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Alterations in Lymphocyte Phenotype and Function in Children with Shigellosis Who Develop Complications

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This study was designed to see whether alterations occur in peripheral blood mononuclear cell phenotype and function in children with *Shigella dysenteriae* 1 infection with complications (leukemoid reaction and/or hemolytic-uremic syndrome) and whether there are any alterations prior to the development of complications. The following groups of children (ages, 12 to 60 months) were compared: children without any infection ($n = 51$), children with uncomplicated shigellosis ($n = 65$), children admitted with complicated shigellosis (leukemoid reaction and/or hemolytic-uremic syndrome) ($n = 29$), and children with shigellosis who developed complications after enrollment (subsequently complicated shigellosis) ($n = 12$). Tests for the peripheral blood mononuclear cell phenotype (CD3, CD4, CD8, CD16, CD20, and CD25), spontaneous proliferation, and the proliferative response to phytohemagglutinin, pokeweed mitogen, and the lipopolysaccharide of *S. dysenteriae* 1 were performed, as were skin tests for delayed-type hypersensitivity (DTH). Children who subsequently developed complications differed from other groups of children as follows: (i) the numbers of $CD3^+$ and $CD4^+$ cells were lower than in uninfected children ($P < 0.05$), (ii) the CD4/CD8 ratio was lower than in children with uncomplicated shigellosis ($P < 0.05$) and in uninfected children ($P < 0.05$), and (iii) the levels of spontaneous proliferation of peripheral blood mononuclear cells were higher and DTH responses were lower than those in children with uncomplicated shigellosis ($P < 0.05$ and $P < 0.017$, respectively). Children with complications differed by having (i) increased numbers of $CD3^-$ $CD16^-$ $CD20^-$ cells ($P < 0.05$) compared with those in other groups of children and (ii) lower CD4/CD8 ratios ($P < 0.05$), higher levels of spontaneous proliferation ($P < 0.05$), and lower DTH responses ($P = 0.005$) than children with uncomplicated shigellosis. Three to five days after enrollment, the number of $CD4^+$ cells increased in children who subsequently developed complications ($P = 0.025$), i.e., when they developed complications and at this time their $CD4^+$ cell number was similar to that of other groups of children. Thus, lymphocyte phenotype and function are altered prior to the development of complications in children with shigellosis, and once complications develop, the pattern of alterations changes. Whether these alterations have a role in precipitating complications or whether they reflect early events underlying the development of complications remains to be elucidated.

Protection from shigellosis appears to be mediated by antibodies specific to *Shigella* antigens (5, 6) as well as by *Shigella*-specific T cells. The evidence for the involvement of T cells is provided by patients with AIDS in whom illness with shigellosis is very severe (2, 10), by the increased numbers of $CD4^+$ and $CD8^+$ cells in the guts of adults with shigellosis (13), and by the generation of a *Shigella*-specific T-cell clone from the peripheral blood of a patient with reactive arthritis (21). Infection with *Shigella dysenteriae* 1 in children under 5 years of age can lead to life-threatening complications, such as a leukemoid reaction (blood leukocyte count of ≥40,000/μl, granulocytosis, and an increase in immature granulocytes) (4) and hemolytic-uremic syndrome (HUS) (hemolytic anemia, thrombocytopenia, and acute renal failure) (12). Although a leukemoid reaction is not serious in itself, its presence is a bad prognostic indicator (17). The factors precipitating these complications are not well understood.

Children with leukemoid reactions in shigellosis have been shown to have more peripheral blood mononuclear cells (PBMs) that are neither E rosette positive (T cells) nor EAC rosette positive (B cells) (9). Addition of thymopoietin to these cells results in an increase in the numbers of E rosette-positive cells in some of these children, suggesting a block in maturation of T cells (8). However, the overall numbers of T and B cells are normal in those children. It therefore remains unclear whether there are alterations in lymphocyte populations in children with complicated shigellosis (CS), and if there are, whether those alterations are a consequence of the complications or whether they predispose the children to the complications. We therefore designed a study to determine lymphocyte phenotype and function in children with shigellosis who develop leukemoid reactions and/or HUS compared with those in uninfected children, children with shigellosis in whom these complications had already developed, and children with shigellosis who never develop them.

