Early activation of mucosal dendritic cells and macrophages in acute Campylobacter colitis and cholera: An in vivo study

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Abstract

Background and Aim: Macrophages and dendritic cells are closely related mononuclear phagocytic cells. Little is known about their in vivo role in acute intestinal bacterial infections in humans. We undertook to evaluate these cells in rectal mucosal biopsies of patients with acute colitis.

Methods: All mucosal mononuclear phagocytic cells in rectal biopsies of patients with acute Campylobacter colitis (n = 5), shigellosis (n = 5), and cholera (n = 10) were evaluated ultrastructurally and compared with those in controls (n = 5).

Results: Mononuclear phagocytic cells in the superficial rectal mucosa showed a higher prevalence of ultrastructural features of activation in Campylobacter colitis and cholera than in controls. A lower prevalence of features of activation with increased monocytes was seen in shigellosis. Cells with the ultrastructural morphology of activated dendritic cells constituted 41% and 45% of all mononuclear phagocytic cells in two of five patients with Campylobacter colitis and 4–22% of cells in four of 10 patients with cholera. Their presence in patients with Campylobacter colitis was associated with significant surface epithelial damage and prominent acute inflammatory changes in the mucosa.

Conclusions: This is the first ultrastructural study to show activated macrophages and dendritic cells in vivo in acute Campylobacter colitis and cholera. Dendritic cell activation occurred early in the clinical course of these infections. Surface epithelial damage may play a role in the activation of dendritic cells.

Introduction

Macrophages and dendritic cells are closely related mononuclear phagocytic cells that play a key role in developing immune responses. Both are present in gut associated lymphoid tissue and the lamina propria and lie in close proximity to the intestinal lumen with its large antigenic load. Increased numbers of activated macrophages and dendritic cells are seen in the intestinal mucosa in chronic inflammatory disorders. Both cell types are known to play a critical role in the immune response to bacterial agents, but little is known about their in vivo response to acute bacterial infections in the human intestinal tract.

The majority of lamina propria dendritic cells share a common myeloid etiology with macrophages. It is known that mucosal macrophages are derived from monocytes migrating in from the blood, but it is not clear whether dendritic cells are directly recruited from the bone marrow or whether they are derived from local differentiation of monocytes. Macrophages undergo morphological changes when activated. Activated dendritic cells also show alterations in shape and surface processes, prominence of macropinocytosis, and the presence of convoluted nuclei that can be recognized by ultrastructural study and that parallel changing antigenic expression and functional changes. We therefore undertook an ultrastructural evaluation of the mononuclear phagocytic cell (MPC) population of macrophages, activated dendritic cells, and monocytes, in the rectal mucosa of individuals with acute colitis due to Campylobacter, Shigella, and cholera, to try to understand their contribution to the mucosal response to these infections. Although cholera is primarily an infection of the small intestine, acute inflammatory changes indistinguishable from those due to other bacterial pathogens have also been found in the rectal mucosa of these patients.

Methods

Patients

Rectal mucosal biopsies from 20 patients with acute diarrhoea and faecal cultures positive for bacterial pathogens were studied. The pathogens identified included Campylobacter spp. (n = 5),

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Biopsies

Biopsies from all patients were fixed in chilled 2.5% glutaraldehyde, postfixed in osmium tetroxide, and embedded in araldite.

Light microscopy

One-micron sections stained with toluidine blue were used for light microscopic assessment.

Acute colitis score

A simple histological score was computed to assess the severity of mucosal changes due to acute colitis by scoring the following histological variables as mild (1), moderate (2), or severe (3): mucin depletion, surface epithelial damage, cryptitis, crypt abscess formation, neutrophil infiltration of the lamina propria, congestion, edema, hemorrhage, and neutrophil margination in blood vessels. The sum of the scores for all these variables was taken as the final acute colitis score.

Ultrastructural study

Ultra thin sections were cut on well-oriented blocks with a Leica ultracut UCT (Vienna, Austria) and a diamond knife (Diatome, Berks, Switzerland). Sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 201C electron microscope (Eindhoven, the Netherlands).

