Intra-abdominal sepsis: an immunocytochemical study of the small intestine mucosa


Abstract

**Aim**—To investigate immunocytochemical changes in intestinal tissues from patients with intra-abdominal sepsis, and to relate the changes to the possibility of enhanced bacterial adhesion and translocation.

**Methods**—Tissues from 17 patients suffering from intra-abdominal sepsis and from controls were sectioned and stained immunocytochemically for IgA, IgM, secretory component, J chain, and HLA-DR. Differences in the distribution and characteristics of positively staining cells between the patient groups were assessed.

**Results**—Patients with intra-abdominal sepsis had noticeable reductions in numbers of IgA and IgM plasma cells, reduced J chain staining, and had little immunoglobulin on the surfaces of enterocytes. In contrast, HLA-DR positive cells were increased in the sepsis compared with the control group. The plasma cells present showed cytological changes suggestive of apoptosis.

**Conclusions**—Stress associated with sepsis and its immediate causes might result in increased plasma glucocorticoid levels that bring about apoptosis of mucosal plasma cells (or their precursors). The consequent reduction in expression of IgA and IgM may favour bacterial adhesion to the enterocytes and facilitate bacterial translocation into the tissues.

(J Clin Pathol 1997;50:294–298)

Keywords: intra-abdominal sepsis; bacterial adhesion; bacterial translocation; apoptosis

The concept that organisms from the gastrointestinal tract play a major role in the genesis and perpetuation of sepsis in critically ill patients is supported by the correlation between the microbial species colonising the proximal intestine and the organisms causing endogenous infection in intensive care units. This phenomenon reflects bacterial translocation across the mucosa, which can be attributed to alterations in the barrier function of the small intestine.

The immunological defences of the small intestine include substantial numbers of lamina propria plasma cells, the majority of which produce IgA. In its dimeric form, linked by J chain, this immunoglobulin passes through the overlying epithelium where secretory component is added, and reaches the luminal surface to be trapped in the mucosal layer. Smaller amounts of IgM reach the lumen in a similar manner.

Studies on this immunological component in critically ill patients are limited by ethical and therapeutic constraints; however, suitable tissues may be obtained from patients whose condition necessitates surgical removal of part of the small bowel. A series of such specimens was retrieved for the present study. Tissues from near the excision margins—considered viable by the surgeons and judged to be relatively remote from the primary intra-abdominal damage by the pathologists—were selected for immunohistochemical studies.

**Methods**

**PATIENTS AND TISSUES**

Blocks from the small intestines of 17 patients were taken from the files of the Department of Pathology, Aberdeen Royal Infirmary, Scotland (12 patients: six males, aged 54–78; six females, aged 34–79), and the Department of Surgery, Universidade Federal de Pernambuco, Recife, Brazil (five patients: one male, aged 39; four females, aged 57–79). All of the Aberdeen cases had presented with an acute abdomen; 11 had infection of the small bowel attributed to a variety of thorobtic or mechanical causes. The Recife cases met the same criteria for selection. One Aberdeen case had a perforated ulcer. Controls were normal small intestine from two transplant donors. Tissues had been fixed in 10% neutral buffered formalin and processed to paraffin wax. Sections of 4 µm were cut onto 3-aminopropyltriethoxysilane-subbed slides and dried at 60°C for 30 minutes.

**IMUNOHISTOLOGY**

Sections were first dewaxed in xylene and endogenous peroxidase blocked using 1.5% hydrogen peroxide in methanol. Optimal unmasking of antigens other than IgM was obtained by placing sections in 10 mM citrate buffer, pH 6.0, in an 800 W microwave oven operated on full power for 20 minutes, maintaining the level of buffer at 60 ml. Sections were left in hot buffer on the bench for a further 20 minutes prior to transferring to Tris buffered saline (TBS). IgM was exposed by digestion in 0.1% trypsin in 0.1% calcium chloride at 37°C, pH 7.8, for 30 minutes, followed by five-minute washes in running water (once) and TBS (twice).

Murine monoclonal antibodies against IgM (Oxoid, Basingstoke, UK, MF02) and HLA-DR (Dako, High Wycombe, UK, M0646) were used at dilutions of 1:160 and...
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1:40, respectively, and incubated for 60 minutes. Rabbit polyclonal antibodies against IgA (Behring, Lewes, UK, ORCl 03/05), J chain (Nordic, Maidenhead, UK, 9-480), and secretory component (Dako A0187) were used at dilutions of 1:2000, 1:600, and 1:1000, respectively, and incubated for 30 minutes. Following TBS washes, biotin labelled species specific secondary antibodies were applied for 30 minutes. For mouse primary antibodies the secondary antibody was rabbit antimouse/biotin (Dako E0343) at a dilution of 1:150 in TBS containing 4% heat inactivated normal human AB serum, and for rabbit primary antibodies, swine antirabbit/biotin (Dako E0345) at a dilution of 1:200 containing 4% heat inactivated normal human AB serum. Slides were then washed in TBS after which sABC (Dako K0377), diluted as per kit instructions, was applied for 90 minutes (for polyclonal primary antibodies), or 30 minutes (for monoclonal primary antibodies), followed by TBS washes. Demonstration of HRP was with the substrate 3,3-diaminobenzidine tetrahydrochloride, immediately following which the sections were placed into 0.5% cupric sulphate in saline to darken the DAB reaction product to brown/black. Sections were then counterstained in Harris haematoxylin, dehydrated, cleared, and mounted in DPX.