MATERIALS AND METHODS

Study population. Children 12 to 60 months of age attending the Clinical Research and Service Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), with acute dysentery (visible blood in stool and presence or absence of fever) were initially enrolled in the study. Stools were examined microscopically and cultured for enteric bacteria (19). Only children who were culture positive for *Shigella* organisms were included in the study. Informed consent was obtained from the guardian(s) of each child before enrollment, and the study was approved by the Ethical Review Committee of the ICDDR,B.

Patients were clinically evaluated by their medical history, daily physical ex-

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amination, and laboratory investigations which included determination of hematocrit; total and differential counts of leukocytes (WBCs), platelets, and fragmented erythrocytes (RBCs); and serum electrolyte and creatinine concentrations. All patients were treated with pivmecillinam or ciprofloxacin. Some children received additional antibiotics because of the presence of concomitant infections, such as respiratory tract infection, middle ear infection, or septicemia. Children matched for age and without any infection attending the Nutritional Follow-Up Unit of ICDDR,B were also enrolled. Of the 157 children who were enrolled in the study, 106 had *S. dysenteriae* 1 infection and 51 were children with no infection (NI) at the time of enrollment. Children with *S. dysenteriae* 1 infection were divided into three groups: those who had HUS or leukemoid reactions upon enrollment (complicated shigellosis [CS]; $n = 29$), those who developed leukemoid reactions and/or HUS following enrollment (subsequently complicated shigellosis [SCS]; $n = 12$), and those who had no such complications (uncomplicated shigellosis [US]; $n = 65$). In children with SCS, complications developed from 1 to 6 days after enrollment (mean, 2.8 days). As the number of PBMs varied among the children, not all assays could be done for all children.

Venous blood (5 ml) was collected aseptically in sterile, heparinized Vacutainer tubes (Becton Dickinson, Rutherford, N.J.). Blood was collected from patients upon enrollment and 3 to 5 days later, except from those who had received blood transfusions or were transferred to other hospitals in the interim period. A single sample of blood was collected from each uninfected child.

PBM separation. Fresh blood was separated on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) by centrifugation at $500 \times g$ for 25 min. PBMs which formed a band at the interface were collected, washed, and counted.

Phenotypes of PBMs. Phenotyping of PBMs was done by indirect immunofluorescence for CD3, CD4, CD8, CD16, CD20, CD25, and leukocyte common antigen (CD45) by using the monoclonal antibodies UCHT1 (anti-CD3), QS4120 (anti-CD4), UCHT4 (anti-CD8), HNK1 (anti-CD16), and 2D1 (anti-CD45) (gifts of P. C. L. Beverley, Tumor Immunology Group, Imperial Cancer Research Fund, London, United Kingdom); B1 (Coulter Immunology, Hialeah, Fla.) (anti-CD20); and anti-CD25 (interleukin 2R) (Dakopatts, Glostrup, Denmark). B1 and anti-CD25 were used at dilutions of 1:5 in minimal essential medium with 2% fetal bovine serum (FBS) (GIBCO, Grand Island, N.Y.); the other monoclonal antibodies were not diluted. A positive control monoclonal antibody (2DI) and a negative control of medium alone (minimal essential medium plus 2% FBS) were used in all assays. Briefly, 3×10^5 PBMs were incubated with each monoclonal antibody for 30 min at 4°C, washed three times, and then incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Dakopatts) at a dilution of 1:40 in minimal essential medium with 2% FBS for 30 min at 4°C. The cells were then washed three times and fixed by using phosphate-buffered saline (10 mM, pH 7.2) with 2% FBS and 1% formaldehyde. The cells were analyzed within 3 days of being fixed by using a UV microscope (BH-2; Olympus) with a 40× objective; at least 100 cells were counted.

Proliferation assays. The proliferative response of PBMs was assessed with resting cells, for which spontaneous DNA synthesis was measured, and with cells stimulated with mitogens or antigens for several days. In all cases, PBMs were cultured at 5×10^5/ml of RPMI 1640 (GIBCO) supplemented with 10% FBS, 2 mM glutamine (Flow Laboratories, Rickmansworth, Herts, United Kingdom), 50 IU of penicillin (Flow), and 50 μg of streptomycin (Flow) per ml in 96-well U-bottomed microtiter plates (Flow) and incubated in a humidified atmosphere containing 5% CO_2. All cultures were done in triplicate.