All MPCs in each biopsy were studied. They were identified by their nuclear characteristics and their small, membrane bound, round, electron-dense, lysosomes. Their location in the mucosa and relation to the surface or crypt epithelium was noted. The nuclear-cytoplasmic ratio and morphological features of each cell were evaluated.

MPCs were sub-classified as monocytes if the nuclear-cytoplasmic ratio was ≤1 and only minimal cytoplasmic organelles were present. They were considered to be activated dendritic cells if they had a convoluted nucleus, prominent surface dendritic processes, and prominent macropinosomes (pinosomes more than 1 micrometer in diameter). All other MPCs were considered macrophages.

Immunohistochemistry

Immunohistochemical studies were done on frozen and formalin-fixed tissue from all the controls and a subset of patients with cholera. Monoclonal antibodies to CD68 (PG-M1); the major histocompatibility complex (MHC)–class antigens HLADP,DQ,DR; the macrophage scavenger receptor CD163; the monocyte lipopolysaccharide receptor CD16; and the dendritic cell marker CD11c were obtained from Dako (Glostrup, Denmark). Five-μm-thick frozen sections were fixed in acetone prior to immunostaining with CD163, CD14, and CD11c. Formalin-fixed, paraffin-embedded sections were used for staining with CD68 and HLADP,DQ,DR. Antigen retrieval was carried out with proteinase K. Rabbit antimouse was used as the secondary antibody and the Envision kit for labeling (both from Dako, Glostrup, Denmark).

Positive cells were quantitated using an eye-piece graticule moved from the surface epithelium down to the muscularis mucosa at 1000× magnification in five adjacent but non-overlapping areas. The results were expressed as the mean counts of positive cells per square mm of upper and lower mucosa.

Statistical analysis

The ultrastructural features of MPCs in the different groups and their association with clinical features and acute colitis scores were compared using the χ², Fisher’s exact probability, and Mann–Whitney U-tests in the SPSS package version 9.05 (SPSS, Chicago, IL, USA).

Results

Light microscopy

Acute colitis score

The mean acute colitis score was 5.4 for patients with Campylobacter infection, 7.2 for patients with shigellosis, and 3 for patients with cholera. Surface epithelial damage with shortening of cells, loss of nuclear polarity, mucin depletion, cytoplasmic vacuolation, and intercellular edema was seen in four of five patients with Campylobacter colitis, three of six patients with shigellosis, and to a mild degree in seven of 10 patients with cholera. Mild architectural alteration was noted in one biopsy from a patient with acute Campylobacter infection. Granulomas were not seen in any of the biopsies.

Ultrastructural study

The mean number of MPCs studied in each biopsy was 22.8 in the controls, 16.8 in Campylobacter colitis, 22 in shigellosis, and 21.2 in cholera.

Mononuclear phagocytic cells in controls

A total of 88.6% of the MPCs seen in controls had the morphology of macrophages (Fig. 1) and the remaining had the morphology of monocytes (Table 1). Activated dendritic cells were not seen.
Prominent phagocytic activity was seen in 58% of all mononuclear phagocytic cells, increased primary lysosomes in 11%, cytoplasmic lipid bodies in 32%, and prominent pseudopodia in 22%.

A total of 76% of MPCs in the control biopsies were located in the superficial or upper third of the rectal mucosa. Some of the subepithelial MPCs were seen to insert pseudopodia between the surface epithelial cells.

Mononuclear phagocytic cells in acute Campylobacter colitis, cholera, and shigellosis

The distribution of MPCs in the rectal mucosa of patients with acute Campylobacter colitis and cholera was not different from that in controls. However, the proportion of MPCs showing morphological features of activation in the upper third of the mucosa was significantly increased. These features included a significantly higher number of lipid bodies in each cell (Fig. 2) ($P = 0.018$), presence of increased numbers of lysosomes ($P = 0.000$), and prominent phagocytic activity ($P = 0.000$). None of the cells had phagocytosed bacteria and none appeared to be undergoing apoptosis.

