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Cytokines in the Stools of Children with Complicated Shigellosis

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The pathogenesis of the systemic complications, leukemoid reaction and hemolytic uremic syndrome, associated with *Shigella dysenteriae* type 1 infection is not well understood. The excessive production of proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), has been suggested as a possible factor. We measured IL-6 and TNF-α in stools of 56 children with *S. dysenteriae* 1 infection and 29 children without any apparent infection, all age 12 to 60 months. Sixteen children with *S. dysenteriae* 1 infection had leukemoid reaction or hemolytic uremic syndrome (complicated shigellosis), while the others did not (uncomplicated shigellosis). Stool IL-6 and TNF-α concentrations were higher in children with uncomplicated shigellosis than in children with complicated shigellosis ($P = 0.009$ and < 0.001, respectively) or in uninfected children ($P < 0.001$). It is concluded that complicated infection is not associated with higher concentrations of the proinflammatory cytokines IL-6 and TNF-α in stool.

Systemic complications such as leukemoid reaction (blood leukocyte count of $\geq$40,000/mm^3, granulocytosis, and an increase in immature granulocytes) (3) and hemolytic uremic syndrome (hemolytic anemia, thrombocytopenia, and acute renal failure) (7) may be associated with *Shigella dysenteriae* type 1 infection. These severe, often fatal complications are usually seen in children below 5 years of age. The pathogenesis of these complications remains to be understood, but the excessive release of proinflammatory cytokines may be one of the associated factors. High concentrations of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been found in the plasma and stool of patients with acute shigellosis (4, 6, 8). Concentrations in stool, however, are 100 times higher than concentrations in plasma, suggesting that the response is, to some extent, compartmentalized (8). Children with systemic complications associated with *S. dysenteriae* 1 infection have higher serum IL-6 and TNF-α concentrations than children with uncomplicated infection (4); concentrations in stool have not been measured. In these children, although the initial infection is in the gut, the complications are no longer localized to the gut, and the source of cytokines in the serum may therefore be from sites other than the gut. Since stool TNF-α is considered to be a reliable marker for intestinal inflammation (2), we compared stool TNF-α and IL-6 concentrations in children with complicated shigellosis (i.e., those with hemolytic uremic syndrome or leukemoid reaction), children infected with *S. dysenteriae* 1 without systemic complications (uncomplicated shigellosis), and children without any infection at the time (uninfected children).

Children between 12 and 60 months of age attending the Diarrhea Treatment Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, were enrolled in the study. Stools were examined microscopically for the number of leukocytes (WBC) and erythrocytes (RBC) per high-powered field (HPF) and cultured for shigellae (9). Fifty-six children were culture positive for *S. dysenteriae* 1, and 29 children were from the Nutrition Follow-up Unit of the International Centre for Diarrhoeal Disease Research, Bangladesh, and had no infection at the time of enrollment (uninfected children). Of the children with *S. dysenteriae* 1 infection, 16 had complications such as leukemoid reaction or hemolytic uremic syndrome (complicated shigellosis), while 40 had no such complications (uncomplicated shigellosis). Freshly passed stools were collected from children with shigellosis on the day of enrollment and 3 to 5 days later. From uninfected children, single stool samples were collected. One gram of stool was thoroughly mixed with 1 ml of sterile phosphate-buffered saline (pH 7.2) and centrifuged at 20,000 × *g*, and the supernatant was filtered through a 0.45-μm-pore-size filter (Sartorius, Goettingen, Germany). Aliquots of filtrates were stored at −70°C until tested. TNF-α and IL-6 were measured, in duplicate, with enzyme-linked immunosorbent assay kits (Endogen Inc., Boston, Mass.). The detection limits for TNF-α and IL-6 were 5 and 4 pg/ml, respectively. Concentrations were calculated by extrapolation with a standard curve and were expressed as picograms per gram of stool.

The significances of differences between two groups of continuous variables were assessed by using the two-tailed Mann-Whitney U test. Sets of more than two groups were compared by the Kruskal-Wallis test. The chi-square statistic was used to test the significance of differences in proportions. Comparisons between the two study periods were made with paired samples by using the Wilcoxon matched-pairs signed rank test and the McNemar test. Correlations between variables were assessed by using Pearson's correlation coefficient. Multiple regression analyses were done to determine the effects of the duration of diarrhea before enrollment, nutritional status, and presence of concomitant infections on stool IL-6 and TNF-α concentrations. Differences were considered significant when P was $\leq$0.05. Data entry and analyses were carried out with the Statistical Package for Social Sciences (version 6.0 for Windows; SPSS Inc., Chicago, Ill.).