For spontaneous DNA synthesis, PBMs were incubated with 1 μCi of [^{3}H]thymidine (Amersham International, Aylesbury, United Kingdom) per well of a microtiter plate (Flow) for 3 h. The cells were then harvested onto glass fiber filter strips with a cell harvester (Automash 2000; Dynatech, Billingshurst, Sussex, United Kingdom). The amount of [^{3}H]thymidine incorporated was determined as counts per minute by liquid scintillation counting in a beta counter (Beckman Instruments Inc., Fullerton, Calif.).

For the proliferative response to mitogens and antigens, PBMs were cultured with medium alone, phytohemagglutinin (PHA) (Wellcome Diagnostics) at 1.25 μg/ml, pokeweed mitogen (PWM; Sigma) at 10 μg/ml, and the lipopolysaccharide (LPS) of *S. dysenteriae* 1 (18). The LPS of *S. dysenteriae* 1 was used at concentrations of 160 to 5 μg/ml at doubling dilutions in subsets of children from all four study groups. As no proliferation was observed with LPS at any of the concentrations used, a single concentration of 10 μg/ml was used in all experiments. Cells were incubated for 3 days with PHA and for 5 days with other stimulants. At the end of the incubation, the cells were pulsed with [^{3}H]thymidine at 1 μCi per well for 6 h; they were then harvested onto glass fiber filter strips and counted in a beta counter. Results were expressed as net proliferation, which was the difference between the mean counts per minute of cells with stimulants and those without stimulants.

DTH responses. Delayed-type hypersensitivity (DTH) responses were measured by skin tests with the Multitest CMI kit (Pasteur Mérieux, Lyon, France), whereby seven antigens and a glycerine control solution were introduced into the skin of the paravertebral area of the back of each child and the induration was measured at the end of 48 h. An induration of 2 mm or more was considered a positive response. The antigens present in the kit included tetanus (550,000 Mérieux units per ml), diphtheria (1,100,000 Mérieux units per ml), streptococcus (group C) (2,000 Mérieux units per ml), tuberculin (300,000 IU/ml), *Candida*

TABLE 1. Clinical characteristics of children upon enrollment[a]

Study group	Age, mo	Wt for age, % of the NCHS[b] median	No. (%) Male	Duration of diarrhea before enrollment, days	Stool frequency/24 h	No. (%) with:		Total WBC count/μl of blood	% Lymphocytes	Total no. of lymphocytes/μl of blood
						>20 WBCs/HPF in stool[c]	>20 RBCs/HPF in stool[d]			
NI	33.9 ± 15.8	69.7 ± 10.9	24.0 (47.1)	NA[e]	NR[f]	1.0 (1.9)	0	11,992 ± 3,467	55.7 ± 11.7	6,607 ± 2,257
US	32.1 ± 13.5	65.6 ± 11.7	35.0 (53.8)	8.0 ± 5.2	24.3 ± 16.0	57.0 (93.4)	43.0 (69.4)	17,852 ± 7,859	41.9 ± 16.7	6,944 ± 2,720
SCS	31.1 ± 15.5	73.3 ± 8.8	7.0 (58.3)	5.7 ± 1.8	27.2 ± 21.2	7.0 (58.3)	5.0 (41.7)	25,393 ± 7,943	22.4 ± 13.5	6,175 ± 4,370
CS	26.9 ± 11.6 (NS[g])	69.7 ± 11.9 (NS)	18.0 (62.1) (NS)	8.2 ± 4.1 (NS)	23.0 ± 15.2 (NS)	19.0 (67.9) ($P < 0.001$)	7.0 (25.0) ($P = 0.002$)	43,567 ± 19,150 ($P < 0.001$)	28.1 ± 14.4 ($P < 0.001$)	10,475 ± 5,576 ($P = 0.005$)

[a] All values are means ± SDs unless otherwise stated. The Kruskal-Wallis or chi-square test was used to determine the differences in results for ≥3 groups of children; the differences are in parentheses.
[b] NCHS, National Center for Health Statistics.
[c] WBC counts of >20 per HPF are considered indicative of inflammation.
[d] RBC counts of >20 per HPF are considered indicative of inflammation.
[e] NA, not applicable.
[f] NR, not relevant.
[g] NS, not significant.

