Common Biostructure of the Colonic Microbiota in Neuroendocrine Tumors and Crohn's Disease and the Effect of Therapy

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Background: The aims were to comparatively investigate the biostructure of colonic microbiota in patients with neuroendocrine tumors and Crohn's disease (CD) and to study the response of the microbiota to therapy.

Methods: Sections of fecal cylinders from 66 patients with neuroendocrine tumors (NET; 25 foregut, 30 midgut, 11 hindgut), 50 patients with CD (Crohn's Disease Activity Index [CDAI] $\geq$150), and 30 patients with chronic idiopathic diarrhea seen at the Charité Hospital and 25 healthy controls were investigated using fluorescence in situ hybridization with probes specific for five bacterial groups: *Faecalibacterium prausnitzii, Clostridium* group XIVa / *Roseburia* group, *Bacteroides, Enterobacteriaceae*, and *Bifidobacteriaceae*.

Results: We found a striking *F. prausnitzii* (Fprau) depletion in the stool of patients with NET of the midgut and patients with CD. The changes of the microbiota in the two other NET groups were uncharacteristic and similar to those observed in patients with chronic idiopathic diarrhea. Fprau depletion was reversible with chemotherapy and with interferon alpha-2b treatment in patients with midgut NET. Somatostatin analogs had no influence on Fprau concentrations.

Conclusions: Patients with NET and CD show similarities in their abnormalities of the fecal biostructure. Interferon alpha and systemic chemotherapy significantly improved the fecal biostructure in patients with midgut NET.

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Key Words: biostructure, colonic microbiota, neuroendocrine tumors, Crohn's disease

Neuroendocrine tumors (NET) of the digestive tract are relatively rare benign or malignant tumors. The WHO classification system is based on differences in morphology, function, and clinical behavior.[1] According to the German Neuroendocrine Tumor Registry (2004–2007), the location of the primary tumor was in the digestive system in 79%, 14% were cancers of unknown primary, 5% in the lungs, and in 3% the location was not reported.[2]

The impact of NET on colonic microbiota has not been systematically investigated thus far. Within the large intestine, the bacterial growth is facilitated and concentrations of 10^{12}/mL are usual. In disease, however, bacterial growth and fermentation are impaired.[3–6]

Although the composition of the colonic microbiota is extremely diverse, three groups of bacteria, *Faecalibacterium prausnitzii* (Fprau), *Clostridium* group XIVa / *Roseburia* group (Erec), and *Bacteroides* (Bac) compose together 60%–90% of the bacterial mass.[5,7] *F. prausnitzii, Bacteroides*, and *Clostridium* group XIVa / *Roseburia* group are obligatorily present and distributed web-like throughout the stool cylinder; therefore, we called these groups habitual bacteria.[5] All other bacterial groups are present only occasionally. The composition of occasional bacterial groups is individual for each person.

A depletion of *F. prausnitzii* (Fprau; $<1 \times 10^9$/mL) was found in patients with Crohn's disease (CD), in patients with untreated celiac disease with severe enteropathy, and 12 patients with carcinoid tumors, but in none of the other disease controls.[6,8] Because of this depletion, we investigated whether the concentrations of Fprau and four other colonic microbiota differ in patients with NET, depending on the primary location or are influenced by therapy.

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MATERIALS AND METHODS

Subjects

Sixty-six inpatients and outpatients with neuroendocrine tumors were recruited at the Charité University Hospital in Berlin, Germany. The diagnosis of NET was confirmed by clinical and biochemical parameters and typical histology including immunohistochemistry.[1] Neuroendocrine differentiation was substantiated in all cases by immunohistochemical positivity for synaptophysin and in 81.8% for chromogranin A. Fourteen NETs were histopathologically classified according to the revised Capella classification as well-differentiated NET with benign or uncertain behavior, 46 as well-differentiated neuroendocrine carcinoma (NEC) with low-grade malignant behavior, and six as poorly-differentiated NEC with high-grade malignant behavior.[9]

The histopathological diagnosis was made from the primary tumor (surgical specimen or biopsy) in 49 and from the biopsy of metastases in 17 cases. The 66 patients were divided into three groups according to the origin of their primary tumor in the embryonic gut: 25 with NET of the foregut, 30 with NET of the midgut, and 11 with NET of the hindgut. The clinical data for the organ specific locations are shown in Table 1.

