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Original contribution

Megacolon in Chagas disease: a study of inflammatory cells, enteric nerves, and glial cells☆

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Summary After acute infestation with the Chagas disease parasite, *Trypanosoma cruzi*, some patients who are serologically positive develop chronic megacolon and megaesophagus, whereas others are symptom-free. Chagas disease with gastrointestinal involvement involves an inflammatory invasion of the enteric plexuses and degeneration of enteric neurons. It is known that glial cells can be involved in enteric inflammatory responses. The aims were to determine the nature of any difference in lymphocytic invasion, enteric neurons, and enteric glial cells in seropositive individuals with and without megacolon. We have compared colonic tissue from serologically positive individuals with and without symptoms and from seronegative controls. Subjects with megacolon had significantly more CD-57 natural killer cells and TIA-1 cytotoxic lymphocytes within enteric ganglia, but numbers of CD-3 and CD-20 immunoreactive cells were not significantly elevated. The innervation of the muscle was substantially reduced to about 20% in megacolon, but asymptomatic seropositive subjects were not different to seronegative controls. Glial cell loss occurred equally in symptomatic and unaffected seropositive subjects, although the proportion with glial fibrillary acidic protein was greater in seropositive, nonsymptomatic subjects. Development of megacolon after acute infection with *T cruzi* is associated with maintained invasion of enteric ganglia with cytotoxic T cells and loss of muscle innervation, but changes in glial cell numbers are not associated with progression of enteric neuropathy.

1. Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is an endemic cause of morbidity and mortality in areas of Central and South America [1]. There are many immigrants to the United States and other

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countries from these areas who are chronically infected. A current estimate suggests that there are approximately 100 000 infected individuals in the United States [2].

Acute Chagas disease is characterized by fever and myocarditis related to intracellular parasitism. Usually, these symptoms subside spontaneously. Most patients then remain serologically positive, but asymptomatic, for the rest of their lives. However, some patients, after a prolonged asymptomatic period (20-30 years), can exhibit chronic Chagas disease, which is usually manifested by cardiomyopathy and gross enlargement of the tubular structures of the gastrointestinal system, particularly megaesophagus and megacolon [3,4].

In megacolon, motility disturbances are associated with the enlargement of the colon and constipation. The rectum and the sigmoid colon are the most compromised segments [5], which exhibit striking luminal enlargement and muscular hypertrophy. Inflammatory lesions in the enteric nervous system (ENS) are associated with a substantial reduction in the number of neurons. This loss of neurons has been thought to underlie the clinical findings in megasyndromes [6].

Information is still scarce regarding changes in the ENS after *T cruzi* infection and the potential role of immune cells in the development of chagasic megacolon. It is possible that there is a chronic inflammation of enteric ganglia in the infected subjects who develop late gastrointestinal symptoms, but this possibility has not been tested. Furthermore, because of the reaction of enteric glia when the enteric ganglia are inflamed [7], these cells are a potential intermediate in the effects on enteric neurons. We have therefore compared, quantitatively and qualitatively, the presence of inflammatory cells in enteric ganglia, changes in enteric innervation of the muscle, and enteric glial cell numbers in chagasic patients with and without megacolon.

2. Materials and methods

2.1. Patients and samples

Samples of colon wall tissue were obtained from 8 chagasic patients with megacolon, 8 chagasic patients without megacolon, and 10 control individuals submitted to necropsy or surgical procedures at Faculdade de Medicina do Triângulo Mineiro (Uberaba, Minas Gerais, Brazil). The affected area of colon samples from chagasic patients with megacolon was selected (rectum-sigmoid region), whereas in the other groups, the equivalent region was collected. Patients did not receive any parasite-specific treatment. Informed consent was obtained from the patient or family members before tissue procurement. This work was approved by the Universidade Federal de Minas Gerais Research Ethics Committee.

Results of serological tests indicative of Chagas disease (complement fixation, hemagglutination, and immunofluorescence tests) were positive in all patients studied. All of the patients had left the endemic area more than 20 years before the tissue collection; and during that time, patients without megacolon did not present any symptom related to digestive disease. All patients originated from Uberaba, Minas Gerais, Brazil, where the natural transmission of Chagas disease was interrupted more than 20 years ago; and they had never received blood transfusions. The patients with and without megacolon had mean ages of 57 $\pm$ 10 and 55 $\pm$ 14 years, respectively. The presence of megacolon was established based in clinical data reporting colon obstruction and from radiological studies. Manometric studies of megacolon demonstrated decreased peristalsis and incomplete relaxation of the anal sphincter. These abnormalities precede clinical symptoms and dilatation seen by radiographic studies.

The control group was composed of noninfected individuals, as indicated by negative serology specific for Chagas disease. Noninfected individuals were also from the state of Minas Gerais and had a mean age of 54 $\pm$ 20 years.

2.2. Histology and peroxidase immunohistochemistry

Tissue samples were collected from the rectosigmoid region. Each specimen was fixed in 4% neutral buffered formaldehyde solution and embedded in paraffin for immunohistochemistry studies. Sections of 7 μm were deparaffinized using xylene and rehydrated through graded alcohols. Some sections were then stained for standard histology using hematoxylin and eosin (H&E), whereas others were prepared for immunohistochemistry. For immunohistochemistry, endogenous peroxidase was inhibited by incubation with 1% hydrogen peroxide and 30% absolute methanol for 30 minutes. The slides were then incubated with 2% normal swine serum (Sigma, St. Louis, MO) in phosphate buffered saline for 15 minutes and subsequently with the following monoclonal antibodies: anti–PGP 9.5 (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1000) for neural fibers, anti–S-100 and anti-GFAP (Santa Cruz Biotechnology, 1:2000; DAKO, Carpinteria, CA; 1:500) for glial cells, anti-CD3 for T lymphocytes, anti-CD20 (DAKO, USA, 1:100) for B lymphocytes, anti-CD57 (Santa Cruz Biotechnology, clone sc-6261, 1:200) for natural killer (NK) cells, and anti–TIA-1 (Santa Cruz Biotechnology, clone sc-1751, 1:200). After this, the tissue sections were incubated with peroxidase-conjugated rabbit antimouse antibodies (DAKO) for 45 minutes; and peroxidase activity was detected by incubation with 3,3'-diaminobenzidine (Sigma) and hydrogen peroxide for 10 minutes. Slides were counterstained with Gill hematoxylin (Sigma), dehydrated in graded alcohols, and mounted in synthetic mounting media. Negative control slides without primary antibody were performed for each case.

2.3. Cell quantification and morphometric studies

Enumeration of glial cells was performed in the submucosal and myenteric plexuses, whereas the counting