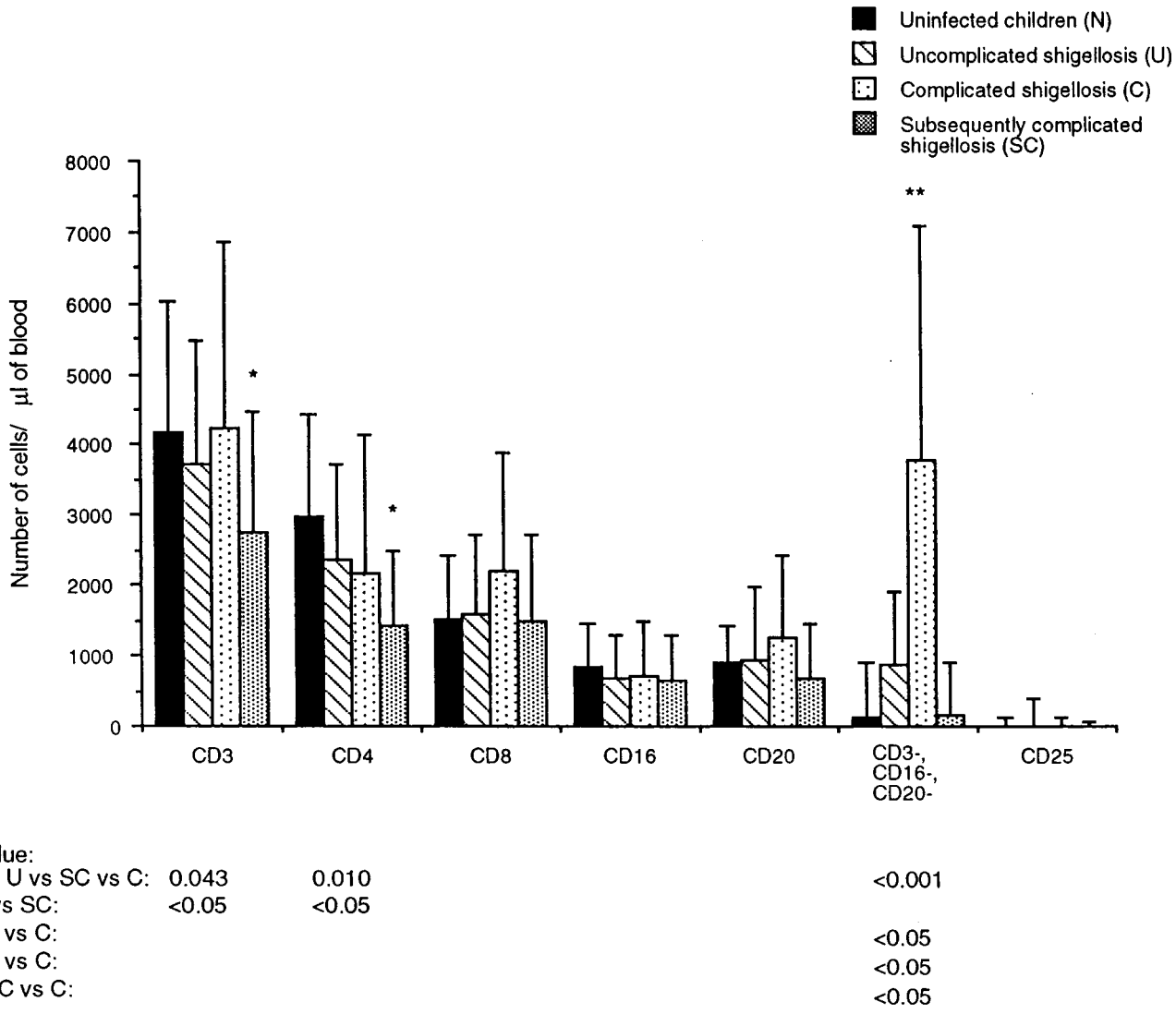

FIG. 1. Lymphocyte phenotype, upon enrollment, in children with NI (N), US (U), CS (C), and SCS (SC). Values are medians, and error bars represent SDs. Comparisons between the four study groups were done by the Kruskal-Wallis test; when the differences were significant by this test, this test was followed by the Dunn test for comparison of any two groups. *P* values are shown only for significant differences.

albicans (2,000 Mérieux units per ml), *Trichophyton mentagrophytes* (150 Mérieux units per ml), and *Proteus mirabilis* (150 Mérieux units per ml) antigens.

