Online Case Report

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Antibiotic-Associated Apoptotic Enterocolitis in the Absence of a Defined Pathogen: The Role of Intestinal Microbiota Depletion*

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Objective : Antibiotic therapy is a major risk factor for the development of diarrhea and colitis with varying severity. Often the origin of antibiotic-associated gastrointestinal deterioration remains elusive and no specific infectious agents could be discerned.

Patients: We represent three cases of intractable high-volume diarrhea associated with combined antibiotic and steroid therapy in critically ill patients not fitting into established disease entities. Cases presented with severe apoptotic enterocolitis resembling acute intestinal graft-versus-host-disease. Microbiologic workup precluded known enteropathogens, but microbiota analysis revealed a severely depleted gut microbiota with concomitant opportunistic pathogen overgrowth.

Interventions: Fecal microbiota transplantation, performed in one patient, was associated with correction of dysbiosis, rapid clinical improvement, and healing of enterocolitis.

Conclusions: Our series represents a severe form of antibiotic-associated colitis in critically ill patients signified by microbiota depletion, and reestablishment of a physiologic gastrointestinal microbiota might be beneficial for this condition. (*Crit Care Med*; 45:e600–e606)

Key Words: antibiotic-associated colitis; epithelial apoptosis; fecal microbiota transplantation; high-volume diarrhea; microbiota depletion

Diarrhea and colitis are frequent complications of antibiotic treatment, particularly in the critically ill (1, 2). Besides direct drug-induced toxicity of antibiotics, depletion of the endogenous microbiota and subsequent pathogen overgrowth are major disease causes, exemplified by colitis due to *Clostridium difficile* and *Klebsiella oxytoca* (3, 4). Importantly, in many cases of antibiotic-associated colitis (AAC), no causal pathogens can be identified (5). In addition, immunosuppression sets individuals at risk for diarrhea and gastrointestinal inflammation (6, 7). A balanced interaction between the intestinal microbiota and mucosal immune

system is required for homeostasis of the gastrointestinal tract (8). Thus, combined antibiotic and immunosuppressant therapies may disturb microbiota-gut homeostasis even more than individual treatments. In this context, we report on a series of severe apoptotic enterocolitis of critically ill patients following combined treatment with broad-spectrum antibiotics and steroids. Cases showed a severe depletion of the endogenous gastrointestinal microbiota, and the condition might be improved by restoration of a physiologic microbiota, for example, by means of fecal microbiota transplantation (FMT).

CASE DESCRIPTIONS

Case A, a 28-year-old woman was hospitalized for fever of unknown origin and subsequently admitted to the medical ICU for systemic inflammatory response syndrome (SIRS). Prior to ICU admission, 100 mg/d prednisolone was initiated because of suspected Still's disease. SIRS persisted and several empiric courses of antibiotics were given. No infectious etiology for SIRS was detectable. Diarrhea started on ICU day 15, the patient still being under steroid therapy, and aggravated to peak stool volumes of up to 4.4 L/d. Diarrhea was accompanied by severe vomiting resulting in inability for oral nutritional intake for 14 days. Cessation of antibiotics and steroid tapering was accompanied by a decrease of diarrhea. The patient clinically improved and was transferred to the normal ward to receive fluid and electrolyte replacement therapy for 2 further weeks. Diarrhea subsided after 45 days.

Case B, a 46-year-old man with rheumatoid arthritis was hospitalized for methotrexate-induced pneumonitis. He was admitted to the medical ICU due to acute respiratory distress syndrome (ARDS) and SIRS to receive mechanical ventilation and antibiotic therapy. No infectious etiology for ARDS or SIRS was detectable. Glucocorticoid therapy initiated 8 months ahead was stopped at hospital admission but recommenced to 50 mg/d prednisolone upon ICU admission. On ICU day 25, massive vomiting and watery diarrhea developed, which reached peak volumes of up to 6 L/d. Despite discontinuation of steroids and changes in the antibiotic therapy regimen, severe diarrhea persisted and the patient died from multiple organ failure at ICU day 66.

