Quantitative assessment of IgG and IgA subclass producing cells in rectal mucosa during shigellosis

Dilara Islam, Béla Veress, Pradip Kumar Bardhan, Alf A Lindberg, Birger Christensson

Abstract

Aims—To assess quantitatively both the morphological changes in the rectal mucosa and the changes in the relative frequency of IgA and IgG subclass producing cells found in the rectal mucosa during the acute phase of shigellosis and at convalescence.

Methods—Rectal biopsies from 25 *Shigella dysenteriae* 1 infected patients, 10 *Shigella flexneri* infected patients, and 40 uninfected controls were studied. Morphological changes in the mucosa were graded. The frequency of IgA and IgG subclass producing cells was assessed. In addition, immunostaining for secretory component in epithelial cells was analysed.

Results—Using morphological grading, 20% of the 35 patients studied had advanced inflammation (grade 3) in the acute phase of the disease. At convalescence, grade 1 inflammation was seen in 37% of the patients and in 10% of the controls. In the acute phase, as well as at convalescence, the number of IgA₁, IgA₂, and IgG₁ positive cells was significantly higher than in the controls. The results were related to the histopathological degree of inflammation.

Conclusions—In shigellosis, there is evidence for a prolonged humoral response residing in the mucosa long after the clinical symptoms have resolved, suggesting that shigellosis induces persisting mucosal humoral immune and inflammatory responses, remaining at least until 30 days after the infection.

(J Clin Pathol 1997;50:513-520)

Keywords: shigellosis; degree of inflammation; immunoglobulin producing cells

Shigellosis is a significant cause of morbidity and mortality in developing countries, such as Bangladesh, particularly in children under the age of 5 years.¹ Of the four species of *Shigella* causing diarrhoeal diseases, the most severe disease found in Bangladesh is associated with *S dysenteriae* 1 and *S flexneri*.

In most cases, the infection is limited to the intestinal mucosa, and systemic infections are rare, especially in adults. Invasion, multiplication, and the spread of shigella (a complex series of events)²⁻⁴ causes an inflammatory enteritis. The most pronounced lesions are seen in the rectosigmoid area and the intensity of the inflammation decreases in the proximal

direction.^{5,6} Colitis and ulceration in severe shigellosis result in the loss of the protective mucosal barrier, allowing bacteria and bacterial antigens to enter into the circulation.^{7,8} The histological features of shigella colitis during its acute phase are indistinguishable from those of other forms of acute infectious colitis. In addition, during shigellosis, excessive epithelial hyperplasia, distortion, and branching of crypts are seen, features that are commonly seen in patients with chronic ulcerative colitis and in Crohn's disease.^{7,8}

The most well defined part of mucosal immunity is the secretory antibody system; it depends on local production and selective epithelial transport of polymeric IgA (pIgA) and pIgM.⁹ Mucosal surface protection afforded by secretory IgA (sIgA) antibodies depends on the local number of IgA producing cells, their subclass distribution (IgA₁ and IgA₂), and their ability to express joining (J) chain. Different classes and subclasses of immunoglobulins exhibit distinct functions and the subclass response to an infection may be of biological significance. In various gut diseases, local immunoglobulin production is altered, both with regard to class and subclass.

Disease associated alterations of mucosal IgA subclass production may be of biological importance. Enhanced secretory immunity may accelerate the pathogenesis of the disease. In the large intestine, the number of immunoglobulin containing cells seems to be lower in the rectum than in the sigmoid colon and ascending colon.¹⁰ IgA producing cells are the most frequent immunoglobulin producing cells in the large intestine. IgG producing cells normally constitute 3-5% of the immunocytes in the intestinal mucosa. IgG producing cells are more numerous in the appendix than in the colon and accumulate preferentially adjacent to lymphoid follicles, suggesting that IgG⁺ cells most likely represent follicle derived B cells that have reached terminal maturation locally.^{11,12} IgG producing cells were found to increase strikingly with increasing degrees of inflammation in the gastric mucosa, particularly in the basal part of mucosa.^{13,14}

There is a disproportionate increase in IgG producing cells in inflammatory bowel disease.¹² Local production of IgG₁ is particularly marked in ulcerative colitis.¹⁵⁻¹⁷ Conversely, lesions in Crohn's disease contain relatively more IgG₂ cells than in ulcerative colitis. In both diseases, there is a shift from a predominance of local IgA₁ producing cells to IgA₁ producing cells, and less of the IgA is dimeric. In addition, epithelial expression of

Laboratory Sciences
Division, International
Centre for Diarrhoeal
Disease Research,
Bangladesh
D Islam

Division of Pathology,
Karolinska Institute,
Huddinge University
Hospital, Sweden
B Veress