Statistical analyses. For continuous, nonparametric data, the Mann-Whitney U test was used for comparison of two groups and the Kruskal-Wallis test was used for comparison of three or more groups. When significant differences were recorded with the Kruskal-Wallis test, the Dunn test was used to assess the significance of differences between any two groups. For comparisons between proportions, the chi-square statistic was used. Differences of paired samples from the two study periods were assessed by the Wilcoxon matched-pair signed-rank test (for continuous variables) and the McNemar test (for categorical variables). Multiple-regression analysis was carried out to see the effects of nutritional status, sex, concomitant infection, and duration of diarrhea on lymphocyte phenotype and function. Differences were considered significant when the *P* value was ≤ 0.05. Data analyses were carried out with the Statistical Package for Social Sciences (version 6.0 for Windows; SPSS Inc., Chicago, Ill.) and SigmaStat (version 1.0 for Windows; Jandel Scientific, San Rafael, Calif.).

RESULTS

Study groups. Table 1 shows the clinical characteristics of the children upon enrollment. Children of all four groups were comparable for age, nutritional status (as determined by weight for age as a percentage of the National Center for Health Statistics median), and sex. The three groups of children with shigellosis had similar durations of diarrhea before enrollment and similar stool frequencies in 24 h upon enrollment. Total WBC counts ($10^3/\mu l$ of blood) were higher in children with shigellosis (whether US, SCS, or CS) than in children with NI ($P < 0.05$ for all comparisons). The percentage of lymphocytes was lower in children with shigellosis (US, SCS, or CS) than in children with NI ($P < 0.05$ for all three). Children with CS had more WBCs than those with US ($P < 0.05$), a lower percentage of lymphocytes than children with US ($P < 0.05$), but higher numbers of lymphocytes than all other groups of children ($P < 0.05$ for all three). Lymphocyte numbers were similar in children with NI, US, and SCS (Table 1). The numbers of children with >20 WBCs per high-powered microscopic field (HPF) and >20 RBCs per HPF of stool were greater among children with shigellosis (whether US, SCS, or CS) than among children with NI ($P < 0.001$ and $P = 0.002$, respectively) (Table 1).

Phenotypes of PBMs. Upon enrollment, the four groups of children had significantly different numbers of $CD3^+$ and

$CD4^+$ cells ($P = 0.043$ and 0.010, respectively) (Fig. 1). The numbers of $CD3^+$ and $CD4^+$ cells were both lower in children with SCS than in those with NI ($P < 0.05$); they were similar to those of other groups of children (Fig. 1). The numbers of $CD8^+$, $CD16^+$, $CD20^+$, and $CD25^+$ cells were similar in all four groups of children. The CD4/CD8 ratio was significantly lower in children with SCS (median, 1.0; standard deviation [SD], 0.4) and CS (median, 1.1; SD, 1.1) than in children with US (median, 1.6; SD, 0.9) or NI (median, 1.8; SD, 1.0) ($P <$ 0.05 for all comparisons). The ratios were similar in children with SCS and CS and children with US and NI. Children with CS had more cells that were negative for CD3, CD16, or CD20 than children in the other three groups ($P < 0.05$ for all comparisons) (Fig. 1).

Comparison of paired samples between the day of enrollment (day 0) and 3 to 5 days later (Table 2) showed that the numbers of $CD3^+$ and $CD4^+$ cells increased in children with SCS ($P = 0.017$ and 0.025, respectively). Although the $CD8^+$ cell numbers also increased, the difference was not statistically significant. The phenotype was unchanged between the two study periods in children with US and CS (Table 2). The CD4/CD8 ratios were unchanged between the day of enrollment and 3 to 5 days later in all four groups of children (data not shown). There were not enough paired samples for comparison of $CD16^+$ and $CD3^-$ $CD16^-$ $CD20^-$ cells between the two study periods in children with SCS and CS.