Case C, a 16-year-old girl was admitted to the neurosurgical ICU after multiple trauma including severe head injury subsequent to a car accident. Dexamethasone (80 mg/d) and antibiotic therapy were initiated. On ICU day 11, diarrhea started and culminated in stool volumes of 7.2 L/d on ICU day 39 accompanied by high gastric residue with inability of adequate enteral nutrition. Conservative approaches including probiotic supplementation administered over 42 days did neither lead to reduced diarrheal volumes nor to endoscopic or histologic improvement. Seventy-two days after onset of diarrhea, FMT was performed according to a recently described protocol on compassionate use basis (9). Donor feces was provided by the patient's mother, and a total of 400 mL of fecal solution was instilled into the ileum and colon by ileocolonoscopy. Two days after FMT, diarrhea improved with significantly decreased stool volumes (1 L/d), leading finally to full clinical and histologic recovery (last follow-up 97 wk after FMT).

Detailed descriptions of the individual disease courses and therapies applied are shown in **Fig. S1** (Supplemental Digital Content 1, http://links.lww.com/CCM/C408) and **Tables S1** and **S2** (Supplemental Digital Content 2, http://links.lww.com/CCM/C409). Microbiologic workup included repeated testing for *C. difficile* (polymerase chain reaction for *C. difficile* toxin A and B, enzyme-linked immunosorbent assay *C. difficile* toxin A), *K. oxytoca*, campylobacter, salmonella, yersinia, shigella, Enterohemorrhagic *Escherichia coli* in stools of all cases and testing for viruses in biopsies and blood (**Tables S3** and **S4**, Supplemental Digital Content 2, http://links.lww.com/CCM/C409). All these tests did not reveal any enteropathogen in any case. Additional microbiologic investigations done are shown in **Table S5** (Supplemental Digital Content 2, http://links.lww.com/CCM/C409).

METHODS

Details for methods used are given in the **supplemental material** (Supplemental Digital Content 1, http://links.lww.com/CCM/C408). Mucosal biopsies and stool samples for microbiota analyses (cases B and C) were frozen at –20°C until further processing. 16S rRNA gene pyrosequencing data have been deposited in the European Nucleotide Archive under the accession number ERP013256. Investigations were approved by the review board of the Medical University of Graz (EK-23-212-ex-10/11). Informed consent for FMT (compassionate use) was obtained from the patient's guardian.

RESULTS

Endoscopic and Histopathologic Findings in Antibiotic-Associated Apoptotic (AAA) Enterocolitis

All patients underwent repeated gastro-duodenoscopies and colonoscopies. At presentation, a severe inflammation was evident in the small and large intestines (**Fig. 1**) (**Fig. S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/C408). Macroscopically, segmental loss of folds and large erosions were seen in the duodenum and terminal ileum of all patients, whereas colonic manifestations varied from mosaic-like patterns to large "geographic" ulcerations and areas with a completely denuded mucosa. The histopathologic hallmark feature was epithelial mass apoptosis reminiscent of severe acute graft-versus-host-disease (GvHD) leading to (sub)total crypt losses and moderate-to-severe segmental villus blunting in the small intestines, colonic features ranged from individual crypt losses to large areas with completely lost epithelium (Table S4, Supplemental Digital Content 2, http://links.lww.com/CCM/C409). Goblet and Paneth cells were detectable in all cases, no signs suggestive of viral infection were evident, and repeated testing of biopsies for viruses was negative (Table S4, Supplemental Digital Content 2, http://links.lww.com/CCM/C409). The lamina propria showed a moderately dense mixed infiltrate, $CD8^+$ T cells were significantly increased in the colon, and $CD4^+$ T cells concomitantly reduced (CD4-to-CD8, 1:19; normal, 3:1). Regulatory T cells were not changed compared