The distribution of MPCs in the rectal mucosa of patients with acute shigellosis was different from that of controls. Forty-three percent were located in the upper mucosa (vs 76% in controls; $P = 0.0000$), 33% in the middle third (vs 14% in controls), and 25% in the lower third (vs 10% in controls). Morphological features of activation were reduced in shigellosis: the number of lipid bodies per cell and the proportion of cells with prominent phagocytic activity was significantly less than in controls ($P = 0.041$ and $P = 0.000$, respectively). Cells with the morphology of monocytes constituted 25% of MPCs in shigellosis versus 11.4% in controls ($P = 0.0167$) (Fig. 3). None of the cells had phagocytosed bacteria and none appeared to be undergoing apoptosis.

Activated dendritic cells

Activated dendritic cells were identified by their prominent surface processes, convoluted nuclei, and macropinosomes (Fig. 4). They were often arranged in clusters and were seen in the rectal mucosa of patients with acute Campylobacter colitis and cholera (Fig. 5), but in none of the controls or patients with acute shigellosis. In two patients with acute Campylobacter infection, activated dendritic cells constituted 41% and 45% of the total MPCs seen. They were located in the upper third of the mucosa (Fig. 5) except for one activated dendritic cell that was marginated within a vascular channel in the deep mucosa (Fig. 6). Two-thirds of the activated dendritic cells showed evidence of phagocytic activity, but the phagosomes were never large. A total of 20% had lipid bodies. A total of 89% of dendritic cells from one patient had prominent lysosomes, although these were not seen in the other patient.

Table 1 Proportion of macrophages, monocytes, and dendritic cells among the mononuclear phagocytic cells in rectal biopsies of controls and patients with acute colitis on ultrastructural study

<table>
<thead>
<tr>
<th></th>
<th>Macrophages</th>
<th>Monocytes</th>
<th>Activated dendritic cells</th>
<th>Total mononuclear phagocytic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>101</td>
<td>13</td>
<td>0</td>
<td>114</td>
</tr>
<tr>
<td>Campylobacter colitis</td>
<td>68</td>
<td>2</td>
<td>14</td>
<td>84</td>
</tr>
<tr>
<td>Shigellosis</td>
<td>83</td>
<td>27</td>
<td>0</td>
<td>110</td>
</tr>
<tr>
<td>Cholera</td>
<td>188</td>
<td>14</td>
<td>9</td>
<td>211</td>
</tr>
</tbody>
</table>

Figure 1 Minimally activated macrophage with few cytoplasmic organelles (uranyl acetate and lead citrate; Bar 2 μm).

Figure 2 Activated macrophage with increased primary lysosomes (arrows) and lipid bodies (arrow heads) (uranyl acetate and lead citrate; Bar 2 μm).
Activated dendritic cells were seen in four of 10 patients with acute cholera, but were far less frequent than in Campylobacter colitis, constituting 4–22% of all MPCs. They were seen both in the superficial and in the deep mucosa.

None of the activated dendritic cells seen had dendritic processes extending between surface epithelial cells.

**Correlation of macrophage and dendritic cell activation with histological scores and clinical features**

Biopsies of the two patients with Campylobacter colitis and numerous activated dendritic cells were obtained within 4 h and 24 h of the onset of clinical illness. The total duration of clinical illness in both these patients was only 2 days, whereas the total duration in the three cases without activated dendritic cells was 4–5 days (mean 4.6 days). The two patients with activated dendritic cells also had more prominent surface epithelial damage (moderate damage in one, and severe damage in the other, Fig. 5) and higher acute colitis scores (scores of 8 and 11) in their biopsies than the three patients in whose biopsies activated dendritic cells were not seen (surface epithelial damage absent or mild; acute colitis scores 0, 2, and 4).

The mean duration of clinical illness at the time of biopsy was 1.2 days among the cholera cases with activated dendritic cells. There was no correlation between the prevalence of activated
dendritic cells and surface epithelial damage, acute colitis scores or other clinical features in these patients with cholera.

**Immunohistochemistry**

Table 2 shows the mean numbers of positive cells per square mm of mucosa in controls and acute cholera. Cells staining positive for CD68, MHC antigens, CD163, and CD11c were large and stellate, oval, or spindle-shaped. Positive cells in the deep lamina propria were more often spindle-shaped and oriented perpendicular to the surface epithelium or located pericryptally. Some CD11c cells appeared small and lymphoid. All cells staining positive for CD14 were small and rounded. Some subepithelial macrophages were seen to insert pseudopodia between surface epithelial cells, but this was not seen among the CD11c positive cells.

