The involvement of IL-6 and IL-8 in acute invasive gastroenteritis of children

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Abstract

The involvement of the proinflammatory cytokines, interleukin 8 (IL-8) and 6 (IL-6), was studied during the first 72 h of acute invasive gastroenteritis. Study population included 33 infants and young children aged six months to six years and seven age-matched controls. As a group, patients with acute invasive gastroenteritis had an increased serum level of IL-8 and IL-6 as compared with healthy controls (p < 0.002 and p < 0.001, respectively). Subjects were then divided into two groups based on stool cultures (proven and non-proven bacterial cultures). Patients with bacterial-proven acute invasive gastroenteritis tended to have increased IL-8 serum concentrations (p < 0.07) as compared with those with non-proven bacterial etiologies and IL-6 levels were only detected in subjects with positive bacterial cultures (p < 0.05). When dividing each sub-group into early and late blood drawing with respect to disease onset, no statistical differences were found in each group but subjects with bacterial-proven etiologies had significant higher IL-6 levels as compared with non-proven etiologies at the two time points (p < 0.019 and p < 0.015, respectively).

In conclusion, the proinflammatory cytokines, IL-6 and IL-8, are involved in acute invasive gastroenteritis. The difference in IL-6, and to a lesser degree IL-8, between proven and non-proven bacterial etiologies, needs further investigation.

Keywords: AIGE; IL-6; IL-8

Abbreviations: AIGE; acute invasive gastroenteritis; IL-6; interleukin 6; IL-8; interleukin 8; IFN-γ; interferon gamma

1. Introduction

Intestinal epithelial cells have been shown to secrete various cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-8 (IL-8), interleukin-10 (IL-10), monocyte chemotactic protein 1 (MCP1) and granulocyte-macrophage colony-stimulating factor (GM-CSF), either constitutively [1] or in response to stimuli such as viruses [2], invasive bacteria [3–5] or chronic inflammation [6]. The proinflammatory cytokines, TNF-α, IL-1β, IL-6 and IL-8, all play a major role in coordinating mechanisms which control inflammation [7]. TNF-α and IL-1β mediate the septic state by influencing vascular resistance and permeability, cardiac and bone marrow function and metabolic parameters [8]. TNF-α increases neutrophil migration and activates the antimicrobial activity of monocytes, macrophages, neutrophils and eosinophils [9]. As the release of TNF-α and IL-1β is generally a short-lived phenomenon, these cytokines may be undetectable in the serum of patients during the early phase of disease. However, these cytokines stimulate the production of other inflammatory cytokines that perpetuate the sepsis cascade. Particularly important is the local release of IL-8 which is a potent chemotactic and an activating peptide for leukocytes, macrophages and intraepithelial lymphocytes [10]. It may also play a role in the expansion of B cells and in host defense mechanisms in bacterial infections [11]. Recently, it was suggested to also be involved in gastroenteritis caused by rotavirus [12]. TNF-α and IL-1β also induce the secretion of other
mediators such as IL-6. IL-6 also plays a central role in various host defense mechanisms such as immune response, hematopoiesis and acute-phase reactions [13]. In adults and in neonates, serum IL-6 levels rapidly increase during sepsis and they appear to be more stable and to correlate with the disease severity [14].

A significant body of information is known regarding cytokine production and interaction in inflammatory bowel disease [15], but few investigators have studied cytokine production during acute bacterial or viral gastroenteritis.

The present study was designed to study the production of IL-6 and IL-8 during the acute phase (72 h) of acute invasive gastroenteritis (AIGE) and also to investigate a possible correlation between their production and the disease severity.

2. Materials and methods

2.1. Study population

Study population consisted of 33 infants and young children, aged six months to six years, with community-acquired AIGE, who had been referred to the Pediatric Emergency room at the Soroka Medical Center, Beer Sheva. Subjects were included in the study if they had a history of soft or liquid stools with a frequency of more than three times within the last 24 h and of less than 72 h duration, fever above 38 °C (per rectum) and more than 15 white blood cells and/or 15 red blood cells per high power microscopic field (×40) in stool sample. For controls we have used age-matched healthy infants and young children who came for routine follow-up to our out-patient clinics and had no history of recent disease, fever and diarrhea and were not on any medication.

Upon enrolment, a detailed history was taken and subjects underwent physical examination. Rectal temperature was recorded and blood was drawn for a complete white blood count and serum cytokine concentrations. Stool cultures were performed for isolation of Shigella spp., Salmonella spp., Campylobacter jejuni and Escherichia coli. In addition DNA probe methods were used for identification of various diarrheagenic E. coli [16].