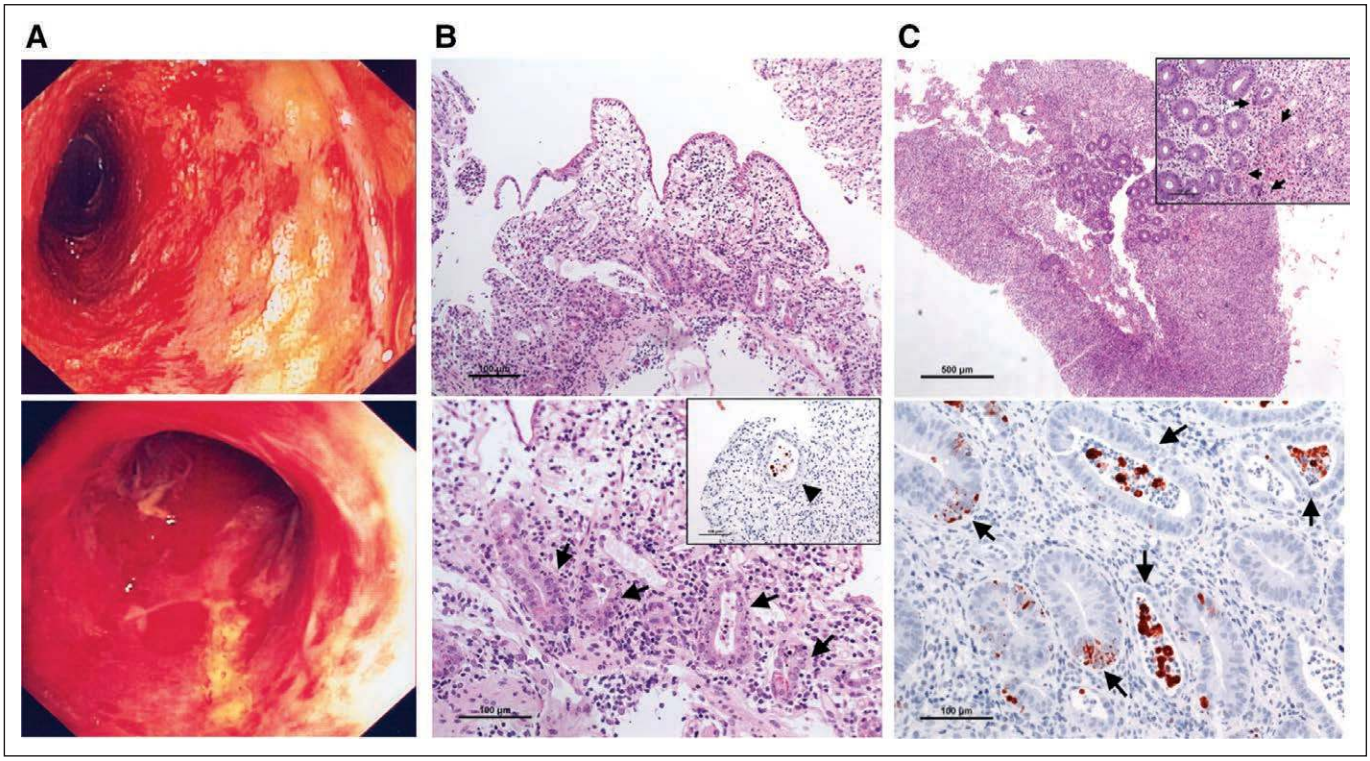

Figure 1. Endoscopy and histology in antibiotic-associated apoptotic (AAA) enterocolitis. (**A**) Hemorrhagic inflammation with loss of folds in the duodenum (*top*). Complete loss of vascularization, "geographic" ulcerations with abundant fibrin and denudation in the colon (*bottom*). (**B**) Segmental loss of crypts and villi and abundant apoptoses (*arrows*) in the duodenal mucosa (inset: activated caspase-3 positivity). (**C**) Large areas completely devoid of crypts and surface epithelium in the colon (inset: crypt epithelial apoptosis). Activated caspase-3 positive crypt epithelial cells and cell debris (*bottom*). Images: A (*top*) and B, case A onset of diarrhea; A (*bottom*), case C 45 d after diarrhea onset; C (*top*), case C 15 d after diarrhea onset; C (*bottom*) and inset C, case C 17 d after onset of diarrhea.

with healthy controls (**Fig. S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/C408).