T1

A total of 28 NET patients (42%) suffered from functional syndromes at the initial diagnosis. The most frequent functional syndrome was carcinoid syndrome. Metastases were observed in 52 patients (79%). Distant metastases were present in 43 patients (65%), while a single local-regional lymph node metastasis was found in nine patients (14%). Fifty-one patients underwent at least one surgical intervention of their primary tumor and/or metastases. Twenty-seven patients received somatostatin analogs, 13 systemic chemotherapy, three tyrosine kinase blockers, seven radiofrequency ablation, and three selective arterial chemoembolization of liver metastases. Eleven patients with NET of the midgut received five million units interferon alpha-2b three times per week or PEG-interferon 80–100 μg once a week for at least 6 months.

The healthy control group consisted of 25 volunteers. They were either laboratory or medical staff and their relatives without intestinal complaints or known diseases. A noninflammatory control group consisted of 30 patients with chronic idiopathic diarrhea.

The study included 50 patients with CD of the small intestine and/or colon with moderate activity (Crohn's Disease Activity Index [CDAI] $\geq$150 to $\leq$400). The CD activity score was calculated as previously published.[10] Each CD patient had a complete gastroenterological diagnostic work-up including colonoscopy, gastroscopy, ultrasound, and laboratory investigation. CD was diagnosed according to accepted criteria.[11] Twenty-five patients with CD had stool collected prior to therapy and 25 while receiving oral therapy with azathioprine for >12 weeks at the time of stool sample collection.

TABLE 1. Clinical Data

	Healthy Controls	Chronic Idiopathic Diarrhea	Neuro-endocrine Tumors (all)	Foregut NET	Midgut NET	Hindgut NET	Crohn's Disease (all)	Crohn's Disease Prior to Therapy	Crohn's Disease on Azathioprine Treatment	*P*
Number	25	30	66	25	30	11	50	25	25	
Male/female	9/16	12/18	27/39	10/15	13/17	4/7	20/30	9/16	11/14	
Mean age	47.9	46.1	58.5	57.8	59.9	54.7	39.0	35.4	42.5	
range (years)	18–63	21–77	27–85	30–85	27–79	37–77	19–68	19–47	27–68	
Primary location				12 pancreas 5 stomach 4 duodenum 4 lung	22 ileum 5 jejunum 3 cecum	6 colon 5 rectum				
Ki67			5.35 ± 6.82	8.92 ± 14.29	2.33 ± 2.45	5.72 ± 6.77				F/M = 0.016 M/H = 0.045 F/H ns

Ki67, quantifies the proliferation fraction, expressed as the percentage of neoplastic cells showing nuclear labeling; F, foregut NET; M, midgut NET; H, hindgut NET.

Sample Collection (Stool)

Each participant delivered at least three fecal samples. Twenty-six of the NET patients delivered ≥3 fecal samples prior to therapy and during therapy. Eleven of the patients who suffered from midgut NEC delivered at least six fecal samples prior to and during interferon alpha-2b treatment.

Only samples of CD patients without change in therapy were selected. Stools were either dropped on cleansing tissue or on the dry flat surface part of the toilet, which is typically used in Germany. Pieces of feces (2–5 g) were taken from the stool using a plastic drinking straw with a 3-mm inside diameter (Schlecker, Germany) and dropped into 50-mL Falcon tubes filled with 30 mL of Carnoy solution (6/6/1 vol. ethanol/glacial acetic acid/chloroform). Straws were given to the participants together with instructions how to take the stool and drop it into the Falcon tubes filled with Carnoy solution.[5] Vaseline was used to close the lower end of the straw if the stool was very loose. The upper end of the straw needed to stay open so the solution could reach the stool sample. The fixed stools were kept at room temperature until delivered to the laboratory.

Sample Handling in the Laboratory

In the laboratory the straws with the enclosed fecal cylinder were removed from the Carnoy solution. Then the stool cylinder was removed from the straw and embedded in paraffin using standard techniques, cut into 4-μm sections, and placed on SuperFrost slides (R. Langenbrinck, Emmendingen, Germany) for microscopic examination and fluorescence in situ hybridization (FISH) studies.

FISH

Microscopy was performed using a Nikon e600 fluorescence microscope. The images were photo documented using a Nikon DXM 1200F color camera and software (Nikon, Tokyo, Japan). Hybridizations were performed in multicolor FISH according to previously described protocols for evaluation of tissue specimens and the criteria for identification of bacteria.[12,13]

For each group-specific FISH probe, high-power (×1000 magnification) images were made. Highly concentrated bacteria were counted within a 100 μm^2 area of the microscopic field representative of the region of interest. Bacteria with uneven distribution or overall low concentrations were enumerated within larger areas of 100 × 100 μm^2 or within all microscopic fields. The conversion of the numbers within a microscopic field to concentrations of bacteria per mL was based on the calculation that a 10-μL sample with a cell concentration of 10^7 cells per mL has 40 cells per average microscopic field at a magnification of 1000. The details of this conversion were previously described.[13] The following criteria were evaluated for each sample and group of bacteria: morphologic appearance, species identification, occurrence, concentration and distribution of bacteria, the differences in intensity of the fluorescence signal of single bacterial groups, and the stability in concentrations between the first and the following investigations (range divided by minimum).

