Brief Definitive Report

TUMOR NECROSIS FACTOR mRNA LOCALIZED TO PANETH CELLS OF NORMAL MURINE INTESTINAL EPITHELIUM BY IN SITU HYBRIDIZATION

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Paneth cells are granular secretory cells in the epithelium of intestinal crypts (1, 2). They have an antibacterial function (3, 4), and may also be involved in digestive and other processes (2, 5). Paneth cells contain the antibacterial enzyme lysozyme (3, 6), and reports of intracellular bacterial debris suggest that they may be phagocytic (4).

We have previously demonstrated that Paneth cells contain large amounts of lysozyme mRNA, whereas resident macrophages in the lamina propria do not (7). We therefore compared the capacity of Paneth cells and macrophages to synthesize other characteristic macrophage products. Using in situ hybridization, we found that Paneth cells in the small intestine of normal mice contain appreciable quantities of RNA for TNF, and by Northern blot analysis, we found that the RNA detected in small bowel is similar to that found in activated macrophages.

Recent reports that mRNA for α_1-antitrypsin and for a defensin-like peptide are present in Paneth cells (8, 9), together with our findings, suggest that Paneth cells are a significant exocrine source of immunoregulatory peptides.

Materials and Methods

Mice. 3-mo-old female C57/Bl6 mice were obtained from the mouse breeding unit at the Sir William Dunn School of Pathology.

In Situ Hybridization. Tissue samples were obtained from animals after perfusion fixation with 4% paraformaldehyde in PBS, and 10-μm cryostat sections were prepared as described (7). Subsequent treatment and in situ hybridization were carried out with minor modifications of a standard protocol (10).

The murine TNF probe used was obtained from Genentech Inc. (San Francisco, CA). We subcloned a 330-bp Sac I/Xba I fragment from the pmuTNFtrp plasmid into the multiple cloning site of the pT3T7 plasmid (Pharmacia Fine Chemicals, Uppsala, Sweden). The subcloned fragment encodes amino acids 1-110 of the mature murine TNF protein (11). Sense and antisense probes were transcribed from linearized plasmids in the presence of ^{35}S-UTP (10).

Northern Blot Hybridization. Organs were removed into liquid nitrogen, and RNA was pre-

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pared by a standard method (12). Agarose-formaldehyde gels were processed according to standard protocols, and ^{32}P-labeled DNA probes were prepared using the random oligonucleotide priming method (12).

Immunohistochemistry. Tissue samples were obtained from animals after perfusion fixation with periodate-lysine-paraformaldehyde (13). Immunohistochemistry was performed on 10-μm cryostat sections, as described (13). F4/80 antiserum was prepared by Dr. P. Dri in our laboratory (Sir William Dunn School of Pathology). mAb 2.4G2 was a gift from Dr. J. Unkeless (Mt. Sinai Hospital, New York), mAb M1/70 was from Dr. T. Springer (Harvard Medical School, Boston, MA) and mAb Tib122 was from Dr. H. Waldman (Cambridge University, Cambridge, England).

Results

Hybridization of the murine TNF probe to sections of normal mouse small intestine is shown in Fig. 1. At low magnification, using dark-field microscopy (Fig. 1, *A*), a clear positive hybridization signal is seen at the base of the intestinal crypts, where Paneth cells are found. The remaining tissue shows only a background level of autoradiographic signal, also present with the sense strand probe (Fig. 1 *B*). The

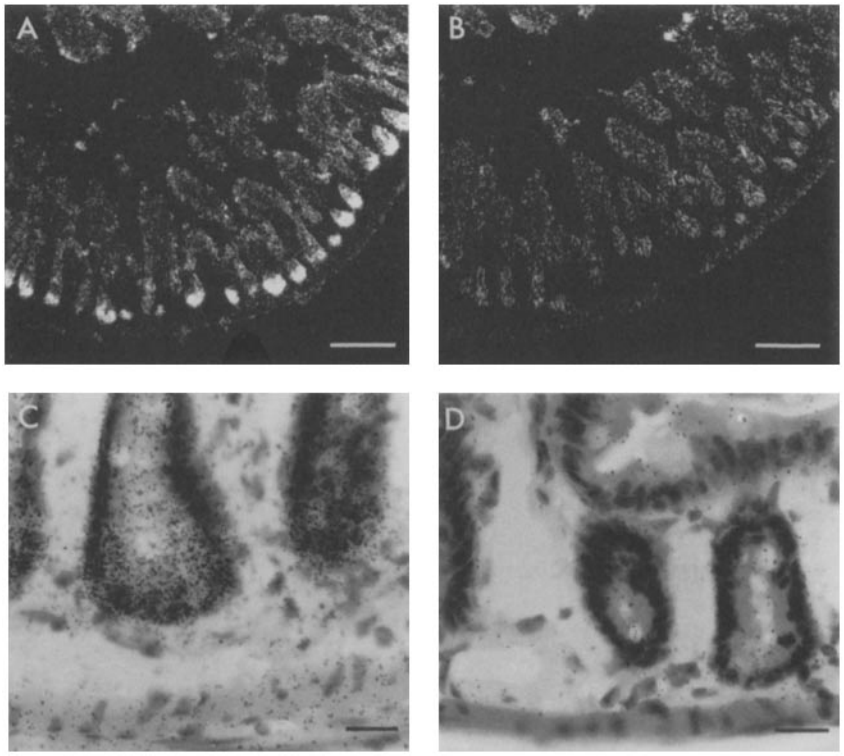

FIGURE 1. In situ hybridization of TNF mRNA in murine Paneth cells. Autoradiographs of sections of normal small intestine hybridized to the ^{35}S-labeled murine TNF probe (antisense, *A* and *C*) and the control probe (sense strand, *B* and *D*). *A* and *B* are dark-field photomicrographs at low magnification, and *C* and *D* are higher magnification views of intestinal crypts counterstained with haematoxylin and eosin. Paneth cells were identified by their basal nuclei and characteristic apical granules. Resident macrophages found in abundance in adjacent lamina propria show no such labeling. Autoradiographs were exposed at 4°C for 4 d. Scale bar: *A* and *B*, 100 μm; *C* and *D*, 20 μm.

lamina propria, which contains many macrophages, lymphocytes, and other cells, shows little or no specific signal. At high magnification, autoradiographic grains are concentrated over the most basal cells of the crypt (Fig. 1, *C* vs. *D*). Other cells in the vicinity of the crypt are clearly negative. The positive cells were identified as Paneth cells by their position and morphology (1, 2).

To establish that the in situ hybridization signal was from normally transcribed TNF mRNA, we extracted RNA from the small intestine and stomach of normal mice and compared it with RNA from the liver of a mouse that had been infected 2 wk earlier with Bacille Calmette Guerin (BCG) and subsequently challenged with an intraperitoneal injection of endotoxin for 2 h (14). Total small intestine contains low levels of TNF mRNA with the same electrophoretic mobility as the more abundant TNF mRNA present in BCG-infected liver, while normal stomach gave no TNF signal (result not shown).

To investigate whether Paneth cells produced TNF protein, we used a sensitive

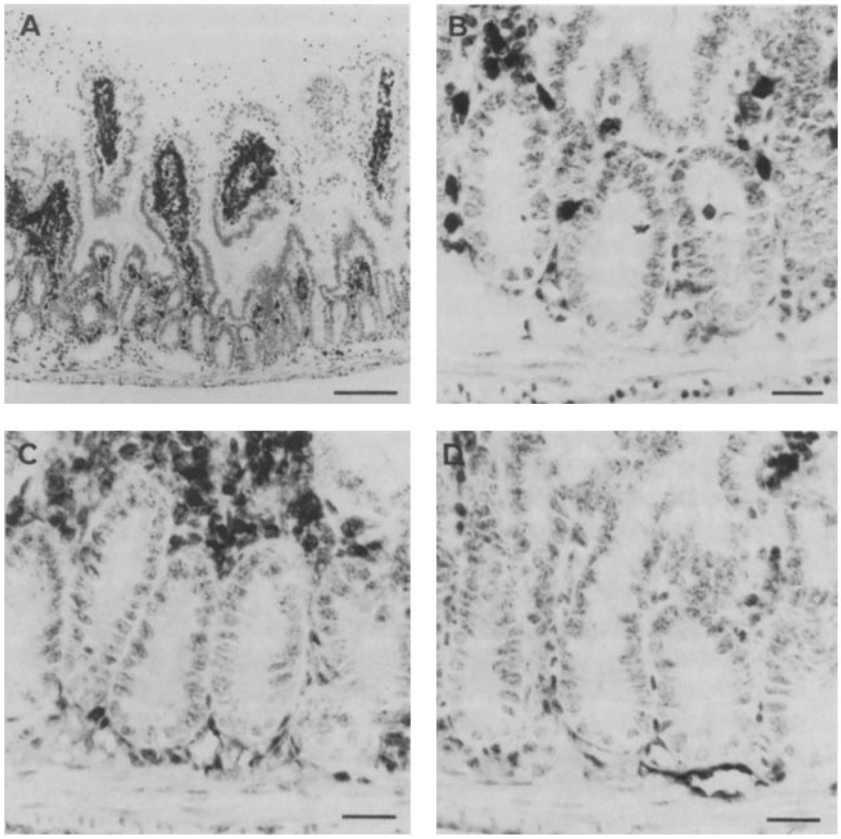

FIGURE 2. Paneth cells lack leukocyte surface markers. Avidin biotin-peroxidase immunohistochemistry for leukocyte cell surface antigens in sections of mouse small intestine viewed at low (*A*) and higher (*B–D*) magnification. (*A* and *B*) F4/80 polyclonal antiserum directed against a murine macrophage-specific antigen; (*C*) mAb TiB122 against leukocyte common antigen; (*D*) mAb 2.4G2 against IgG Fc receptors. With all these markers, Paneth cells were unlabeled, whereas resident macrophages in lamina propria were strongly immunoreactive. Scale bar: *A*, 100 μm, *B–D*, 20 μm.

cytotoxicity assay (14) and detected low levels of TNF activity in extracts of mouse small intestine (not shown). TNF antigen was not, however, detectable in this tissue by Western blotting or immunohistochemistry using a number of mAbs and polyclonal antisera. With the same immunohistochemical methods, lysozyme was easily detected in Paneth cells, and it appears that TNF mRNA in normal small intestine is translated at a low rate or not at all.

Because Paneth cells are able to produce several leukocyte secretory proteins (3, 6–9), and because of unusual features of their proliferation in intestinal crypts (2, 15), we examined the possible relationship of Paneth cells to bone marrow–derived cells by analysis of various leukocyte differentiation antigens (16). Unlike macrophages in the lamina propria, Paneth cells did not express the murine macrophage-specific antigen F4/80, here probed with a highly sensitive and specific polyclonal antiserum (Fig. 2, *A* and *B*). Paneth cells also lacked the leukocyte common antigen (LCA; mAb TIB22), an excellent marker for cells of known haematopoietic origin (Fig. 2 *C*). Similar labeling patterns were observed with mAb 2.4G2 against a macrophage type FcR (Fig. 2 *D*) and M1/70, against the type 3 complement receptor (not shown). Paneth cells also did not contain endogenous peroxidase activity (not shown).

Discussion

Paneth cells constitute a large body of cells in humans and rodents, with estimates of 20–30 cells per intestinal crypt (2). The evidence that they may regulate the intestinal flora is twofold. First, it is known that Paneth cells contain lysozyme (3, 6), and we recently demonstrated that they contain large amounts of lysozyme mRNA (7) and are capable of synthesizing the enyme. Other workers have reported bacterial debris within Paneth cell phagolysosomes (4), and recently, a cDNA with the consensus sequence for the antibacterial defensin family of peptides was localized to Paneth cells (9). Indirect evidence for a host defense function comes from experiments that show that germ-free mice and rats contain few Paneth cells and that these cells degranulate and proliferate in response to bacterial recolonization (17).

We have found that Paneth cells in the small intestine of mice contain readily detectable TNF mRNA, unlike resident macrophages in the surrounding lamina propria. Northern blot analysis showed that the TNF mRNA found in normal small intestine comigrates with TNF mRNA from activated macrophages, although the specific concentration in total intestine was much lower than in BCG-infected liver. Taken with the in situ hybridization analysis, this result is compatible with a Paneth cell source of TNF mRNA.

The TNF gene in macrophages can be transcribed and steady-state levels of mRNA maintained without translation into protein (14). TNF protein could not be detected in normal Paneth cells by immunohistochemistry, nor did extracts of whole small intestine contain TNF antigen by Western blotting, although low levels of cytolytic activity were present. TNF levels in resting Paneth cells are therefore probably also post-transcriptionally regulated. Further studies on intact intestine and isolated cells are needed to identify the stimuli required for translation and secretion of TNF.

Paneth cells are thought to arise from the same stem cells as other intestinal epithelial cells, but they are replaced at a lower rate and migrate and differentiate toward the bases of the crypts rather than toward the tips of the villi (15). They lack

typical murine leukocyte markers, such as F4/80 antigen, FcR, CR3, and LCA, and apart from their ability to perform leukocyte-like functions, there is no evidence that they have an extra-intestinal or bone marrow origin. The absence of specialized leukocyte endocytic receptors suggests that endocytic activity in Paneth cells and professional phagocytes proceed by different mechanisms.

Paneth cells are specialized for secretion into the intestinal lumen, whereas other sources of TNF are directed into the vascular or interstitial compartments (14). Under conditions in which TNF mRNA is translated, TNF may be secreted baso-laterally, away from the Paneth cell granules, or with the granules into the intestinal lumen. Although there is no evidence for a direct effect of TNF on bacteria or parasites, lumenal TNF could contribute to local defence by modulating the growth and differentiation of rapidly dividing intestinal epithelial cells. Reabsorption of released TNF might have significant effects on other local cellular targets in small intestine, and if absorbed into lymph or blood TNF, could exert potent effects on systemic targets in pathological conditions.

Summary

Paneth cells in normal murine small intestine contain TNF mRNA that is readily detectable by in situ hybridization, unlike resident macrophages in lamina propria, which are negative. Northern blot analysis of whole tissue shows the presence of mRNA that has the same electrophoretic mobility as TNF mRNA from activated macrophages. A low level of TNF bioactivity, but no immunoreactivity, was detected in normal small intestine, and TNF production in resting Paneth cells appears to be post-transcriptionally controlled. Typical leukocyte surface membrane markers were not found on Paneth cells, but were expressed by the surrounding lamina propria macrophages. Paneth cells are thus epithelial cells with leukocyte-like secretory potential that may be important in intestinal physiology and pathology.

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