Microbiota Profile in AAA Enterocolitis

The clinical findings in the index patient (case A) prompted us to perform microbiota analyses in cases B and C subsequently. Fecal and mucosal specimens collected during acute disease showed a severely depleted microbiota characterized by significantly reduced richness, diversity, and microbial load, as well as an overabundance of Proteobacteria (99.9% ± 0.1% in case B, 98.6% ± 1.9% in case C), and minimal fractions of Firmicutes (0.02% ± 0.03%) and Bacteroidetes (0.01% ± 0.02%) (**Fig. 2**, ***A–C***) (**Fig. S4**, Supplemental Digital Content 1, http://links.lww.com/CCM/C408). Interestingly, microbiota underwent extreme compositional shifts over time. In case B, concentrations of a single *Pseudomonas* taxon shifted from 98.8% on day 2 to 2% ± 0.5% on day 24, with the latter time point being dominated by an unclassified *Enterobacteriaceae* (97.5% ± 0.9%). In case C, samples were dominated by different strains from an administered probiotic (*Lactobacillus* species, 60.4% ± 18.7% on day 33; *Enterococcus* species, 52.6% ± 9.5% on day 46), as well as typical skin commensals (*Staphylococcus epidermidis* 34.5% ± 10.4% and *Propionibacterium acnes* 1.8% ± 3.2%). After cessation of probiotics *Haemophilus parainfluenzae*, a Proteobacterium typically colonizing the oropharynx, accounted for 74.2% ± 21.2% (Fig. 2*A*). Taken together, these dramatic and apparently random fluctuations in gut microbiota composition are suggestive of a lost intrinsic colonization resistance of the gut microbial ecosystem (10).

Attenuation of Severe Dysbiosis by FMT Was Associated With Resolution of Symptoms and Mucosal Healing in a Patient With AAA Enterocolitis

Case C clinically deteriorated, severe diarrhea persisted (2–7 L/d), and endoscopy showed persistent severe inflammation predominantly in the left colon. Given the severe microbiota depletion, FMT was performed on compassionate use basis 72 days after diarrhea onset. The transplant represented a physiologic fecal microbiota (61.7% Bacteroidetes and 37.3% Firmicutes; Fig. 2***D***). After FMT stool volumes continuously declined and sigmoidoscopy showed improvement as early as 7 days after FMT, histologically confirmed by regenerating crypts and a reestablished epithelial lining (**Fig. 3*A***). By day 1 after FMT, *H. parainfluenzae* was considerably reduced in the feces (1.5%). Initially, the patient's fecal microbiota was dominated by *Enterobacteriaceae*, originating from the transplant, followed by reestablishment of a physiologic colonic microbiota dominated by Bacteroidetes and Firmicutes (Fig. 2*D*). Bacterial load in stools significantly increased over time (Fig. 2*C*) although levels of microbial richness and diversity remained reduced compared with controls (Fig. 2*B*) (Fig. S4, Supplemental Digital Content 1, http://links.lww.com/CCM/C408). During the acute phase of disease, short-chain fatty acids (SCFAs) in stools were reduced. After FMT, acetate and