Table 1 shows the clinical characteristics of the children studied. The age, nutritional status (weight for age as a percentage of the National Center for Health Statistics median),

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TABLE 1. Clinical characteristics of the children on the day of enrollment

Group	Age (mo)[a]	No. (%) male	Wt for age (% of NCHS median)[a,b]	Duration of diarrhea (days)[a]	Stool frequency (per 24 h)[a]	No. (%) with:	
						>20 WBC/HPF in stool	>20 RBC/HPF in stool
Uninfected children	27.3 ± 14.3	16 (46.7)	64.3 ± 6.9	NA[c]	NA	NA	NA
Shigellosis							
Uncomplicated	32.9 ± 13.4	21 (48.8)	65.4 ± 13.3	8.6 ± 6.3	28.1 ± 17.4	36.0 (90.0)	29.0 (72.5)
Complicated	26.2 ± 12.5	7 (41.2)	7.2 ± 12.1	6.7 ± 2.4	25.2 ± 16.8	11.0 (68.8)	4.0 (25.0)
P	0.079	0.459	0.136	0.814	0.481	0.072	0.002

[a] Expressed as mean ± standard deviation.
[b] NCHS, National Center for Health Statistics.
[c] NA, not applicable.

and proportion of males and females were comparable among the study groups ($P = 0.079$, 0.136, and 0.459, respectively). The duration of diarrhea before enrollment and the stool frequency were similar in children with uncomplicated and complicated shigellosis ($P = 0.814$ and 0.481, respectively). In addition, multiple regression analyses showed no effect of any of these parameters on stool IL-6 and TNF-α concentrations. However, the numbers of RBC in stool per HPF were higher in children with uncomplicated infection than in those with complicated infection ($P = 0.002$). The numbers of stool WBC per HPF were also higher in children with uncomplicated shigellosis, but the difference was not statistically significant ($P = 0.072$).

Concentrations of both IL-6 (Fig. 1A) and TNF-α (Fig. 1B) in stool were higher in children with uncomplicated shigellosis than in those with complicated shigellosis ($P = 0.009$ and <0.001 for IL-6 and TNF-α, respectively) or in uninfected children ($P < 0.001$ for both IL-6 and TNF-α). Concentrations in children with complicated shigellosis were similar to those in uninfected children ($P = 0.065$ and 0.100 for IL-6 and TNF-α, respectively). The reason(s) for the lower levels of cytokines in children with complicated shigellosis is not clear. Sources of