The highest counts were seen in sections stained with CD68 and the lowest counts with CD14. There was no significant difference in the numbers or distribution of positive cells between biopsies from patients with cholera and the controls.

**Discussion**

**Dendritic cell activation**

This is the first ultrastructural study of dendritic cells in vivo in the intestinal mucosa. We have shown that dendritic cell activation occurs early in the clinical course of acute bacterial colitis and that activated dendritic cells may constitute a high proportion of mucosal MPCs in the rectal mucosa in acute infections.

In the normal rectal mucosa, dendritic cells constitute only 0.6% of isolated mononuclear cells. Mildly higher numbers are seen in chronically inflamed tissue (146/mg vs 115/mg in controls). A 3.5-fold increase in MHC class II positive cells with ‘dendritic’ morphology has been described in the bronchial mucosa of rats within 6–24 h of challenge with heat-killed Moraxella catarrhalis, but the time-scale or magnitude of dendritic cell response in acute colitis in animals or humans has not been evaluated. Stimulation of dendritic cells is known to induce maturation with the development of prominent processes and a decreased ability to take up more antigens. The dendritic cells we identified, however, showed evidence of both prominent surface processes and macrophagocytosis.

In our study, all biopsies with activated dendritic cells were obtained within 48 h of onset of clinical illness. Activated dendritic cells constituted approximately 40% of all MPCs in the biopsies of two patients with Campylobacter infection. This increase in activated dendritic cells in the mucosa could occur by recruitment from the bone marrow or by local differentiation of monocytes. Campylobacter jejuni has been shown to induce maturation of dendritic cells in vitro as indicated by the up-regulation of cell surface proteins CD40, CD80, and CD86, in addition to the activation of NF-kappa-B and the production of interleukin-beta-1 (IL-1β), IL-6, IL-8, IL-10, IL-12, gamma interferon, and tumor necrosis factor-alpha (TNF-α). The presence of an activated dendritic cell within a vascular channel of one of the biopsies from a patient with Campylobacter colitis could indicate that activation can occur prior to or during the process of recruitment.

The prominent surface epithelial damage seen in both biopsies with numerous activated dendritic cells may have been responsible for entry of luminal antigens that facilitated activation of these cells.

It is interesting that the two patients with Campylobacter colitis and large numbers of dendritic cells in this study were also the ones with the shortest course of clinical illness, suggesting that dendritic cells may play a role in limiting illness.

In addition to their surface processes and macrophagocytic vesicles, activated dendritic cells also showed evidence of

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**Table 2** Mean number of cells staining positive for macrophage markers per square mm of upper and lower rectal mucosa in controls and cholera

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Controls (n = 5)</th>
<th>Cholera (n = 5)</th>
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<tbody>
<tr>
<td></td>
<td>Upper mucosa</td>
<td>Lower mucosa</td>
</tr>
<tr>
<td></td>
<td>mean, SEM</td>
<td>mean, SEM</td>
</tr>
<tr>
<td>CD68</td>
<td>1093, 159</td>
<td>608, 84</td>
</tr>
<tr>
<td>HLA-DP,DQ,DR</td>
<td>993,141</td>
<td>701,101</td>
</tr>
<tr>
<td>CD163</td>
<td>714,110</td>
<td>510,90</td>
</tr>
<tr>
<td>CD11c</td>
<td>550,46</td>
<td>110,90</td>
</tr>
<tr>
<td>CD14</td>
<td>185,56</td>
<td>33,28</td>
</tr>
</tbody>
</table>
phagocytic activity and increased numbers of lysosomes and lipid bodies, suggestive of an active role in innate immune responses. Studies on rat intestines have shown dendritic cells inserting processes between surface epithelial cells to sample luminal antigens. We did not observe this however, although many macrophages were seen to insert pseudopodia between surface epithelial cells. Dendritic cells are known to develop prominent surface processes only when antigenically stimulated and it is possible that the inactive or immature dendritic cells normally present in mucosa may be morphologically indistinguishable from macrophages, with which they share a common myeloid etiology.

The presence of activated dendritic cells in the rectal mucosa in cholera is an unexpected finding. It suggests a more widespread immune response than would be expected with an infection primarily targeting the small intestine. Experimental studies show that cholera toxin has various stimulatory and inhibitory effects on dendritic cells including the up regulation of MHC class II antigens, induction of migration within Peyer’s patches, and inhibition of the production of IL12, TNF-α, and MIP-1α. Exposure to endotoxin and cholera toxin also induces dendritic cell maturation and migration to regional lymph nodes. Endotoxin or cholera toxin entering the mucosa from the lumen may have been responsible for the mucosal dendritic cell activation noted in our study.

Shigella infection is associated with the activation of epithelial cells, T cells, and macrophages in the colon mucosa. However Shigella spp. are also known to inhibit immune responses and induce apoptosis of dendritic cells. This could account for the absence of activated dendritic cells in the rectal mucosa of patients with acute shigellosis in this study. Sampling error or the slightly later time periods at which these biopsies were obtained (3.6 days vs 1.2 for cholera and 1.4 for Campylobacter) could also, however, have contributed to the lack of dendritic cells in these biopsies.

Macrophage activation and monocyte recruitment

This is the first study to show macrophage activation in vivo in the rectal mucosa in acute bacterial colitis in humans. The increased numbers of primary lysosomes and lipid bodies, where digestive enzymes and eicosanoid mediators localize, in MPCs of patients with cholera and Campylobacter suggests heightened ‘preparedness’ to tackle any invading pathogens. The increased phagocytic activity noted could be an indication of ‘mopping up activity’ following damage to surface epithelial cells by luminal and proinflammatory factors. The presence of macrophocytosis in some of these cells suggests enhanced soluble antigen uptake as well, implicating a role in antigen presentation.

Cholera toxin, a potent mucosal adjuvant, has been shown to enhance antigen presenting function, archicadonic acid metabolism, and inflammatory cytokine expression in macrophages in vitro, and could be responsible for activating rectal macrophages in patients with acute cholera. Campylobacter has also been shown to activate macrophages in vitro.

Intriguingly, the MPC population in shigellosis showed less prominent phagocytic activity, lipid bodies, and lysosomes than in controls, Campylobacter coliitis, or cholera. The high proportion of monocytes in shigellosis suggests recent recruitment of these cells from the blood stream. This possibility is supported by the findings of a study on rabbit ileal loops injected with Shigella flexneri in which large numbers of macrophages/monocytes were found in the subepithelial dome region over lymphoid follicles within 2–4 h of exposure to the pathogen. The increased proportion of MPCs we saw in the deep mucosa of patients with acute shigellosis could be due to their migration from the superficial mucosa or due to their entry from submucosal vessels: similar to the way neutrophils enter the mucosa in rabbit ileal loops injected with Shigella flexneri.

The relative paucity of activated macrophages in the biopsies of acute shigellosis is more difficult to explain, but could be due to down-regulation of innate immune responses by Shigella spp. Encounter with live shigellae is known to induce apoptosis or necrosis of macrophages and dendritic cells in vitro, but no invading bacteria or apoptotic cells were seen in these biopsies.

Our immunohistochemical studies suggest that there is a major overlap between markers of macrophages and lamina propria dendritic cells. The numbers of cells staining positive for HLA DR (thought to be highly expressed in dendritic cells but not in macrophages) were at least twice as high as the numbers of cells staining positive with CD11c. Immunohistochemical studies alone may therefore not be entirely accurate, and it is our opinion that immunoelectron microscopy is the ideal technique to correlate the ultrastructural morphology of these cells with their antigen expression.

In conclusion, we found increased morphological features of activation in MPCs in the superficial rectal mucosa of patients with Campylobacter colitis and cholera and decreased features of activation in shigellosis. Numerous activated dendritic cells were found in a few patients with Campylobacter coliitis and lesser numbers were found in cholera. Only biopsies with prominent surface epithelial damage were associated with significant dendritic cell activation.

Acknowledgment

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References

6. Hart AL, Al-Hassi HO, Rigby RJ et al. Characteristics of intestinal...
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