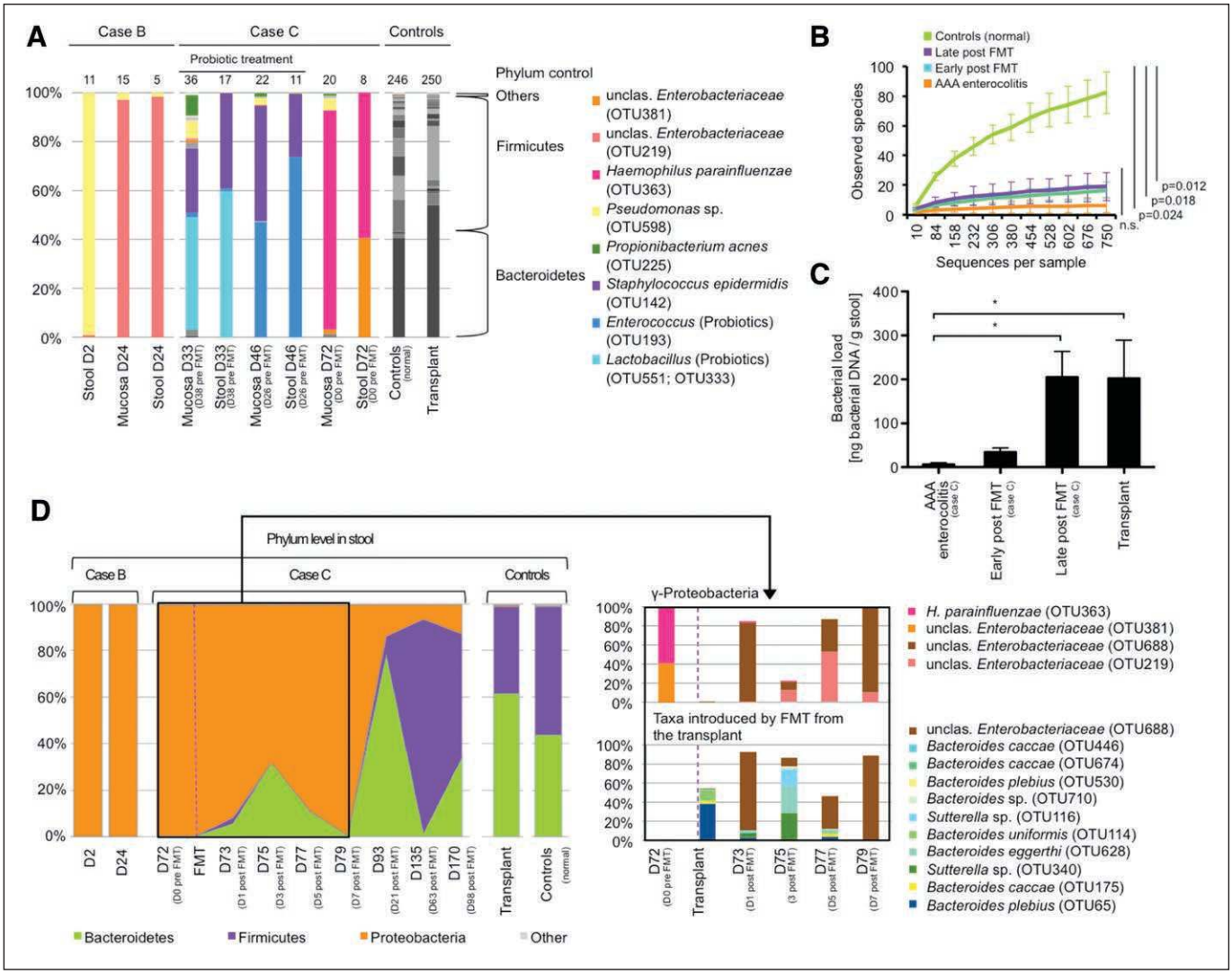

Figure 2. Microbiota depletion and effects of fecal microbiota transplantation (FMT) in antibiotic-associated apoptotic (AAA) enterocolitis. (**A**) Fluctuating taxa during acute disease in cases B and C. A normal colonic microbiota is shown as control (*gray bars*). The numbers above bars indicate number of prevalent taxa. (**B**) Microbial richness is impaired in AAA enterocolitis. Neither probiotic treatment nor FMT is able to restore reduced richness (Two-sample *t* test, 999 Monte-Carlo permutations, Bonferroni-correction, samples were rarefied to 750 sequences per sample; sample designations are given in Table S4). (**C**) Microbial load is significantly reduced and increases after FMT (one-way analysis of variance [ANOVA], Bonferroni posttest; * $p < 0{,}05$). (**D**) Phylum level microbiota dynamics in case C before and after FMT compared with case B and controls (*left*). Operational taxonomic unit (OTU) level analysis indicates removal of *Haemophilus parainfluenzae* after FMT and recolonization with Gammaproteobacteria followed by Bacteroidetes originating from the transplant (*right*).

subsequently propionate levels increased (**Fig. S5**, Supplemental Digital Content 1, http://links.lww.com/CCM/C408) and butyrate levels remained low, consistent with reduced amounts of butyrate-producing genes in the fecal metagenome (**Fig. S6**, Supplemental Digital Content 1, http://links.lww.com/CCM/C408). The CD4-to-CD8 ratio reversed to normal levels in colonic biopsies after FMT; no significant changes in regulatory T-cell counts were detected (Fig. 3***B***). Ninety-seven days after FMT, the gastrointestinal mucosa showed a normal gross appearance (**Fig. S7**, Supplemental Digital Content 1, http://links.lww.com/CCM/C408).

DISCUSSION

We herein describe severe antibiotic-associated enterocolitis in patients treated at the ICU because of a severe underlying disease. All three patients exhibited several common features: 1) all patients were concomitantly treated with broad-spectrum antibiotics and steroids when intractable watery high-volume diarrhea and gastrointestinal failure started with sudden onset; 2) histopathology revealed severe apoptotic enteropathy, reminiscent of acute GvHD in the absence of transplantations; and 3) despite extensive microbiologic workup, no established enteropathogens could be found. The disease course was death in one patient, spontaneous recovery after discharge from the ICU after 2 months in the second patient, and improvement to FMT in the third patient.

Several recent reports indicate that critical illness is often accompanied by gastrointestinal microbiota depletion and severe insult or trauma alone, already favor this condition (11, 12). In our cases, it is likely that the prolonged antibiotic