Known positive control sections were included in each batch of immunostained specimens. Negative controls were provided by incubating sections from the same block with TBS in place of mouse monoclonal primary antibody, or normal rabbit immunoglobulin fraction (Dako X0903) at a dilution of 1:1000 in place of rabbit polyclonal primary antibody.

Results

IgA Controls IgA was present in the cytoplasm of cytologically well preserved plasma cells located in the lamina propria of the villi or in the loose connective tissue around the intestinal crypts. Small granules of IgA positivity were observed throughout the cytoplasm of the crypt cells and concentrated at the apical poles. The apical surface of the enterocytes lining the villi was also IgA positive (fig 1A).

Intra-abdominal sepsis There was a striking absence of morphologically normal IgA positive plasma cells. However, rare small cells could be detected immunocytochemically, the IgA within these cells being detected in the form of coarse cytoplasmic granules. These cells lacked the classical chromatoid pattern and conspicuous nucleoli associated with plasma cells, showing instead a dense chromatin nucleus. No IgA was present in the cytoplasm of the crypt cells or lining the enterocyte surfaces (fig 1B).

IgM Controls The apparent number of IgM positive plasma cells was fewer than those which were IgA positive. They were located in the lamina propria of the villi adjacent to intestinal crypts. The apical poles of the crypt cells were rich in deeply stained IgM granules. In contrast to IgA, enterocytes presenting a superficial lining of sIgM were observed only in the basal third of the villi (fig 1C).

Intra-abdominal sepsis IgM positive cells with normal plasma cell morphology were not detectable. However, with IgA, immunocytochemistry revealed rare IgM positivity in cells with dense chromatin nuclei that were devoid of nucleoli. No IgM positivity was noted in the crypt cells or on the enterocytes (fig 1D).

J CHAIN

Controls The lamina propria of the villi and the loose connective tissue around the intestinal crypts contained numerous J chain positive plasma cells (fig 2A).

Intra-abdominal sepsis As with IgA and IgM, morphologically normal plasma cells with J chain positivity were not detectable. The slight J chain positive staining of the apical pole of the crypt cells in normal individuals was absent in septic patients (fig 2B).

SECRETORY COMPONENT

Controls Secretory component was noted in the crypt cells of normal human jejunum, concentrated in the Golgi area. Small secretory component positive granules were observed on the surface of the enterocytes located at the basal third of the villi (fig 2C).

Intra-abdominal sepsis Cytoplasmic secretory component was noted in the whole crypt area. Beside the conspicuous staining of the Golgi area, cytoplasmic secretory component positive granules were noted in the epithelium of the entire villus (fig 2D).

HLA-DR

In both controls and sepsis cases the apical poles of the enterocytes showed linear HLA-DR positive small granules. In septic patients HLA-DR positive cells were strikingly more numerous in the intestinal lamina propria (figs 2E and 2F). These cells presumably included antigen presenting cells and many had the morphology of macrophages.

Discussion

These results indicate a drastic reduction of sIgA and sIgM on the apical surfaces of jejunal enterocytes during intra-abdominal sepsis. There were also morphological indications that this lack of luminal sIg may have resulted from the death of plasma cells located in the neighbourhood of the intestinal crypts, the cells of which are known to be responsible for secretory component mediated transfer of these immunoglobulins from the lamina propria to the crypt lumen. Tissues from sepsis patients showed fewer IgA positive, IgM positive, and J chain positive plasma cells than those from the normal controls.

In normal specimens, IgA and IgM plasma cells had cytoplasm filled with small granules of positivity staining IgA and IgM, and nuclei with well defined chromatin granules and conspicuous nucleoli. Conversely, in tissues from patients with intra-abdominal sepsis, morpho-
logically normal plasma cells were not detected. However, the rare cells detected with immunocytochemical positivity for IgA and IgM were reduced in size, their immunoglobulins were in the form of compact, coarse granules, the nuclei were devoid of nucleoli and without apparent chromatin granules. These changes are equivalent to some of the features associated with cellular apoptosis. Presumably these were degenerating cells which had not yet been phagocytosed. Whereas in degeneration by necrosis cells become vacuolated, in apoptotic cells DNA cleavage leads to a reduced cell volume and chromatin compaction, often followed by nuclear fragmentation and phagocytosis. Apoptotic cells are commonly recognised and removed by macrophages. Apoptosis in B lymphocytes is thought to result from programmed cell death or to be induced by an increase in plasma glucocorticoid levels. The decrease in the number and percentage of blood lymphocytes and eosinophils seen during stress may be the result of apoptosis.

Bacterial translocation, the passage of viable bacteria through the epithelial intestinal barrier into the lamina propria is a significant factor in the aetiology of sepsis. It requires initial attachment of bacteria to intestinal enterocytes, the cell membranes of which are then ruptured, allowing the bacteria to penetrate and reach the basal membrane. Once this is achieved, intestinal lymphatic drainage carries bacteria to the mesenteric lymph nodes for dissemination to other organs and tissues. This dissemination may lead to sepsis syndrome, multiple organ failure, and death of the patient. Bacterial translocation has been reported in a variety of pathological conditions including intestinal obstruction, surgical trauma, drug cytotoxicity, thermal injury, intravenous feeding, haemorrhagic shock, parenterally administered endotoxin, and hyperpyrexia. These have in common an association with trauma that induces severe patient stress.

Following trauma, both humoral and cell mediated immunity are affected adversely. Stress triggers the hypothalamic–pituitary–adrenal axis with a subsequent rise in serum levels of many hormones including somatostatin, corticotrophin, angiotensin, insulin, substance P, adrenaline, glucagon, and glucocorticoids. Stress and sepsis have a strong impact on the blood levels of lymphocytes, effects related to the release of glucocorticoids. Glucocorticoids have a variety of immunosuppressive effects, including downregulation of macrophage function, prevention of monocyte differentiation, inhibition of synthesis of interferon-γ, interleukin (IL)-1, IL-6, and tumour necrosis factor, and suppression of B cell maturation. In rats, the number and

Figure 1  Immunocytochemistry of small intestinal tissues stained for IgA and IgM. (A, C) Normal tissues. (B, D) Tissues from patients with intra-abdominal sepsis. (A) IgA positive plasma cells are present in the lamina propria and IgA positive granules are concentrated in the apical poles of crypt cells; (B) only apoptotic plasma cells are present, IgA positive granules are absent from crypt cells; (C) IgM positive plasma cells are located near intestinal crypts and IgM positive granules are accumulated in the apical poles of crypt cells; (D) one apoptotic plasma cell is shown, crypt cells are IgM negative.
percentage of lymphocytes decreases when the animals experience a mild acute stress. Lymphopenia and increase of plasmatic glucocorticoid level have also been noted during human sepsis.

We surmise that the stress related increases in glucocorticoid levels resulting from the aforementioned pathologies may promote apoptosis of the mucosal plasma cells (or their B cell lineage progenitors) responsible for secretion of IgA and IgM. Such a reduction of secreted immunoglobulins would interfere with the normal barrier mechanism and facilitate adhesion of intestinal bacteria to the surface of the enterocytes—the initial step in the chain of events that may evolve to sepsis and multiple organ failure. It is well established that sIgA present in mucus located on the enterocyte glycocalyx prevents bacterial adhesion. Albanese et al., using the Ussing model, have shown that *Escherichia coli* bound to sIgA could not cross a morphologically intact segment of viable intestinal tissue.

Cytoplasmic secretory component was increased in the crypt cells and the enterocytes of septic patients compared with normal individuals (figs 2C and 2D). This may indicate that increased glucocorticoid levels had not

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**Figure 2** Immunocytochemistry of small intestinal tissues stained for J chain, secretory component, and HLA-DR. (A, C, E) Normal tissues. (B, D, F) Tissues from patients with intra-abdominal sepsis. (A) Numerous J chain positive plasma cells are present in the lamina propria; (B) J chain positive plasma cells are greatly reduced; (C) secretory component is expressed in the Golgi of crypt cells; (D) secretory component positive granules are noted in crypt cells and enterocytes; (E) HLA-DR granules are present in the apical poles of enterocytes and in macrophages; (F) the macrophage population is increased in the lamina propria.
interfered with secretory component synthesis, and may indeed have led to an increase in secretory component. Although secretory component molecules normally leave the crypt cells attached to dimeric IgA or pentameric IgM to form the respective sIgs, the absence of IgA and IgM may result in the cytoplasmic accumulation of secretory component in crypt cells and crypt derived enterocytes by a positive feedback process.

Ayala et al. reported a decrease in antigen presenting cells associated with the loss of major histocompatibility class II following haemorrhage. In contrast, we noted an increase in the number of HLA-DR positive cells, probably macrophages (figs 2E and 2F). There was also an apparent increase of the cytoplasmic content of HLA-DR in lamina propria macrophages and dendritic cells. These findings appear consistent with well documented reports that macrophages participate in removing cellular debris resulting from cellular apoptosis.

This work was supported by The British Council/FACEPE, European Union Contract T33–CT93.0227, Bank of Brazil Foundation, and Conselho Nacional de Pesquisas.

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