS Clinical Trial Group and Neurological AIDS Research Consortium study team. *AIDS.* 1999;13:1677–1685

14. Starace F, Dijkgraaf M, Houweling H, Postma M, Tramarin A: HIV-associated dementia: clinical, epidemiological and resource utilization issues. *AIDS Care.* 1998;10(suppl 2):S113–S121

15. Gurbindo D, Resino S, Sánchez-Ramón S, León JA, Muñoz-Fernández MA. Correlation of viral load and CD8 T-lymphocytes with development of neurological manifestations in vertically HIV-1-infected infants. A prospective longitudinal study. *Neuropediatrics.* 1999;30:197–204

16. Muñoz-Fernández MA, Obregón E, Navarro J, et al. Relationship of virologic, immunologic, and clinical parameters in infants with vertically acquired human immunodeficiency virus type 1 infection. *Pediatr Res.* 1996;40:597–602

17. Centers for Diseases Control Prevention. Revised classification system for HIV-1 infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep.* 1994;43:1–13

18. Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. Report of a Working Group of the American Academy of Neurology AIDS Task Force. *Neurology.* 1991;41:778–785

19. Muñoz-Fernández MA, Navarro J, Obregón E, et al. Immunological and virological markers of disease progression in HIV-1 infected children. *Acta Paediatr Suppl.* 1997;421:46–51

20. Resino S, Gurbindo D, Navarro J, et al. Prognostic markers in naive infants younger than two months of age with HIV-1 infection. *Pediatr Relat Top (Padiatr Grenzgeb).* 1999;38:431–446

21. Resino S, Gurbindo D, Cano JM, Sánchez-Ramón S, Muñoz-Fernández MA. Predictive markers of clinical outcome in vertically HIV-1-infected infants. A prospective longitudinal study. *Pediatr Res.* 2000;47:509–515

22. Chase C, Ware J, Hittelman J, et al. Early cognitive and motor development among infants born to women infected with human immunodeficiency virus. Women and Infants Transmission Study Group. *Pediatrics.* 2000;106(2). Available at: www.pediatrics.org/cgi/content/full/106/2/e25

23. Sánchez-Ramón S, Bellón JM, Resino S, et al. Low CD8+ T-lymphocyte counts predict HIV-1-associated progressive encephalopathy in children and may trigger CNS spread of HIV-1-activated monocytes. Presented at the 9th Conference on Retroviruses and Opportunistic Infections; February 24–28, 2002; Seattle, WA

24. Pulliam L, Gascon R, Stubblebine M, McGuire D, McGrath MS. Unique monocyte subset in patients with AIDS dementia. *Lancet.* 1997;349:692–695

25. Gartner S. HIV infection and dementia. *Science.* 2000;287:602–604

26. Epstein LG, Gendelman HE. Human immunodeficiency virus type 1 infection of the nervous system: pathogenetic mechanisms. *Ann Neurol.* 1993;33:429–436

27. Glass JD, Fedor H, Wesselingh SL, McArthur JC. Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann Neurol.* 1995;38:755–762

28. McClernon DR, Lanier R, Gartner S, et al. RNA levels and patterns of zidovudine resistance. *Neurology.* 2001;57:1396–1401

29. Rouzioux C, Costagliola D, Burgard M, et al: Timing of mother-to-child HIV-1 transmission depends on maternal status. The HIV Infection in Newborns French Collaborative Study Group. *AIDS.* 1993;7(suppl 2):S49–S52

30. Newell ML. Mechanisms and timing of mother-to-child transmission of HIV-1. *AIDS.* 1998;12:831–837

31. Maldonado Y. Human immunodeficiency virus HIV and acquired immunodeficiency syndrome. In: Long SS, ed. *Principle and Practice of Pediatric Infectious Disease.* 4th ed. New York, NY: Churchill Livingstone; 1997:2169–2226

32. Margolick JB, Munoz A, Donnenberg AD, et al. Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. The Multicenter AIDS Cohort Study. *Nat Med.* 1995;1:674–680

33. Phillips AN, Sabin CA, Elford J, et al. CD8 lymphocyte counts and serum immunoglobulin A levels early in HIV infection as predictors of CD4 lymphocyte depletion during 8 years of follow-up. *AIDS.* 1993;7:975–980

34. Anderson RE, Shiboski SC, Royce R, et al. CD8+ T lymphocytes and progression to AIDS in HIV-infected men: some observations. *AIDS.* 1991;5:213–215

35. Buseyne F, Janvier G, Teglas JP, et al. Impact of heterozygosity for the chemokine receptor CCR5 32-bp-deleted allele on plasma virus load and CD4 T lymphocytes in perinatally human immunodeficiency virus-infected children at 8 years of age. *J Infect Dis.* 1998;178:1019–1023