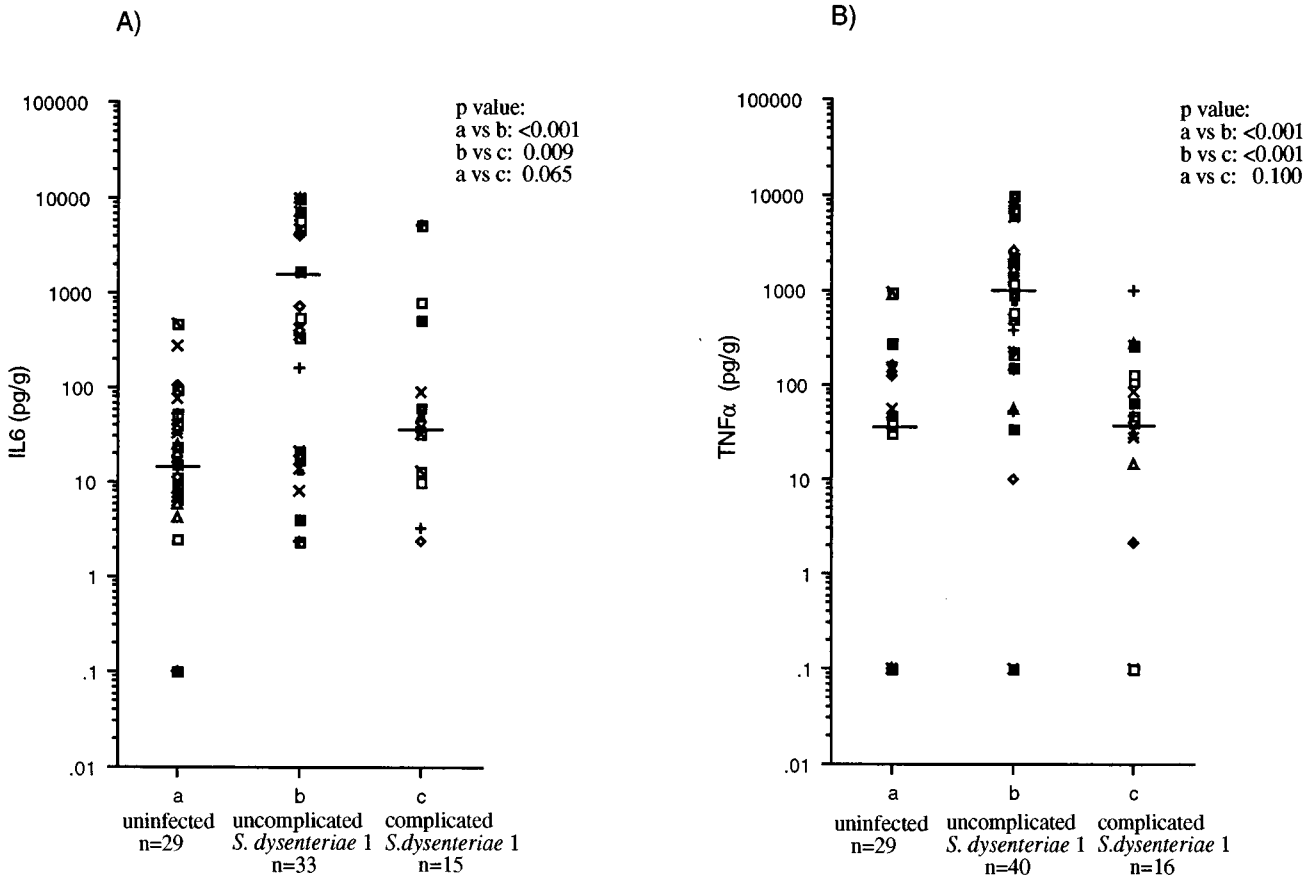

FIG. 1. Concentrations of IL-6 (A) and TNF-α (B) in stool at the time of enrollment in uninfected children (a) and in children with uncomplicated (b) and complicated (c) *S. dysenteriae* 1 infection. Horizontal bars represent median values. (Numbers of symbols do not match numbers of children because some values are similar or so close that they appear as single symbols.)

