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Original contribution

Crypt apoptotic body counts in normal ileal biopsies overlap with graft-versus-host disease and acute cellular rejection of small bowel allografts☆

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Summary Crypt apoptosis in intestinal epithelium is an important diagnostic feature of graft-versus-host disease (GVHD) and acute cellular rejection (ACR) of intestinal transplants (ITx). In ITx pathology, 2 or fewer apoptotic bodies in 10 consecutive crypts are considered normal, whereas 6 or more is consistent with mild ACR. The presence of 3 to 5 apoptotic bodies is problematic and is often classified as indeterminate for ACR. The minimum diagnostic threshold for GVHD is controversial but also depends on the apoptotic body count (ABC). We investigated how many crypt apoptotic bodies could be identified in histologically normal ileal biopsies from healthy subjects (native intestines, no bone marrow transplant) who underwent screening colonoscopy and had ileal biopsy to confirm complete colonoscopy. We recorded the number of biopsy pieces per specimen and the maximum ABC in 10 consecutive crypts. Twenty-six of 40 patients (65%) had an ABC of 3 or more in 10 crypts, thus only 35% were "normal." Four (10%) had an ABC of ≥6 (positive for ACR). Twenty-two (55%) had 3–5 (indefinite for ACR). Depending on the criteria, up to 60% of the cases could be diagnosed as positive for GVHD.

1. Introduction

Crypt apoptotic bodies in intestinal epithelium may be a rare finding, owing to the crypts being a proliferative zone. They are often focal, small, and histologically subtle, requiring a meticulous high-power examination of multiple levels. Furthermore, their importance in diagnostic pathology is limited to a few rare situations, particularly acute cellular rejection (ACR) of an intestinal allograft, graft-versus-host disease (GVHD), and some medication reactions (eg, mycophenolic acid) [1]. So, considering that crypt apoptotic bodies are both difficult to identify and usually irrelevant, most pathologists do not search for them unless there is a specific indication to do so, and thus our experience with them may be limited, and we may underestimate their prevalence. However, in cases

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of ACR and GVHD, their identification in a biopsy specimen can have a determinative impact on patient care.

Intestinal transplantation (ITx) is a useful therapeutic option for patients with intestinal failure (IF). Based on the Organ Procurement and Transplantation Network annual report data, 106 ITx and multivisceral transplants were performed within the United States in 2012 [2]. ITx is indicated when the continued use of parenteral nutrition, the mainstay of therapy for IF, has resulted in life-threatening complications, such as multiple blood stream microbial infections, loss of venous access, and intestinal failure–associated liver disease [3]. The major causes of IF for adults include short gut syndrome due to ischemia (porto-mesenteric venous thrombosis), volvulus, Crohn's disease, trauma, low-grade neoplasms (tumors involving the mesenteric root, ie, desmoid tumors) and motility disorders. The major causes of IF for children include motility disorders and short gut syndrome due to gastroschisis, necrotizing enterocolitis, volvulus and intestinal atresia. Rare diseases such as microvillous inclusion disease and tufting enteropathy may also be treated by ITx [4]. Retransplantion after graft failure is an emerging indication [5].

Acute cellular rejection is a major complication following ITx, affecting 44% of recipients within 2 years of transplantation [2]. The diagnosis of ACR is partially based on clinical symptoms and endoscopic findings; however, the main component is histopathologic evaluation of allograft biopsies. The guidelines for histopathologic diagnosis of ACR in the intestinal allograft include three components: crypt epithelial cell apoptosis, epithelial cell injury, and inflammation [6]. The grading system proposed at the VIII International Small Bowel Transplant Symposium is widely used today and ranges from Grade 0 (no evidence of ACR) to Grade 3 (severe ACR). These grades encompass a spectrum of changes in each component, with each grade indicating increased severity. Up to 2 apoptotic bodies in 10 crypts is considered normal, while 6 or more is compatible with mild ACR. Three to 5 is considered indeterminate [6]. While epithelial injury may be subtle or focal and mixed inflammation can be difficult to assess in a heavily immunosuppressed population (particularly in an immunologically active part of the body), crypt apoptosis is fairly objective and quantifiable [7]. Therefore, crypt apoptosis tends to be treated as a cardinal feature of ACR (authors' personal observations:S.M.L., H.E.R.).

A study published by Lee et al. found a mean apoptotic body count (ABC) per 10 crypts of 0.2 (with a range of 0–2) in native small bowel mucosa from 45 patients who underwent orthotopic small bowel transplantation [8]; however, when evaluating clinical biopsies for apoptotic bodies, one searches for the region with the highest density of ABC [9]. In practice, one can often identify at least focal areas with an ABC higher than the strict criteria for normalcy [3], but below the threshold for grade 1 ACR [8]. In these instances, one is left with the diagnosis of indeterminate for ACR.

Graft-versus-host disease is another entity in which the histopathologic diagnosis relies heavily on the ABC. GVHD is seen predominantly in patients following bone marrow transplantation and may show variable crypt epithelial apoptosis, crypt or basilar gland destruction, and mucosal denudation of the gastrointestinal tract. The minimal criteria for diagnosis is controversial, however, National Institute of Health (NIH) guidelines recommend a value greater than or equal to one crypt apoptotic body per biopsy piece [10]. Others (Lin et al.) have argued that 6 apoptotic bodies in 10 consecutive crypts are much more specific for GVHD [11]. In between the liberal NIH criteria and the more restrictive Lin criteria, is the German-Austrian-Swiss GVHD Consortium, which defined the minimum diagnostic threshold as 2 apoptotic bodies in one 10× field [7].

The purpose of this study is to carefully examine histologically normal terminal ileal biopsies from outpatients who had a normal screening colonoscopy (biopsied to ensure complete colonoscopy) to quantify the maximal crypt ABC in terminal ileal mucosa.

2. Materials and methods

Following approval of the Columbia University Institutional Review Board, our pathology database was searched from 2013–2015 for terminal ileal biopsies. Forty consecutive outpatients undergoing screening colonoscopy for colorectal cancer with no known gastrointestinal diseases, no reported gastrointestinal complaints, and histologically normal biopsies (by original diagnosis and re-review by a gastrointestinal pathologist, S.M.L.) were included in the study. Several cases were excluded secondary to focal, likely non-specific, neutrophilia and replaced with the next case chronologically. The minimum criteria for an apoptotic body were defined similar to the German-Austrian-Swiss Consortium definition (shrunken cells with condensed nuclear chromatin and cytoplasmic hyperchromasia, or multiple fragments of karyorrhectic debris in a localized area) [7]. This definition of apoptosis has been termed the "liberal" definition by a group studying reproducibility in small bowel allograft biopsy interpretation [12]. Each case was examined on 6 levels (each with a serial section) for a total of 480 representations from 40 patients. Each piece was screened at 20× magnification. Areas with the greatest concentration of apoptotic cells were evaluated at 40× magnification, and the maximum density of crypt apoptosis in 10 consecutive crypts was counted in 2 areas (maximum ABC and next highest) by an experienced gastrointestinal pathologist (S.M.L.). A second experienced gastrointestinal pathologist (H.E.R.) verified the maximum ABC, and in cases of disagreement the lower value was recorded. As a control group, we calculated the average ABC from ileal biopsies in patients diagnosed with GVHD (7) and indeterminate for ACR (18).

3. Results

Of the 40 cases selected, the average patient age was 58 years with 52% being female. All biopsies had preserved villous architecture, no pseudopyloric metaplasia and no