Clinical Sciences
Division, International
Centre for Diarrhoeal
Disease Research,
Bangladesh
P K Bardhan

Pasteur Mérieux
Connaught Group,
Marcy L'Etoile, France
A A Lindberg

Divisions of Clinical
Immunology and
Pathology, Karolinska
Institute, Huddinge
University Hospital,
Sweden
B Christensson

Correspondence to:
Dr D Islam, Laboratory
Sciences Division,
International Centre for
Diarrhoeal Disease Research,
Bangladesh, GPO Box 128,
Dhaka-1000, Bangladesh.
email: dilara@
cholera.bangla.net

Accepted for publication
25 February 1997

Table 1 Arbitrary scoring system for grading of severity of inflammation in rectal biopsies in shigellosis

Changes in:	Score	Proportion of samples (%) in		
		Patient 1	Patient 2	Control
Mucosal surface				
Normal	0	94	100	100
Irregular	1	6		
Surface epithelium				
Oedema	1	85	45	5
Loss	2	30		
Erosion	3	25		
Ulceration	4	4		
PMN	0	9	72	92
PMN	1–3	91	28	8
Crypt				
PMN	0	57	82	90
PMN	1–3	43	18	10
Abscess absent	0	90	100	100
Abscess present	1	10		
Goblet cells				
Normal number	0	40	100	100
Lowered number	1	60		
Normal size	0	70	90	100
Smaller size	1	30	10	
Lamina propria				
PMN	0	13	67	88
PMN*	1–3	87	33	12
Oedema	1	90	27	10
Plasma cells*	0		7	45
Plasma cells*	1–3	100	93	55
Maximum total score	21			

*Arbitrary frequency estimation, 0 = no increase; 1 = slight; 2 = moderate; 3 = high. Patient 1, patient sample in the acute phase; Patient 2, patient sample at convalescence (day 30). PMN, polymorphonuclear cells.

secretory component is modulated in these diseases: the normally high levels of secretory component seem to be influenced by dysplastic changes, leading to reduced expression.¹⁸ On the other hand, in coeliac disease, secretory immunity is enhanced both in terms of local poly immunoglobulin production and epithelial secretory component expression.¹⁹ It has been suggested that interferon (IFN) γ upregulates the expression of secretory component.¹² In contrast, in large bowel adenoma and metaplastic polyps, the staining intensity for secretory component and epithelial IgA decreases with increasing degrees of dysplasia.^{20–22}

The purposes of this study were to assess quantitatively the influence of shigella induced inflammation on: frequency and distribution of IgA, IgG, and IgM class and subclass expressing immunocytes in rectal mucosal biopsy specimens from patients with shigellosis; and expression of secretory component by cells in the mucosa. These parameters were analysed immunohistochemically in biopsies obtained early and late in the disease and compared with biopsies from healthy age and sex matched controls. The findings were also correlated to the degree of inflammation.

Methods

STUDY POPULATIONS

Thirty five male patients (aged 20–45 years) seen at the International Centre for Diarrhoeal Disease Research in Bangladesh with culture confirmed shigella (the only infecting organism) infection and 40 healthy subjects (control group) matched for sex, age, and socio-economic status were included in the study. Twenty five of 35 patients were infected with *S. dysenteriae* 1 and 10 with *S. flexneri*. No other pathogenic microorganisms were found in

stool culture from the patients. Neither the controls nor the patients had experienced either bloody dysentery in the six months before inclusion in the study or before the current infection. On admission, all patients exhibited clinical symptoms typical of bacillary dysentery including fever, tenesmus, and blood and mucus in their faeces. Patients were given pivmecillinam chloride 200 mg four times daily for at least one week from the day of admission. On average, patients became culture negative and free from dysenteric symptoms within five days after initiation of pivmecillinam treatment. Patients with additional infections, or other major diseases, were not included in the study. Individuals in this study population were also included in studies relating to other aspects of the immune function in shigellosis.

SAMPLES AND THEIR HISTOPATHOLOGY

Rectal mucosal biopsies obtained at proctoscopy were obtained from each patient within 48 hours of admission (after culture confirmation) and 30–35 days thereafter. At convalescence, all patients' stool cultures were negative for infectious organisms. In controls, one set of rectal biopsies were obtained. Biopsy specimens were fixed in 10% formalin (in 50 mM Tris buffered saline pH 7.6 (TBS) for 20 hours at room temperature) and embedded in paraffin. Sections were stained with haematoxylin and eosin for histological evaluation. To assess the degree of inflammation in the biopsy specimens, a number of morphological parameters were documented (table 1). The total score obtained by evaluating the parameters formed the basis for grading the degree of inflammation. The scoring system followed in this study was based on one used previously.^{23 24}