The study was approved by the Human Subject Committee of the Soroka Medical Center, Beer Sheva.

2.2. Cytokine concentrations

Serum concentrations of IL-6 and IL-8 were measured by a solid phase ELISA. A high sensitive immunoassay kit (Quantikine HS R&D system, Minneapolis, MN 55413, USA) was used. This kit uses an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a colored product. The secondary enzyme system consists of alcohol dehydrogenase and diaphorase (Amplifier). Results were expressed as mean±1SE.

2.3. Statistical analysis

Subjects were divided into two groups, according to proven and non-proven bacterial etiologies. Serum cytokine concentrations were compared with those measured in seven age-matched healthy controls by the Kruskal–Wallis non-parametric analysis of variance because of the doubtfulness of normality. Whenever this analysis resulted in a significant difference between groups pairwise comparisons were performed with Bonferroni correction for the significance level. Pearson correlation was calculated between cytokine concentration and other patient’s parameters. Comparison between early and late blood sampling in each diagnosis group regarding each cytokine was performed using the Mann–Whitney non-parametric test.

The box-and-whiskers plot was used to depict the results. The box-and-whiskers diagram summarizes the sample data. The three horizontal lines depict the upper medium and lower quartiles. The ‘box’, therefore, shows the position of the central 50% of the data points and their dispersion. The ‘whiskers’ extend to the 5th and 95th percentiles. The SPSS for Window Version 11.0 was used for statistical analysis. $P < 0.05$ was considered statistically significant.

3. Results

Bacterial pathogens were isolated from 22 of the 33 subjects and 6 of the other 11 subjects had stool cultures positive for rotavirus. Bacterial pathogens included 13 Shigella spp., 4 Salmonella spp., 3 C. jejuni and 2 virulent E. coli. Based on the above, subjects were divided into two groups of proven and non-proven bacterial etiologies. The clinical and laboratory parameters are presented in Table 1. No differences were found between the two sub-groups regarding body temperature on admission, number of stools per day, white blood count or disease duration prior to admission (Table 1).

Polymorphonuclear neutrophil cells percentage in blood WBC count was significantly higher in subjects with bacterial pathogens ($p < 0.04$) while lymphocytes were significantly higher in the non-bacterial group ($p < 0.03$) (Table 1). As most of the subjects with pathogen-negative diarrhea (10/11) were under two years of age, a separate comparison was also made for subjects less than two years of age (10 and 11, respectively).

Serum concentrations of IL-6 and IL-8 were presented in Table 2 and Fig. 1. Serum concentrations of IL-8 were
significantly higher in patients with AIGE as compared with healthy controls \( (p < 0.0002) \). When comparing the sub-groups only patients with bacterial proven diarrhea had significantly higher concentrations as compared with controls. IL-6 was detected only in subjects with bacterial AIGE and were undetectable in patients with non-proven bacterial AIGE or in healthy controls \( (p < 0.0001) \).

In order to control for the possible confounding effect of the day of sampling in relation to the disease onset, we divided the patients into two groups: ‘early’ (0–36 h) and ‘late’ (36–72 h). Separate analyses were carried out in each group comparing between the two time points as well as between groups for each time point separately. Similar results were demonstrated in the concentrations of the different cytokines in both bacterial and non-bacterial origin on the two time points (Table 3). When comparing the two groups in each point in time a significant difference was found only in IL-6 \( (p < 0.019 \text{ and } p < 0.015, \text{ respectively}) \).

Pearson correlation coefficient for the whole group \( (n = 33) \) indicated a positive correlation between blood WBC and disease duration \( (r = 0.541, p < 0.02) \), and between body temperature and percentage of polymorphonuclear neutrophils \( (r = 0.534, p < 0.02) \). No other significant correlations were found.

### 4. Discussion

Our results suggest an involvement of the pro-inflammatory cytokines, IL-6 and IL-8, in the acute phase of AIGE. In accordance with previous works, we found that IL-8 was involved in the acute phase of different pathogens causing AIGE, but IL-6 seems to be involved only in the sub-group of patients with bacterial-proven AIGE. Only 6 of 11 subjects with non-proven bacterial etiology were positive for rotavirus, so the possibility of bacterial etiology in the rest cannot be excluded. Yet, positive bacterial culture corresponded to a very distinctive cut point: presence or absence of IL-6 in the

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Timing</th>
<th>Bacterial proven</th>
<th>Non-proven bacterial</th>
<th>( p &lt; )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>Early</td>
<td>53.22 ± 8.60</td>
<td>35.14 ± 11.46</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>76.41 ± 14.12</td>
<td>37.12 ± 9.6</td>
<td>0.081</td>
</tr>
<tr>
<td>IL-6</td>
<td>Early</td>
<td>63.66 ± 18.57</td>
<td>0.00 ± 0.00</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>16.62 ± 7.63</td>
<td>0.00 ± 0.00</td>
<td>0.015</td>
</tr>
</tbody>
</table>

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Table 1
Patients’ characteristics on admission (mean ± S.E.)