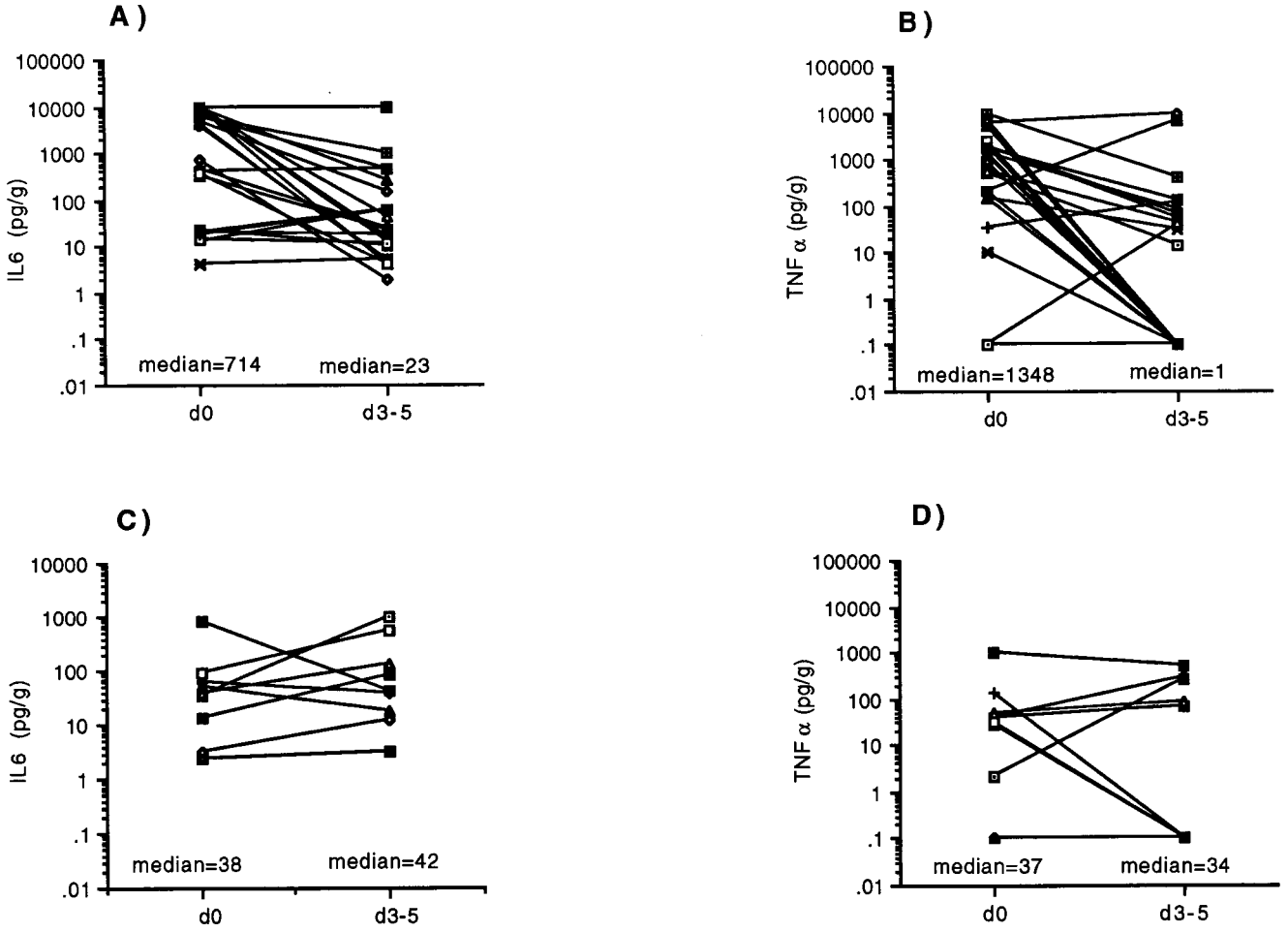

FIG. 2. Stool IL-6 and TNF-α concentrations in children with uncomplicated and complicated *S. dysenteriae* 1 infection for whom samples were available on the day of enrollment (d0) and 3 to 5 days later (d3–5). Each symbol represents a single patient. (A) IL-6 in uncomplicated shigellosis ($n = 23$); (B) TNF-α in uncomplicated shigellosis ($n = 25$); (C) IL-6 in complicated shigellosis ($n = 9$); (D) TNF-α in complicated shigellosis ($n = 10$).

cytokines in stool include (i) WBC present in stool and (ii) leakage across inflamed mucosae. There was a significant direct correlation between stool IL-6 and TNF-α levels and the numbers of stool WBC ($r = 0.4227$ and 0.3025 for IL-6 and TNF-α, respectively; $P < 0.001$) and RBC ($r = 0.5497$ and 0.4321 for IL-6 and TNF-α, respectively; $P < 0.001$). Stool WBC levels, stool RBC levels, and stool frequency are all indicators of the extent of inflammation in the gut. In this study, children with complicated shigellosis had lower stool WBC levels (although the difference was not statistically significant) and lower stool RBC levels ($P = 0.002$) than children with uncomplicated shigellosis, but the stool frequencies were similar in the two groups of patients ($P = 0.481$) (Table 1). In addition, stool RBC levels have been shown to correlate with stool α_1 antitrypsin levels (1) (an indicator of protein loss from the gut and hence of inflammation). Taken together, these findings suggest that the extent of inflammation in the gut was less in children with complicated shigellosis than in children with uncomplicated shigellosis. However, it is not possible, from this study, to rule out effects of other factors, such as cytokine inhibitors in the stool (6) or effects of drugs (5). All children with shigellosis (whether uncomplicated or complicated) were receiving pivmecillinam. Children with complicated infections were receiving additional antibiotics.

Most children with uncomplicated shigellosis showed a decline in stool IL-6 (Fig. 2A) and TNF-α (Fig. 2B) concentrations from the day of enrollment to 3 to 5 days later. Thus, when median concentrations during these study periods were compared, the declines were significant for IL-6 ($P < 0.001$) and TNF-α ($P < 0.001$). Clinically, most children had improved by this time, and their stool frequencies and stool RBC and WBC levels were lower than at enrollment ($P < 0.001$ for each). In children with complicated shigellosis, stool cytokine concentrations remained similar or showed a rise from the day of enrollment to 3 to 5 days later (Fig. 2C and D), and there were no significant differences in median concentrations ($P = 0.323$ and 0.288 for TNF-α and IL-6, respectively). Similarly, the clinical conditions of the children were unchanged.

In summary, stool IL-6 and TNF-α levels correlated with stool WBC and RBC levels, all of which were lower in children with complicated shigellosis than in those with uncomplicated shigellosis. The reasons for these findings are not clear, but they may relate to several factors: (i) systemic complications may correlate more with serum cytokines than with stool cytokines; (ii) there may have been less intensive colitis in children with complicated shigellosis than in children with uncomplicated shigellosis at the time of enrollment, since, in this study, stool WBC and RBC levels were lower in children with

complicated shigellosis than in those with uncomplicated shigellosis; (iii) there was less protein leakage into the gut (since stool RBC levels correlate with protein loss from the gut), resulting in lower levels of serum-derived cytokines in children with complicated shigellosis; (iv) there may have been cytokine inhibitors in the stool; and (v) there may have been drug effects.

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