Spontaneous DNA synthesis. DNA synthesis by unstimulated PBMs of children with shigellosis, whether US, CS, or SCS, upon enrollment was higher than that by PBMs of children with NI ($P < 0.05$) (Table 3). The level of proliferation was higher in children with SCS and CS than in those with US ($P < 0.05$ for both comparisons), and the levels were similar in children with SCS and CS.

Three to five days after enrollment (Table 2), spontaneous DNA synthesis by PBMs in children with US and CS declined significantly from the day of enrollment ($P = 0.001$ and 0.011, respectively). It remained unchanged in children with SCS.

Proliferation of PBMs in response to stimulants. Upon enrollment, the levels of net proliferation of PBMs in response to PHA and PWM were similar in all four groups of children (Table 3). No stimulation with the LPS of *S. dysenteriae* 1 was observed in any of the groups of children (data not shown).

Three to five days after enrollment, the PHA responses were similar to that upon enrollment for all four groups of children (Table 2). With PWM (Table 2), there was a decline in net proliferation between the two study periods in children with US ($P = 0.011$); there were not enough paired samples for comparison in children with CS or SCS.

DTH responses. Skin test results for DTH upon enrollment were significantly lower in children with shigellosis (whether US, CS, or SCS) than in those with NI (Table 4). Responses in children with CS and SCS were diminished compared with the responses of children with US ($P = 0.005$ and 0.017, respectively). However, children with CS and SCS had similar responses. Three to five days after enrollment, DTH responses were unchanged in children with US and CS (data not shown). There were not enough paired samples for comparison between the two study periods in children with SCS.

Effects of clinical parameters on lymphocyte phenotype and function. Multiple-regression analyses showed that PBM phenotype and function were not affected by sex, nutritional status, concomitant infections, or duration of diarrhea before enrollment.

TABLE 2. Alterations in lymphocyte phenotype and proliferation in paired samples from children with shigellosis[a]

Study group	Study day	No. of cells positive for:						Amt of DNA spontaneously synthesized, cpm	Amt of proliferation[b] with:	
		CD3	CD4	CD8	CD16	CD20	$CD3^-$ $CD16^-$ $CD20^-$		PHA	PWM
US	0[c]	3,777 ± 1,709	2,673 ± 1,293	1,604 ± 1,062	691 ± 557	1,013 ± 993	1,439 ± 1,139	2,394 ± 3,739	30,931 ± 20,785	9,166 ± 18,727
	3–5[d]	3,691 ± 1,732	2,520 ± 1,444	1,391 ± 865	571 ± 488	1,016 ± 629	549 ± 926	1,469 ± 2,833	19,426 ± 24,848	7,217 ± 7,441 ($n = 35$; $P = 0.011$)
		($n = 39$; NS[e])	($n = 43$; NS)	($n = 43$; NS)	($n = 24$; NS)	($n = 40$; NS)	($n = 21$; NS)	($n = 53$; $P = 0.001$)	($n = 45$; NS)	
SCS	0	2,444 ± 1,313	1,295 ± 1,098	1,313 ± 1,022	NE[f]	586 ± 696	NE	12,714 ± 5,972	27,734 ± 51,580	NE
	3–5	5,083 ± 2,482	2,841 ± 2,192	2,144 ± 1,576		1,342 ± 449		6,963 ± 3,328	62,776 ± 36,418	
		($n = 8$; $P = 0.017$)	($n = 8$; $P = 0.025$)	($n = 8$; NS)		($n = 8$; NS)		($n = 6$; NS)	($n = 7$; NS)	
CS	0	4,759 ± 1,562	2,929 ± 1,547	2,379 ± 2,041	NE	NE	NE	12,096 ± 671	48,340 ± 46,984	NE
	3–5	3,900 ± 1,378	2,699 ± 1,872	1,699 ± 1,129				3,648 ± 4,970	23,226 ± 46,456	
		($n = 9$; NS)	($n = 9$; NS)	($n = 9$; NS)				($n = 13$; $P = 0.011$)	($n = 10$; NS)	

[a] Samples taken on the day of enrollment were paired with samples taken 3 to 5 days later. The values are medians ± SDs.
[b] Proliferation of PBMs; results are net counts per minute.
[c] Day of enrollment.
[d] Three to five days after enrollment. Wilcoxon matched-pair signed-rank test was used to determine the differences in the results for the children. The differences are in parentheses, after the numbers of children.
[e] NS, not significant.
[f] NE, not enough sample.