<table>
<thead>
<tr>
<th></th>
<th>Bacterial ( (n = 22) )</th>
<th>Non-bacterial ( (n = 11) )</th>
<th>( p &lt; )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>3.76 ± 3.21</td>
<td>1.38 ± 1.58</td>
<td>0.07</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>39.2 ± 0.9</td>
<td>38.8 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>No. of stools (d)</td>
<td>5.4 ± 1.7</td>
<td>5.6 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Duration (h)</td>
<td>32.7 ± 13.9</td>
<td>43.2 ± 18.9</td>
<td>NS</td>
</tr>
<tr>
<td>Blood WBC (cells/mm³)</td>
<td>12135 ± 9025</td>
<td>16895 ± 9383</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>63.45 ± 18.57</td>
<td>43.50 ± 23.98</td>
<td>0.04</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>26.95 ± 17.40</td>
<td>46.50 ± 23.11</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 2
IL-6 and IL-8 serum concentrations in patients with AIGE and healthy controls (mean ± S.E.)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Bacterial proven ( (n = 22) )</th>
<th>Non-proven bacterial ( (n = 11) )</th>
<th>Healthy controls ( (n = 7) )</th>
<th>( p &lt; )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>49.37 ± 14.47</td>
<td>0B</td>
<td>0B</td>
<td>0.0001</td>
</tr>
<tr>
<td>IL-8</td>
<td>62.29 ± 8.50</td>
<td>37.56 ± 7.11</td>
<td>4.23 ± 0.89</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Individual means with different superscript letters are statistically different.
serum. If one assumes, therefore, that all 11 patients were negative for bacterial pathogens, the absence of IL-6 in the serum of patients with AIGE can serve as a way of distinguishing bacterial and non-bacterial AIGE and help in the treatment choices. If, on the other hand, negative stool does not rule out bacterial etiology, then differences in IL-6 may suggest different stimulation of the immunological system.

Previous reports suggested that serum cytokines appear in a temporal sequence after endotoxin injection [17]. Our results did not show differences between those whose blood was drawn earlier (0–36 h) in the course of the disease as compared with those who were sampled later (36–72 h). Very few studies have evaluated cytokine release into the serum during acute gastroenteritis and most of them did not compare viral versus bacterial etiologies. In a study of children with complicated *Shigella dysenteriae* I infection, serum TNF-α and IL-6 were found to be increased during the first two weeks of infection [3]. A significant increase was especially found in subjects with complicated course (hemolytic uremic syndrome, microangiopathic hemolytic anemia, leukemoid reactions, thrombocytopenia and severe colitis). In another study on cytokine secretion in acute shigellosis, cytokine concentrations were higher in stool than in plasma [18]. While TNF-α and IL-6 remained elevated up to 10 days after the onset of the disease, IL-1β, IL-1ra and IL-8 concentrations remained elevated for a longer period of time. IFN-γ concentrations were depressed during the acute phase and increased gradually during the convalescent phase. Plasma concentrations of cytokines were considered to mirror the intestinal inflammation [19]. In children with rotavirus induced gastroenteritis, De Boissieu et al. have found a peak of serum IFN-γ within two days of onset of symptoms [20]. A positive correlation was found between IFN-γ concentrations and the number of vomiting episodes, but not with maximal temperature or fever duration. IFN-γ concentrations tended to be higher in subjects with disease of less than three-day duration than in those with more than four days. As IFN activates macrophages, inflammatory cytokines such as IL-6 and later IL-8, are released in excess during the initiation of the cytokine cascade in inflammation [21].

Our results may suggest, therefore, a possible route for earlier discrimination between bacterial and non-bacterial AIGE based on cytokine serum levels or a possibility of different routes of stimulation of the gut immune system which may correlate with the ability to get positive stool culture. It is suggested, therefore, that more research be conducted to study the concentrations of other proinflammatory serum cytokines with regard to disease duration and various disease etiologies in order to investigate the possibility of incorporating measurement of serum cytokine concentrations in early diagnosis of bacterial versus non-bacterial AIGE.

References