IMMUNOHISTOCHEMISTRY

Immunoperoxidase staining was performed on serial sections (5 μ m) of rectal mucosal biopsies. Sections were deparaffinised, rehydrated, and digested with 0.1% proteinase K (in TBS; Sigma Chemical, St Louis, Missouri, USA) for two to three minutes except where otherwise mentioned. Endogenous peroxidase activity was blocked by incubation in 0.3% H₂O₂ in methanol for 30 minutes. After pre-incubation in TBS for five minutes, the slides were incubated with 20% normal rabbit serum or normal mouse serum (Dako A/S, Glostrup, Denmark) to prevent non-specific binding. The sections were then incubated (20 hours at room temperature) with monoclonal antibodies or rabbit antisera diluted in TBS containing 1% bovine serum albumin (BSA), followed by avidin-biotin horseradish peroxidase complex according to the instructions of the manufacturer (ABC-HRP Kit; Dako A/S). The diaminobenzidine tetra hydrochloride (diaminobenzidine; 0.5 g/l) and H₂O₂ (0.015%) substrate reaction was developed for 10 minutes and the slides were counter stained with haematoxylin. Between the various incubation steps, the slides were washed in TBS. The following murine monoclonal antibodies were used to detect plasma cells in this study: antihuman

Table 2 Grading of rectal biopsies from patients and controls based on the score system for severity of inflammation

Total score	Inflammation grading	Patients infected with		Controls (n=40)
		<i>S dysenteriae</i> 1 (n=25)	<i>S flexneri</i> (n=10)	
Acute phase				
0-2	Normal			36 (90%)
3-5	Grade 1	9 (36%)	4 (40%)	4 (10%)
6-8	Grade 2	11 (44%)	4 (40%)	
9≥	Grade 3	5 (20%)*	2 (20%)†	
Convalescence				
0-2	Normal	14 (56%)	8 (80%)	
3-5	Grade 1	11 (44%)	2 (20%)	

*mean score 13; †mean score 11.

Table 3 Grading of staining intensity for secretory component (SC) in epithelial cells in rectal mucosal biopsies from patients (n=35) and controls (n=40)

SC grading	Patient sample in		Control sample
	Acute phase	Convalescence	
0+	5 (14%)		
1+	13 (37%)		
2+	5 (23%)		
3+	8 (23%)	10 (29%)	
4+	1 (3%)	25 (71%)	40 (100%)

IgG₁ (8c/6-39; Sigma), IgG₂ (HP-6014; Sigma), IgG₃ (HP-6050; Sigma), IgG₄ (HP-6025; Sigma), IgM MB-11; Sigma), IgA₁ (A1-18; Sigma), and IgA₂ (A9604D2; Southern Biotechnology, Birmingham, Alabama, USA); and B-B4 (Syndecan-1; Serotech, Oxford, UK).¹²⁻¹⁵ For the detection of human secretory component, a rabbit antiserum against human secretory component (Dako A/S) was used which detects free or IgA bound secretory component. For B-B4 staining, the sections were microwaved at full power for 3 × 5 minute intervals in a 10 mM citrate buffer (pH 6.0) instead of undergoing protease digestion. The sections were then cooled for 20 minutes, washed in distilled water, and finally washed in TBS. They were then inserted back into the normal staining protocol.

As controls for the staining procedure, the first monoclonal antibody/antiserum was replaced by irrelevant mouse IgG or IgM, or normal rabbit immunoglobulin fraction at the same concentration as the corresponding antigen specific antibody. The biopsy specimens were analysed blindly, without prior knowledge of the diagnosis or inflammatory grading. The number of immunostained cells in the lamina propria was counted in five high power fields (×400 magnification) and the average count was expressed as the number of cells per mm². The intensity of staining was arbitrarily graded as: 0, no staining; 1+, weak; 2+, moderate; 3+, high; 4+, very high. The proportion of each immunoglobulin subclass was calculated in relation to the total number of immunoglobulin

producing cells of the corresponding immunoglobulin class (G or A) present in the evaluated area of the same section. Up to five sections were examined for each subclass because of the scarcity of IgG producing cells in most specimens.

STATISTICAL ANALYSES

Comparison of data between groups and different time points were analysed using non-parametric statistical methods (χ^2 and Mann-Whitney U test), in the JMP statistical software (SAS Institute, Cary, North Carolina, USA). In each case, $p < 0.05$ was considered significant.