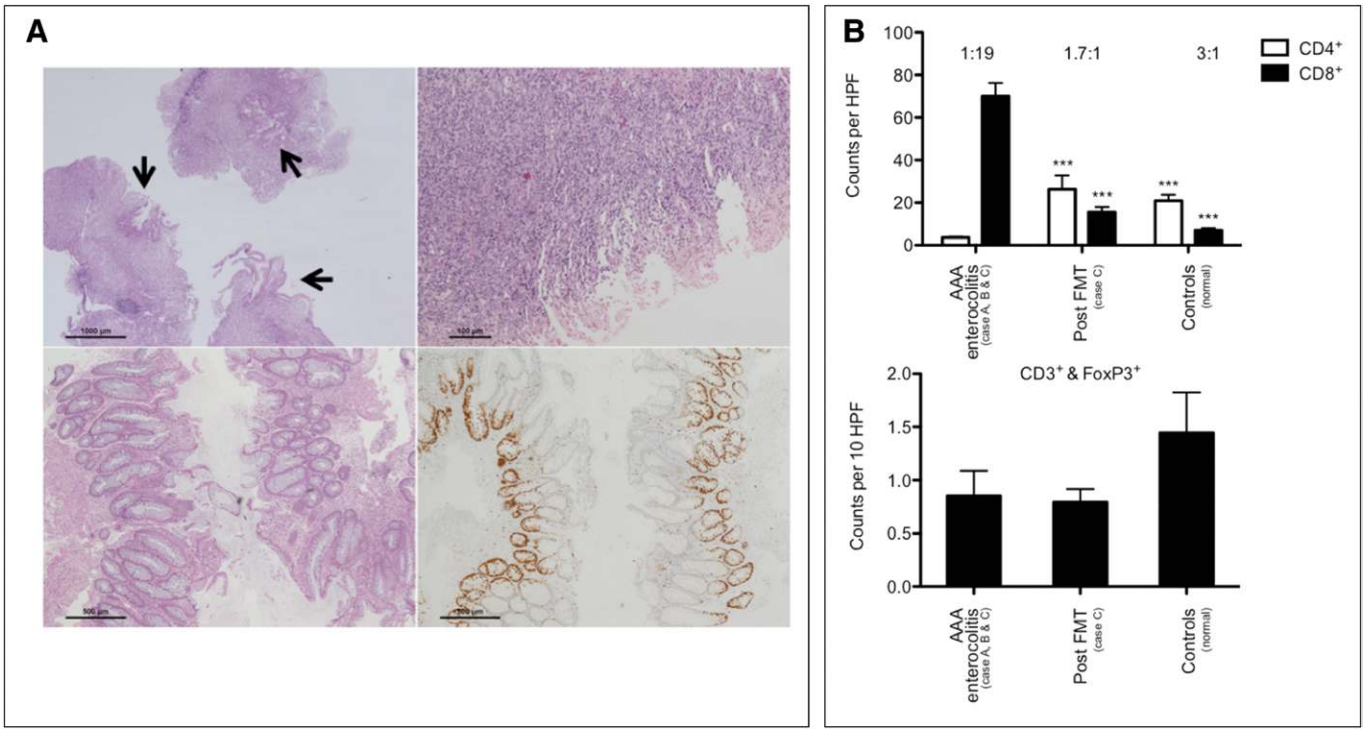

Figure 3. Histopathology and mucosal immunophenotype pre- and post fecal microbiota transplantation (FMT). (**A**) Colon histopathology 12 d ahead (*top left*) and at the day of FMT (*top right*). Only focal residual epithelium is present (*arrows*). Seven days after FMT, the epithelial lining is reestablished (*bottom left*). Ki-67 immunohistochemistry identifies proliferating crypt epithelia (bottom right). (**B**) Significantly increased $CD8^+$ T cells and significantly reduced $CD4^+$ T cells during acute disease in the colon, this immunophenotype is reversible after FMT (*top*); CD4-to-CD8 ratio is given above bars (one-way analysis of variance [ANOVA], Bonferroni-corrected, *** $p < 0.0001$). $CD3^+FoxP3^+$ double-positive Tregs are not significantly altered (*bottom*; one-way ANOVA, $p = 0.2801$). AAA = antibiotic-associated apoptotic, HPF = high power fields.

therapy lead to microbiota depletion but also additional factors like other medications given (e.g., high doses of proton pump inhibitors) might have aggravated the situation (13–15). Importantly, such patients may fail to reestablish a normal microbiota, as they lack direct microbial input from the diet as well as microbial growth substrates (e.g., fiber) due to parenteral nutrition or sterile tube feeding (Table S2, Supplemental Digital Content 2, http://links.lww.com/CCM/C409). A lack of intestinal colonization resistance is especially highlighted by the observation that noncommensal probiotic bacteria could transiently colonize but without altering disease course (16). Depleted ecological niches may predispose for opportunistic pathogen colonization (17). The preferential dominance of facultative anaerobic Proteobacteria might be due to high prevalence of antibiotic resistance in this taxonomic group and due to their ability to proliferate during inflammation and oxidative stress, which has been described also for *Haemophilus*, the opportunist identified in case C (18, 19). Intriguingly, a recent study of antibiotic-induced microbiota depletion in mice showed that the persisting taxon, a *Pseudomonas* similarly as in case B, was able to inhibit mitochondrial gene expression and biogenesis leading to apoptotic epithelial cell death phenotypes similar to our findings (20). In addition, the gastrointestinal pathogenicity of *Pseudomonas* is highlighted also by its ability to cause gut-derived septicemia (21). Furthermore, reduced concentrations of SCFAs, major metabolites of microbial fermentation required for mucosal homeostasis, have been shown to drive mass apoptosis of the gastrointestinal epithelium (22, 23). In case C, SCFAs in stools were highly reduced consistent with reduced amounts of butyrate-producing genes in feces. Thus, direct effects of persisting opportunists like *Haemophilus* or *Pseudomonas*, the lack of SCFAs after microbiota depletion in addition to toxicity of antibiotics might altogether promote epithelial apoptosis and enterocolitis.