TABLE 3. Proliferation of PBMs spontaneously and in response to stimulants upon enrollment[a]

Study group	Amt of DNA spontaneously synthesized, cpm	Net cpm of DNA synthesized in response to:	
		PHA (1.25 μg/ml)	PWM (10 μg/ml)
NI	776 ± 885 ($n = 51$)	21,260 ± 21,086 ($n = 41$)	4,548 ± 9,885 ($n = 31$)
US	2,710 ± 3,804 ($n = 65$)	30,862 ± 22,820 ($n = 62$)	7,992 ± 16,106 ($n = 51$)
SCS	12,714 ± 20,351 ($n = 12$)	25,635 ± 46,498 ($n = 10$)	12,207 ± 13,135 ($n = 10$)
CS	8,694 ± 7,824 ($n = 29$; $P < 0.001$)	33,604 ± 37,879 ($n = 28$; NS)	10,071 ± 11,980 ($n = 19$; NS[b])

[a] Values are medians ± SDs. The Kruskal-Wallis test was used to determine the differences in results for the four groups of children; the differences are at the bottom of each column.
[b] NS, not significant.

DISCUSSION

Several alterations in lymphocyte phenotype and function in children with *S. dysenteriae* 1 infection were identified in this study; these alterations are summarized in Table 5. Alterations in children with SCS and CS can be seen as a continuum, but those in children with US cannot, as those children do not develop complications. Alterations seen in children with US—increased spontaneous proliferation of resting cells and decreased DTH responses—are magnified in children with SCS and CS. Additionally, in SCS, the numbers of $CD3^+$ and $CD4^+$ cells and the CD4/CD8 ratio are decreased, whereas in CS, $CD4^+$ cell numbers are unchanged but the CD4/CD8 ratio is decreased and the numbers of $CD3^-$ $CD16^-$ $CD20^-$ cells are increased. Thus, alterations in lymphocyte phenotype and function do occur prior to the development of complications and a change in the pattern of alterations occurs as children progress from SCS to CS. The possible causes for these alterations are discussed below.

Lower numbers of $CD3^+$ and $CD4^+$ cells with lowering of the CD4/CD8 ratio found in the circulation of children with SCS could be due to (i) loss through the inflamed gut, (ii) maturation block in the thymus (8), and (iii) migration to local sites of inflammation. Shigellosis is a protein-losing enteropathy in which stool RBCs have been shown to correlate with stool α1 antitrypsin (an indicator of loss of protein from the gut) (3). In children with SCS the numbers of stool RBCS and stool WBCs and stool frequency (all indicators of gut inflammation) were higher than in children with NI, and therefore it is possible that lowered $CD3^+$ and $CD4^+$ cell numbers are due to leakage through the inflamed mucosa. However, these indicators of gut inflammation are also elevated in children with US, but the numbers of $CD3^+$ and $CD4^+$ cells were not lowered in this group of children. Therefore, loss through the inflamed mucosa is unlikely to be the only factor responsible for the lowered cell numbers. The second possibility of maturation block of T cells in the thymus appears to be unlikely, as both $CD3^+$ and $CD4^+$ cell numbers increased significantly 3 to 5 days after enrollment in children with SCS; $CD8^+$ cells also increased in number, although the increase was not statistically significant. In children with CS, the numbers of T cells and their subsets were not decreased; however, the numbers of $CD3^-$ $CD16^-$ $CD20^-$ cells were increased. Jackson and Zaman (8) also found increased numbers of cells which were E negative and EAC negative, which they suggested was due to a block in thymic maturation because upon addition of thymopoietin, the cells became E positive. However, this could also be a stress response. The source of the $CD3^-$ $CD16^-$ $CD20^-$ cells in our study is not clear. It is possible that these are immature or stem cells which have poured out into the circulation as overall lymphocyte numbers increased. The third possibility of migration to local sites, i.e., to the gut, therefore remains the most likely explanation, and similar migration has been shown to occur in acute appendicitis (16). However, it is unclear why a selective migration of $CD3^+$ and $CD4^+$ cells should take place, although this has also been shown to occur in other conditions such as appendicitis (16) and intestinal lymphangiectasia (20). Further analyses of subsets of $CD4^+$ cells (naive and memory cells) would shed more light on the nature of cells that are lost from the circulation, by either leakage through the gut or migration to local sites.