Results

HISTOPATHOLOGICAL EVALUATION

Based on an arbitrary score system the grade of inflammatory changes observed in haematoxylin and eosin stained sections was estimated. The grade of inflammation both in *S dysenteriae* 1 and *S flexneri* infected patients and in controls is shown in table 2. In the acute phase, no difference in the grade of inflammation was seen between *S dysenteriae* and *S flexneri*. Among the 35 patients 37% had grade 1 inflammation, 43% had grade 2, and 20% had grade 3. In addition, 10% of the controls had grade 1 inflammation. In contrast, at convalescence, a significantly higher proportion ($p < 0.01$) of the *S dysenteriae* patients (44%) had grade 1 inflammation compared with patients infected with *S flexneri* (20%).

SECRETORY COMPONENT EXPRESSION

The staining intensity for secretory component seen in the control biopsies was considered as normal and was given the arbitrary score of 4+. Decreases in the staining intensity compared with controls were graded as 3+, 2+, 1+ or 0 (virtually negative). A partly granular staining for secretory component was seen in the cytoplasm of crypt epithelium and in surface epithelium at the crypt orifices in the controls. In acute phase biopsies, the staining intensity for secretory component was decreased in the majority of specimens, as shown in fig 1A. The result of the secretory component staining is summarised in table 3. In the acute phase most biopsies from *S dysenteriae* with grade 2 inflammation had 2+ secretory component grading while most biopsies from *S flexneri* with grade 2 inflammation had 3+ secretory component grading. Secretory component grading was 3+ in 29% of the convalescence biopsies; all these samples had grade 1 inflammation and were from *S dysenteriae* patients (fig 1B). The intensity of the secretory component staining was inversely correlated to the degree

Table 4 Immunocyte counts (per mm²) in rectal mucosal biopsy specimens from shigella infected patients and controls

Samples	IgA ₁ + cells	IgA ₂ + cells	IgG ₁ + cells	IgG ₂ + cells	IgG ₃ + cells	IgG ₄ + cells	IgM+ cells	Plasma cells
Control	360 (78) (40%)	532 (118)* (60%)	20 (3) (39%)	13 (6) (25%)	7 (3) (14%)	11 (6) (22%)	53 (13)	992 (39)
Patient 1	841 (184)** (35%)	1562 (411)** (65%)	41 (17)** (28%)	55 (18)** (38%)	11 (6) (8%)	37 (18)** (26%)	69 (24)	2612 (544)**
Patient 2	674 (118)* (40%)	1017 (233)* (60%)	29 (13) (10%)	34 (7)* (36%)	8 (5) (12%)	19 (7) (42%)	62 (17)	1842 (443)*

Values are mean (SD) (percentage of total IgA, IgG, IgM cells as appropriate).

Patient 1, biopsy taken in acute phase (day 1); Patient 2, biopsy taken in convalescence (day 30).

* $p < 0.05$ compared with control; ** $p < 0.05$ compared with patient 2.

Figure 1 Immunohistochemical staining of paraffin sections of rectal mucosal biopsies taken in the acute phase of shigellosis and at convalescence. All sections were counter stained with haematoxylin. (A) Staining with the antihuman secretory component antiserum in acute phase biopsies shows very light staining of crypt epithelial and surface epithelial cells compared with (B) convalescent biopsies, where an intense brown staining is seen in both cell types and in the crypt lumen. (C) Staining of IgA, positive cells in the acute phase shows a high proportion of positive cells in the lamina propria but negative staining in crypt epithelial and surface epithelial cells, whereas in convalescent biopsies (D), it shows fewer IgA, positive cells in the lamina propria and positive staining in crypt epithelial cells, surface epithelial cells, and the crypt lumen. Original magnification $\times 125$.

of inflammation in control and patient biopsies (not shown).

SUBCLASS DISTRIBUTION OF IMMUNOGLOBULIN PRODUCING CELLS IN RECTAL BIOPSIES FROM PATIENTS AND CONTROLS

IgA positive plasma cells were most numerous in the upper two thirds of the lamina propria (fig 1C–F). In the acute phase biopsies, the frequency of IgA₁ (mean (SD) 841 (184)) and IgA₂ (1562 (311)) staining cells was significantly higher than at convalescence and in

control biopsies (table 4). In biopsies from convalescent patients, the number of both IgA₁ and IgA₂ positive cells was significantly higher ($p < 0.01$) than in controls. In the acute phase, 65% of the IgA positive cells were IgA₂ positive compared with 60% both in control and convalescence biopsies (table 4). In the acute phase, the number of IgA₂ positive cells showed a positive correlation with the degree of inflammation, irrespective of whether the patients were infected with *S dysenteriae* or *S flexneri* (not shown). The intra-epithelial stain-

Figure 1 (continued) Immunohistochemical staining of paraffin sections of rectal mucosal biopsies taken in the acute phase of shigellosis and at convalescence. All sections were counterstained with haematoxylin. (E) Staining of IgA₂ positive cells in acute phase biopsies shows a higher proportion of IgA₂ positive cells than IgA₁ positive cells in the lamina propria. (F) At convalescence, there is a decreased number of IgA₂ positive cells in the lamina propria but positive staining is seen in crypt epithelial cells and in the crypt lumen. Original magnification $\times 125$.

ing pattern for IgA closely correlated with that of secretory component.

The number of IgG positive cells in rectal biopsies, both in the acute phase and at convalescence are summarised in table 4. In the acute phase, the IgG subclass pattern was IgG₂ > IgG₁ > IgG₄ >> IgG₃, and the number of IgG₁, IgG₂, and IgG₄ positive cells was significantly higher than in the controls. At convalescence, the IgG subclass pattern was similar to that of acute phase, whereas in controls it was IgG₁ > IgG₂ > IgG₄ > IgG₃. At convalescence, only IgG₂ producing cells were significantly higher than in the controls. The IgG₄ positive cells were more predominant in the lower part of the lamina propria (figs 1G and H). In contrast, IgG₁, IgG₂, and IgG₃ positive cells were more evenly distributed throughout the lamina propria.

The number of IgM positive cells in acute phase biopsies was not significantly different from that in controls (table 4).

DISTRIBUTION OF PLASMA CELLS

As an additional plasma cell marker, biopsy specimens were stained for Syndecan-1 using the monoclonal antibody B-B4. This antibody also stained surface and crypt epithelial cells. In acute phase biopsies, a significantly higher number (mean (SD) 2612 (544), $p < 0.001$) of B-B4 positive plasma cells (fig 1I) was seen than in biopsies from controls or convalescent patients. Also, in convalescence biopsies (fig 1J)

the number of B-B4 positive plasma cells was significantly higher (1842 (443), $p < 0.01$) than in the controls. In the acute phase biopsies, most plasma cells were found just beneath the surface epithelium. However, at convalescence, the B-B4 stained cells were mostly located in the middle part of the lamina propria. Summation of the IgA, IgG, and IgM positive plasma cells deviated not more than 5% from the number of B-B4 positive plasma cells.

Discussion

We have characterised local inflammatory and immunological changes following shigella infection in adult patients. The frequency of immunoglobulin producing cells was positively correlated to the degree of inflammation. Using a detailed scoring system for inflammation, we found that a persistent mucosal inflammation was more frequent in *S dysenteriae* 1 infection (44%) than in *S flexneri* infection (20%). Previously, histopathological findings in the rectal mucosa in shigellosis has been studied mostly in the acute phase.^{7 8 26-29} In this study, we confirmed and extended the observations of a persistent inflammation and have shown that this is more frequent in *S dysenteriae* than in *S flexneri* infection. The local gut humoral immune responses in shigellosis result preferentially in IgA (both IgA₁ and IgA₂) and IgG₂ cell expansion. The IgA producing cell expansion also reflects the mucosal lesion

Figure 1 (continued)
Immunohistochemical staining of paraffin sections of rectal mucosal biopsies taken in the acute phase of shigellosis and at convalescence. All sections were counterstained with haematoxylin. (G) Staining of IgG₁ positive cells in acute phase biopsies shows a higher proportion of IgG₁ positive cells in the lamina propria than (H) at convalescence. (I) Staining of plasma cells with the anti-B-B4 monoclonal antibody (Syndecan-1) in acute phase biopsies shows an increased number of B-B4 positive cells compared with (J) convalescent biopsies. Original magnification: $\times 94$ (G and H), and $\times 187$ (I and J).

more accurately than IgG producing cell expansion in shigellosis.

In shigellosis it is reasonable to assume that the majority of shigella specific antibodies is derived from immunoglobulin producing cells in the infected colonic mucosa. In this study, a higher IgA₂:IgA₁ plasma cell ratio was seen in acute phase biopsies than in controls, although both IgA₁ and IgA₂ producing cells increased significantly in the acute phase biopsies. Previous work has shown that biopsies of patients with acute infectious colitis have a larger

increase in the numbers of IgA producing cells than of cells producing other immunoglobulin classes.³⁰ In addition, we have previously shown an increase in faecal sIgA and serum IgA₂ antibodies specific for shigella antigens in shigellosis,³¹ and others have shown that sIgA antibodies to lipopolysaccharide are mainly of the IgA₂ subclass.³² In this study, the serum level of IgA₂ was also significantly increased in patients compared to controls.³¹ We have also shown increased serum level of IgA₁ antibodies specific for shigella antigens in previous studies

of shigellosis.³¹ This may suggest that there is an early massive increase in mucosal IgA₂ production in response to the shigella infection, at least some of which is shigella antigen specific and may appear in the serum.

We observed a decrease in secretory component and IgA staining in crypt and surface epithelium in the acute phase, correlating with the degree of inflammation, in spite of an increased number of IgA subclass positive cells. Previously, we have also shown that total IgA (tIgA) as well as sIgA is greatly increased in the stools of patients with acute shigellosis.³¹ This was most prominent in patients with severe disease.³³ Thus, in these patients there is an increased local production of IgA in the inflamed mucosa that is transported into the gut lumen both by secretory component dependent and non-dependent mechanisms. Previously we have shown the loss of serum proteins (IgG, albumin, and IgA) into the faeces, probably due to ulceration or exudation through the inflamed mucosal epithelium. The lack of secretory component and IgA staining in gut epithelium in these patients could be due also to a more rapid transepithelial transport, giving less of an intra-epithelial accumulation of secretory component and IgA. The effect of modulation by proinflammatory cytokines on the turnover of secretory component has already been reported.^{9, 34} Recently, analysis of the production of cytokines in the gut mucosa in acute and convalescent phase of shigellosis has revealed a complex pattern of proinflammatory and T helper 1 (Th₁) and Th₂ associated cytokines.³⁵⁻³⁸ However, mucosal levels of IFN γ and IFN γ receptors were lower in the acute phase compared with the convalescent phase of the disease.³⁶⁻³⁸ It is not clear whether this decrease in IFN γ may influence the level of expression of secretory component in shigellosis. In coeliac disease it has been suggested that IFN γ upregulates the expression of secretory component.¹² At convalescence, the still increased frequency of IgA positive cells was associated with a gut epithelium that had regained secretory component and IgA stainability. At the same time, faecal tIgA and sIgA had returned to control levels.³¹ Again, this may suggest that faecal IgA levels are regulated by the rate of transepithelial IgA transport.

We also found an increased number of IgG positive cells in the rectal mucosa as well as alterations in their IgG subclass distribution during shigellosis. A higher number of IgG₂ positive cells was seen in the rectal mucosa of acute phase patients than in the controls. This correlates with our previous findings of an early increase in IgG₂ serum antibodies specific for shigella lipopolysaccharide.³¹ This correlation may suggest that the majority of the shigella specific serum IgG₂ antibodies are derived from the rectal mucosa. In vitro experimental data and in vivo studies indicate a role for specific factors in inducing or enhancing the production of immunoglobulin molecules of particular subclasses. The interleukin (IL)-4 and IL-13 produced by Th₂ and Th₀ cells, respectively, are associated with the enhance-

ment of IgG₁ and IgG₄ production,^{39, 40} whereas IFN γ produced by Th₁ and natural killer cells has been suggested to induce the production of IgG₂ and IgG₃.⁴¹⁻⁴³ However, a complex pattern of proinflammatory and Th₁ and Th₂ associated cytokines³⁵⁻³⁸ would be necessary for the observed IgG and IgA subclass pattern in this study. Our findings of a preferential increase of IgA₂ and IgG₂ positive cells in rectal mucosa in the acute phase of disease is reminiscent of findings in coeliac disease, a disease characterised by an increase of intra-epithelial lymphocytes.¹⁶ Interestingly, in shigellosis an increase in intra-epithelial lymphocytes can be found also (unpublished observation).

Our findings thus indicates that: there is an induction of the gut mucosal humoral immune response in shigellosis, which persists long after the clinical resolution of the disease; at least a proportion of shigella specific IgA and IgG serum antibodies may be derived from the local mucosal immune response; there is a preference for IgA₂ and IgG₂ production; the secretory component mediated transepithelial IgA transport is enhanced in shigellosis; and the local inflammatory response in shigellosis, particularly in *S dysenteriae* 1 infection, is prolonged.

Further studies are needed to evaluate the shigella antigen specificity of the locally immunoglobulin producing cells and the beneficial or detrimental consequences of the marked local IgA responses in shigellosis.

We are indebted to the individuals with shigellosis and healthy individuals from Bangladesh for their participation in the study. We also acknowledge the staff of the study ward of the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). We thank Anita Lindström, Inger Buskas, and Birgitta Axelsson (Divisions of Pathology, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden) for technical assistance. This study was supported by a grant from the Swedish Agency for Research Co-operation with Developing Countries (SAREC), ICDDR,B, the Swedish Medical Research Council (Grant no. 16x 656), and funds of the Karolinska Institute.

- Speelman P, Mc-Glaughlin R, Kabir I, Butler T. Differential clinical features and stool findings in shigellosis and amoebic dysentery. *Am J Trop Med Hyg* 1987;31:549-51.
- Sansonetti PJ, Ryter A, Clerc P, Maurelli AT, Mounier J. Multiplication of *Shigella flexneri* within HeLa cells: lysis of the phagocytic vacuole and plasmid-mediated contact hemolysis. *Infect Immun* 1986;51:461-9.
- Menard R, Sansonetti PJ, Parsot C. Nonpolar mutagenesis of the ipa genes defines IpaB, IpaC, and IpaD as effectors of *Shigella flexneri* entry into epithelial cells. *J Bacteriol* 1993;175:5899-906.
- Gigh N, Mounier J, Prevost MC, Sansonetti PJ. IpaB of *Shigella flexneri* causes entry into epithelial cells and escape from the phagocytic vacuole. *EMBO J* 1992;11:1991-9.
- Speelman P, Kabir I, Islam M. Distribution and spread of colonic lesions in shigellosis: a colonoscopic study. *J Infect Dis* 1984;150:899-903.
- Mathan MM, Mathan VI. Morphology of rectal mucosa of patients with shigellosis. *Rev Infect Dis* 1991;13(Suppl 4):S314-18.
- Streulens MJ, Patte D, Kabir I, Salam A, Nath SK, Butler T. *Shigella* septicemia: prevalence, presentation, risk factor and outcome. *J Infect Dis* 1985;152:784-90.
- Islam MM, Azad AK, Bardhan PK, Raqib R, Islam D. Pathology of shigellosis and its complications. *Histopathology* 1994;24:65-71.
- Brandtzaeg P, Halstensen TS, Kett K, Krajci P, Kvale D, Rognum TO, et al. Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. *Gastroenterology* 1989;97:1562-84.
- Matsueda K, Yamada G, Tsuji T. Immunohistochemical characterization of the lymphocyte and the immunoglobulin-containing cell in the epithelium and the lamina propria of normal human intestines. *Acta Med Okayama* 1991;45:161-9.
- Bjerke K, Brandtzaeg P, Rognum TO. Distribution of immunoglobulin producing cells is different in normal human appendix and colon. *Gut* 1986;27:667-74.