36. Levacher M, Hulstaert F, Tallet S, et al. The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome: staging and prognostic value. *Clin Exp Immunol.* 1992;90:376–382

37. Brouwers P, Civitello L, DeCarli C, Wolters P, Sei S. Cerebrospinal fluid viral load is related to cortical atrophy and not to intracerebral calcifications in children with symptomatic HIV disease. *J Neurovirol.* 2000;6:390–397

38. Sopper S, Sauer U, Hemm S, et al. Protective role of the virus-specific immune response for development of severe neurologic signs in simian immunodeficiency virus-infected macaques. *J Virol.* 1998;72:9940–9947

39. Schutten M, van Baalen CA, Guillon C, et al. Macrophage tropism of human immunodeficiency virus type 1 facilitates in vivo escape from cytotoxic T-lymphocyte pressure. *J Virol.* 2001;75:2706–2709

40. Marcondes MC, Burudi EM, Huitron-Resendiz S, et al. Highly activated CD8+ T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection. *J Immunol.* 2001;167:5429–5438

41. Katsetos CD, Fincke JE, Legido A, et al. Angiocentric CD3+ T-cell infiltrates in human immunodeficiency virus type 1-associated central nervous system disease in children. *Clin Diagn Lab Immunol.* 1999;6:105–114

42. Robertson K, Fiscus S, Kapoor C, et al. CSF, plasma viral load and HIV associated dementia. *J Neurovirol.* 1998;4:90–94

43. Stankoff B, Calvez V, Suarez S, et al. Plasma and cerebrospinal fluid human immunodeficiency virus type-1 (HIV-1) RNA levels in HIV-related cognitive impairment. *Eur J Neurol.* 1999;6:669–675

44. Mofenson LM, Korelitz J, Meyer WA 3rd, et al: The relationship between serum human immunodeficiency virus type 1 (HIV-1) RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1-infected children. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *J Infect Dis.* 1997;175:1029–1038

45. Shearer WT, Quinn TC, LaRussa P, et al. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. *N Engl J Med.* 1997;336:1337–1342

46. Mayaux MJ, Burgard M, Teglas JP, et al. Neonatal characteristics in rapidly progressive perinatally acquired HIV-1 disease. The French Pediatric HIV Infection Study Group. *JAMA.* 1996;275:606–610

47. Blanche S, Newell M, Mayaux M, et al. Morbidity and mortality in European children vertically infected by HIV-1: the French prospective study and the European collaborative study. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1997;14:442–450

48. Resino S, Bellón JM, Sánchez-Ramón S, et al. Impact of antiretroviral protocols on dynamics of AIDS progression markers. *Arch Dis Child.* 2002;86:119–124

49. Jiménez JL, Sánchez-Ramón S, Palomino S, Alcamí J, Muñoz-Fernández MA. Where does free infective HIV-1 rebound come from? *AIDS.* 2001;15:657

50. Spivak JL, Bender BS, Quinn TC. Hematologic abnormalities in the acquired immune deficiency syndrome. *Am J Med.* 1984;77:224–228

51. Poluektova LY, Munn DH, Persidsky Y, Gendelman HE. Generation of cytotoxic T cells against virus-infected human brain macrophages in a murine model of HIV-1 encephalitis. *J Immunol.* 2002;168:3941–3949

52. Ellis RJ, Gamst AC, Capparelli E, et al. Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. *Neurology.* 2000;54:927–936

Updated Information & Services	including high resolution figures, can be found at: http://pediatrics.aappublications.org/content/111/2/e168
References	This article cites 48 articles, 12 of which you can access for free at: http://pediatrics.aappublications.org/content/111/2/e168#BIBL
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): **Infectious Disease** http://www.aappublications.org/cgi/collection/infectious_diseases_sub **HIV/AIDS** http://www.aappublications.org/cgi/collection/hiv:aids_sub **Immunologic Disorders** http://www.aappublications.org/cgi/collection/immunologic_disorders_sub
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.aappublications.org/site/misc/Permissions.xhtml
Reprints	Information about ordering reprints can be found online: http://www.aappublications.org/site/misc/reprints.xhtml

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN®

PEDIATRICS®

Low Blood CD8+ T-Lymphocytes and High Circulating Monocytes Are Predictors of HIV-1-Associated Progressive Encephalopathy in Children

Silvia Sánchez-Ramón, José M? Bellón, Salvador Resino, Carmen Cantó-Nogués, Dolores Gurbindo, José-Tomás Ramos and M?Angeles Muñoz-Fernández

The online version of this article, along with updated information and services, is
located on the World Wide Web at:
http://pediatrics.aappublications.org/content/111/2/e168

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN®