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Interstitial cell of Cajal loss correlates with the degree of inflammation in the human appendix and reverses after inflammation ☆

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Abstract

Background: Normal gut motility relies on the complex interaction between the interstitial cell of Cajal (ICC) and the enteric nerve networks. Inflammation of the gastrointestinal tract adversely affects both ICC and enteric nerves. We aimed to determine the distribution of ICC and nerve networks in patients with appendicitis.

Methods: Specimens from controls and patients with appendicitis were examined with immunohistochemistry (c-Kit for ICC, beta III tubulin [Tuj-1] and neuronal nitric oxide synthase [histochemical diaphorase] for nitrergic neurons) and electron microscopy (EM). Data were quantified using image analysis.

Results: We found a profound decrease in c-Kit immunoreactivity (c-Kit IR) in the advanced inflammatory stages of appendicitis, which correlated with the severity of inflammation. Electron microscopy confirmed ultrastructural injury in both ICC and nerve fiber networks during acute inflammation. After the inflammation resolved, interval appendices displayed a recovery in ICC c-Kit IR to control levels and normal ultrastructure. The neuronal network also displayed ultrastructural recovery; however, neuronal nitric oxide synthase activity did not recover.

Conclusions: Severe inflammation results in significant ultrastructural damage of nerves and ICC networks in appendicitis. The loss of c-Kit IR is likely due to impaired ICC cytophysiology because ICC was still present under EM. After resolution of acute inflammation, ICC recovers their normal ultrastructure and c-Kit IR.

☆ Notes: Data were presented at the 20th International Congress of Pediatric Surgery in Berne, Switzerland 2007 and at the 9th European Pediatric Surgery meeting Istanbul, Turkey. 2008.

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1. Background

Normal gut motility depends on the functional integrity of both the enteric nervous system and the networks of interstitial cell of Cajal (ICC) [1-3]. These 2 networks interact functionally as ICC receives synaptic innervation from enteric nerves [4-6]. The pattern of ICC and neural network distribution seen in the human intestine is also evident in the human appendix [7], making this tissue a useful model for the study of these networks in health and disease. Significant effort has been made to understand the pathophysiology and plasticity of ICC and nerve networks during inflammation in the gastrointestinal (GI) tract. Although animal models and clinical studies have shown that gut inflammation can impair both of these networks [2,8], certain key questions remain to be answered. Acute appendicitis is an inflammatory process independent of the genetics and environmental factors often involved in other inflammatory GI disorders (eg, inflammatory bowel disease). In addition, there are recent reports [7,9] that suggest that the human appendix represents a useful model for the study of motility disorders. Using acutely inflamed appendix as a model for gut inflammation, we attempt to clarify some of the current questions concerning ICC and nerve pathophysiology in acute inflammation and recovery from inflammation. First, we want to determine whether the loss of c-Kit immunoreactivity (c-Kit IR) during inflammation represents a complete loss of ICC or a loss of the c-Kit receptor from the ICC membrane and intracellular compartments [10]. Second, using the different stages of appendix inflammation, we want to quantify the relationship between the degree of inflammation and severity of damage of neuronal and ICC networks. Third, and most important, we want to assess the capacity of ICC to fully recover after the cessation of inflammation.

2. Methods

2.1. Study group

Sections from 61 appendix specimens (10 control/normal: average, 5.7 years old; SD, 5.9; range, 3 days to 16 year-olds; 34 acute appendicitis: average, 8.5 years old; SD, 4.1; range, 3-17 years old; 17 interval appendices: average, 9.5 years old; SD, 4.3 years; range, 3-17 years old) were obtained at the Children's Hospital of Eastern Ontario (CHEO) from November 2006 to June 2008.This study was approved by our institutional review board and the CHEO Research Ethics Committee. Informed consent was obtained from the patient's family to take segments of resected appendix for immunohistologic and ultrastructural evaluation. The specimens were classified into groups. Table 1 shows the sample size for each category (10 controls, 34 acute appendicitis, and 17 interval appendices). In addition, the acute appendicitis group was divided into subgroups according to macroscopic description and histologic (hematoxylin and eosin) findings (Table 2) [11].

Table 1 Appendicitis group characterization

Groups	Diagnosis	n
Normal/control	Intussusception	1
	NEC stricture	2
	Malrotation	3
	Small bowel obstruction	2
	Meconial ileus	1
	Incidental appendectomy	1
Acute appendicitis	Simple	8
	Suppurative	17
	Gangrenous perforated	9
Interval appendices [a]		17
Total		61

Normal/control: male, n = 6; female, n = 4; average, 5.7; SD, 5.9; 3 days to 16 years. Acute appendicitis: male, n = 19; female, n = 15; average, 8.5; SD, 4.1; 3 to 17 years. Interval appendicitis: male, n = 10; female, n = 7; average, 9.5; SD, 4.3; 3 to 17 years.

[a] Patients with acute appendicitis, stable, and with delayed consultation (4-5 days) to surgery. The initial treatment consists of intravenous antibiotics and elective appendectomy 6 weeks or more (according to institutional protocols) after the inflammation has subsided.

2.2. Immunohistochemistry

Cross-sections were prepared from tissue obtained from the base of appendix specimens, immersed for 2 hours in 4% paraformaldehyde fixative containing 7% (vol/vol) saturated picric acid. Tissues were cryoprotected by immersion in 10% sucrose in 100 mmol/L phosphate buffer for at least 48 hours. Ten-micrometer-thick cryostat sections were thaw mounted onto gelatin-coated slides. Sections were stained with

Table 2 Macroscopic criteria for normal and acute appendices classification

Type	Macroscopic criteria
Normal/control	Compressible and non hyperemic surface No periappendicular fluid
Simple	No compressible appendix with a hyperemic surface Periappendicular, thin inflammatory fluid may be present
Suppurative	Appendix surface is covered by a fibrinous exudate with thickness of the mesoappendix Thickening of the fluid periappendicularly and in the Douglas sac
Gangrenous perforated	Green and gray areas on the appendix surface, equivalent to micro perforations (gangrenous) or obvious macroscopic perforation of the appendix (perforated) Presence of frank pus and/or intraabdominal abscesses