- 12 Brandtzaeg P, Bjerke K, Kett K, Kvale D, Rognum TO, Scott H, *et al.* Production and secretion of immunoglobulins in the gastrointestinal tract. *Ann Allergy* 1987;**59**:21–39.
- 13 Valnes K, Brandtzaeg P, Elgjo K, Stave R. Quantitative distribution of immunoglobulin-producing cells in gastric mucosa: relation to chronic gastritis and glandular atrophy. *Gut* 1986;**27**:505–9.
- 14 Valnes K, Brandtzaeg P. Subclass distribution of mucosal IgG-producing cells in gastritis. *Gut* 1989;**30**:322–6.
- 15 Brandtzaeg P, Kett K, Rognum TO. Subclass distribution of IgG- and IgA-producing cells in secretory tissues and alterations related to gut diseases. *Adv Exp Med Biol* 1987;**216A**:321–33.
- 16 Brandtzaeg P, Kett K, Halstensen TS, Helgeland L. Pathogenesis of ulcerative colitis and Crohn's disease: humoral immune mechanisms. *Eur J Gastroenterol Hepatol* 1990;**2**:256–65.
- 17 Scott MG, Nahm MH, Macke K, Nash GS, Bertovich MJ, MacDermott RP. Spontaneous secretion of IgG subclasses by intestinal mononuclear cells: differences between ulcerative colitis, Crohn's disease, and controls. *Clin Exp Immunol* 1986;**66**:209–15.
- 18 Brandtzaeg P, Halstensen TS, Huitfeldt HS, Krajci P, Kvale D, Scott H, *et al.* Epithelial expression of HLA, secretory component (poly-Ig receptor), and adhesion molecules in the human alimentary tract. *Ann N Y Acad Sci* 1992;**664**:157–79.
- 19 Kett K, Scott H, Fausa O, Brandtzaeg P. Secretory immunity in celiac disease: cellular expression of immunoglobulin A subclass and joining chain. *Gastroenterology* 1990;**99**:386–92.
- 20 Rognum TO, Fausa O, Brandtzaeg P. Immunohistochemical evaluation of carcinoembryonic antigen, secretory component, and epithelial IgA in tubular and villous large-bowel adenomas with different grades of dysplasia. *Scand J Gastroenterol* 1982;**17**:341–8.
- 21 Rognum TO, Elgjo K, Fausa O, Brandtzaeg P. Immunohistochemical evaluation of carcinoembryonic antigen, secretory component, and epithelial IgA in ulcerative colitis with dysplasia. *Gut* 1982;**23**:123–33.
- 22 Jass JR, Faludy J. Immunohistochemical demonstration of IgA and secretory component in relation to epithelial cell differentiation in normal colorectal mucosa and metaplastic polyp: a semiquantitative study. *Histochem J* 1985;**17**:373–80.
- 23 Reinholt FP, Veress B, Lindquist K, Liljeqvist L. Qualitative assessment and morphometry in the study of the ileal reservoir after restorative proctocolectomy. *APMIS* 1989;**97**:97–104.
- 24 Veress B, Reinholt FP, Lindquist K, Liljeqvist L. Different types of mucosal adaptation in the ileal reservoir after restorative proctocolectomy. A two-year follow-up study. *APMIS* 1990;**98**:786–96.
- 25 Pellat-Deceunynck C, Bataille R, Robillard N, Harousseau JL, Rapp MJ, Juge-Morineau N, *et al.* Expression of CD28 and CD40 in human myeloma cells: a comparative study with normal plasma cells. *Blood* 1994;**84**:2597–603.
- 26 Mathan MM, Mathan VI. Ultrastructural pathology of the rectal mucosa in Shigella dysentery. *Am J Pathol* 1986;**123**:25–38.
- 27 Mathew M, Mathan MM, Mani K, George R, Jebakumar K, Dharamsi R, *et al.* The relationship of microbial pathogens to acute infectious diarrhoea of childhood. *J Trop Med Hyg* 1991;**94**:253–60.
- 28 Butler T, Dunn D, Dahms B, Islam M. Causes of death and the histopathologic findings in fatal shigellosis. *Pediatr Infect Dis J* 1989;**8**:767–72.
- 29 Sachdev HP, Chadha V, Malhotra V, Verghese A, Puri RK. Rectal histopathology in endemic shigella and salmonella diarrhea. *J Pediatr Gastroenterol Nutr* 1993;**16**:33–8.
- 30 Spreuvel JPV, Lindeman J, Meijer CJLM. A qualitative study of immunoglobulin containing cells in the differential diagnosis of acute colitis. *J Clin Pathol* 1985;**38**:774–7.
- 31 Islam D, Wretling B, Ryd M, Lindberg AA, Christensson B. Immunoglobulin subclass distribution and dynamics of Shigella-specific antibody responses in serum and stool samples in shigellosis. *Infect Immun* 1995;**63**:2054–61.
- 32 Mestecky J, Russell MW. IgA subclasses. *Monogr Allergy* 1986;**19**:277–80.
- 33 Islam D, Wretling B, Hammarström L, Christensson B, Lindberg AA. Semi-quantitative estimation of shigella antigen specific IgG, IgA and IgM antibodies: correlation to disease severity during shigellosis. *APMIS* 1996;**104**:563–74.
- 34 Sartore RB. Viewpoints on digestive disease. *Gastroenterology* 1994;**106**:533–9.
- 35 Raqib R, Lindberg AA, Wretling B, Bardhan PK, Andersson U, Andersson J. Persistence of local cytokine production in shigellosis in acute and convalescent stages. *Infect Immun* 1995;**63**:289–96.
- 36 Raqib R, Ljungdahl A, Lindberg AA, Andersson U, Andersson J. Local entrapment of interferon γ in the recovery from Shigella dysenteriae type 1 infection. *Gut* 1996;**38**:328–36.
- 37 Raqib R, Lindberg AA, Björk L, Bardhan PK, Andersson U, Andersson J. Down-regulation of gamma interferon, tumor necrosis factor type I, interleukin 1 (IL-1) type I, IL-3, IL-4, and transforming growth factor β type I receptors at the local site during the acute phase of shigella infection. *Infect Immun* 1995;**63**:3079–87.
- 38 Raqib R, Wretling B, Andersson J, Lindberg AA. Cytokine secretion in acute shigellosis is correlated to disease activity and directed more to stool than to plasma. *J Infect Dis* 1995;**171**:376–84.
- 39 Punnonen J, Aversa G, Cocks BG, McKenzie ANJ, Menon S, Zurawski G, *et al.* Interleukin 13 induces interleukin 4 independent IgG₁ and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 1993;**90**:3730–4.
- 40 Paul WE. Interleukin-4: prototypic immunoregulatory lymphokine. *Blood* 1991;**77**:1859–70.
- 41 Snapper CM, Paul WE. Interferon γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987;**236**:944–7.
- 42 Snapper CM, McIntyre TM, Mandler R, Pecanha LMT, Finkelman FD, Lees A, *et al.* Induction of IgG₁ by interferon γ : a model for T cell-independent class switching in response to T cell-independent type 2 antigen. *J Exp Med* 1992;**175**:1367–71.
- 43 Amigorena S, Bonnerot C, Fridman WH, Teillaud JL. Recombinant interleukin 2-activated natural killer cells regulate IgG2a production. *Eur J Immunol* 1990;**20**:1781–7.