Of note, the term "AAA" enterocolitis employed in this report is used in a descriptive manner highlighting the histologic hallmark features but is not specifying a distinct entity. Several other conditions like infections (e.g., cytomegalovirus), immunodeficiencies, autoimmune enteropathy, cord colitis, or thymoma could be accompanied by gastrointestinal epithelial apoptosis; however, the severe phenotype reported here is mainly seen in intestinal GvHD (24, 25). Interestingly, cytotoxic $CD8^+$ T cells were significantly increased in the colon, $CD4^+$ T cells concomitantly reduced during acute disease, whereas regulatory T cells were not changed in cases. A similar immune phenotype is reported for GvHD, which is also signified by severe dysbiosis suggesting parallels in the pathogeneses of GvHD and the enterocolitis observed in our cases (26–28). Of note, the CD4-to-CD8 ratio reversed to normal levels in colonic biopsies in case C after FMT.

AAC constitutes one of the most frequent side effects of antimicrobial therapy with variable clinical presentation (1, 5). Often the cause of AAC remains elusive; thus, new clues about disease pathogenesis are needed. Our series and recent reports

point towards a role of microbiota depletion as a possible contributing factor, wherein overgrowth of opportunistic pathogens and also the lack of microbial metabolites, like SCFAs, might support disease development. Attention to new, alternative disease models for AAC are therefore warranted. Our case series, however, is not able to infer causality between reported findings and the pathogenesis of enterocolitis, since other so far not identified factors might have also contributed. For instance, our standard microbiology and 16S rRNA gene-based microbiota investigations might have missed certain pathogens, such as virus or fungi not captured with the applied methods.

Although FMT seemed to have a beneficial effect on the course of patient C, also other factors could have contributed to improvement such as withdrawal of antibiotics, discontinuation of steroids, and continued supportive care as occurred with patient A. FMT is an established treatment for therapy-refractory recurrent *C. difficile* infection (CDI) (29–31) and is also increasingly used for severe and life-threatening CDI (32–35). Despite application of billions of microorganisms into an inflamed gastrointestinal tract, which poses the danger of initiating gut-derived septicemia, short-term infectious complications seem to be relatively rare (36, 37). Even in immunocompromised patients, only few side effects were reported (38). Dangers are related mostly to the application form of FMT (36, 37), like aspiration of fecal suspension after application into the upper gastrointestinal tract, leading to severe pneumonia and death (39, 40). In contrast, application into the lower gastrointestinal tract seems to have a better safety profile (36, 41). Nevertheless, translocation of microorganisms leading to septicemia poses a severe complication that needs to be considered. FMT might also lead to overstimulation of the immune system, which has been recognized as inflammatory bowel disease flares after FMT (42). Although most reported side effects are self-limited, long-term safety data of FMT are missing (34) and physicians should consider this especially if they plan to use FMT as a treatment option in non-CDI indications on a compassionate use basis.

In conclusion, our report outlines severe enterocolitis in critically ill patients associated with antibiotic and steroid treatment. It is characterized by severe apoptosis of the gastrointestinal epithelium that might be the consequence of a deteriorated microbiota-gastrointestinal tract homeostasis. Reestablishment of a physiologic gastrointestinal microbiota, which might not spontaneously happen in the ICU but could be achieved by FMT, possibly relieves this condition (29, 43, 44).

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