This study also shows that a decreased CD4/CD8 ratio may differentiate children with SCS from those with US. Alterations in CD4/CD8 ratios reflect changes in either or both of the CD4 and CD8 T-cell subsets. In children with SCS, decreased CD4/CD8 ratios on enrollment are due to the decreased number of $CD4^+$ cells in the circulation. Three to five days after enrollment, i.e., once complications develop, the CD4/CD8 ratio was still lower despite an increased $CD4^+$ cell number. However, there was also a rise in the $CD8^+$ cell number, although that was not statistically significant. Similarly, the lower CD4/CD8 ratio in children with CS could be due to greater numbers of $CD8^+$ cells (Fig. 1), although the difference in numbers compared with those in other groups of children was not statistically significant.

Increased spontaneous DNA synthesis reflects an increased state of activation of circulating cells in vivo and also occurs in other infections (1). Children with SCS and CS had more preactivated PBMs than those with US. In vivo activation may be due to circulating bacterial components, such as LPS, or products of infection, such as cytokines. Children with HUS associated with *S. dysenteriae* 1 infection have higher concentrations of endotoxin in serum than children with US (11). Although the LPS of *S. dysenteriae* 1 did not induce proliferation of PBMs in this study, an earlier study showed modest proliferation by the lipid-free O-antigenic polysaccharide of *S. dysenteriae* 1 (14). In adults with US, the levels of both Th1 and Th2 cytokines are elevated (15), and in children with CS, concentrations of interleukin 6 and tumor necrosis factor alpha in serum are higher than in those with US (7). It is possible that with complications, the levels of other cytokines, causing T- and B-cell activation, are also elevated.

TABLE 4. DTH responses upon enrollment

Study group	No. (%)[a]:	
	Negative for all antigens	Positive for at least one antigen
NI	12 (25.0)	36 (75.0)
US	29 (51.8)	27 (48.2)
SCS	7 (100.0)	0
CS	16 (88.9)	2 (11.1)

[a] *P* values obtained by using the chi-square statistic: for children with NI and US, 0.006; for children with US and CS, 0.005; for children with US and SCS, 0.017; and for children with CS and SCS, not significant.

TABLE 5. Summary of alterations in lymphocyte phenotype and function in children with shigellosis upon enrollment

Type of shigellosis	Difference[a] in:					
	$CD3^+$ cell no.	$CD4^+$ cell no.	CD4/CD8 ratio	No. of $CD3^-$ $CD16^-$ $CD20^-$ cells	Spontaneous DNA synthesis	DTH response
US					B	A
SCS	A	A	C		D	C
CS			C	E	D	C

[a] A and B, significant decrease and increase, respectively, compared with result for children with NI. C and D, significant decrease and increase, respectively, compared with result for children with US or NI; E, significant increase compared with result for children with SCS or US.

Lowered DTH responses were observed in all groups of children but were more marked in children with shigellosis. Uninfected children were nutritionally similar to children with shigellosis i.e., they were malnourished, which may be the reason for the anergy observed. In children with shigellosis (US, SCS, or CS), lowered DTH responses paralleled increased resting DNA synthesis by PBMs. The in vivo preactivated state of lymphocytes may be a reason for lowered DTH responses in those children.

In summary, alterations in PBM phenotype and function prior to the development of leukemoid reactions and HUS are identifiable in children with *S. dysenteriae* 1 infection. These alterations may have a role in precipitating complications, or they may reflect events that are already underway in the development of complications.

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