FISH Probes

Oligonucleotide probes were synthesized with a fluorescein isothiocyanate, Cy3- or Cy5-reactive fluorescent dye at the 5′ end (MWG Biotech, Ebersberg, Germany). Cy3, Cy5, and DAPI are different fluorescent dyes corresponding to green, orange, dark red, and blue colors.

We selected five group-specific FISH probes for our study. Three represented habitual bacterial groups, which are normally present in each healthy person and contribute each 10 to 50% to the fecal biomass, and together 60%–90% of all fecal bacteria; Fprau (*F. prausnitzii*), Erec482 (*Clostridium* group XIVa / *Roseburia* group), and Bac303 (most *Bacteroidaceae*). In addition, we used two probes representing occasional bacterial groups; Ebac1790 (*Enterobacteriaceae*) and Bif164 (*Bifidobacteriaceae*). Occasional bacterial groups can be absent or can contribute up to 20% of the bacterial biomass in single persons.[5]

Statistical Analysis

All statistical analyses were performed using the statistical software package SPSS v. 15.0 (Chicago, IL), with $P < 0.05$ considered significant. Deviation of each continuous variable from a theoretical normal distribution was assessed through the one-sample Kolmogorov–Smirnov test procedure. Accordingly, because the P value to the Kolmogorov–Smirnov Z-statistic was consistently <0.05, all analyses were performed under the nonparametric assumption. The statistical significance of the differences was tested using the Mann–Whitney U exact test for data following a normal distribution. Statistical significance was accepted if the 2-tailed probability level was <0.05.

Ethical Considerations

All subjects gave informed consent according to the protocol approved by the ethics commission of the Charité University Hospital Berlin, Germany.

RESULTS

The clinical data of the patients and controls are shown in Table 1. The Ki67-labeling index for the proliferating fraction of tumor cells was available in all NET patients. Significantly higher Ki67 indices were seen in patients with foregut NET and hindgut NET compared to midgut NET (Table 1). The patients with NET were significantly older than patients with active CD (mean age 58.5 vs. 39.0 years, $P < 0.01$).

Contribution of Single Bacterial Groups to the Fecal Biomass

Five bacterial groups were evaluated in each of the samples from each of the healthy and diseased persons

TABLE 2A. Bacterial Groups (Mean ± SD Concentrations x10^{10}/mL) and Percent of Patients with Depletion of Fprau

	Healthy Controls	Chronic Idiopathic Diarrhea	All NETs	All Crohn's Disease	
	$n = 25$	$n = 30$	$n = 66$	$n = 50$	P
Fprau	16.4 ± 8.72	8.9 ± 4.5	7.58 ± 7.00	1.14 ± 2.16	Hc/D < 0.001 Hc/NET < 0.001 Hc/CD < 0.001 NET/CD < 0.001
$\leq 0.5 \times 10^{10}$/mL[a]	0%	3%	33.3%	70%	
Erec	25.6 ± 9.70	12.9 ± 7.1	12.42 ± 9.58	16.9 ± 9.11	Hc/D = 0.02 Hc/NET = 0.02
Bac	20.6 ± 8.45	8.8 ± 5.7	14.65 ± 7.13	11.97 ± 7.33	Hc/D = 0.01 Hc/NET = 0.01 Hc/CD = 0.01 Hc/F = 0.033 Hc/M = 0.004
Ebac	0.07 ± 0.18	2.0 ± 3.2	1.56 ± 2.98	1.48 ± 3.21	Hc/D = 0.025 Hc/NET = 0.014 Hc/CD = 0.032
Bif	0.68 ± 0.97	2.0 ± 1.9	2.68 ± 3.26	0.87 ± 1.36	Hc/NET = 0.003 Hc/D = 0.005 NET/CD = 0.0006

Hc, healthy controls; D, chronic idiopathic diarrhea; NETs, neuroendocrine tumors; CD, Crohn's disease.
Nonsignificant differences are not mentioned in the table.
[a]Percent of patients with a mean Fprau concentration of $\leq 0.5 \times 10^{10}$/mL.

using the Fprau (*F. prausnitzii* group), Erec (*Roseburia*/*Clostridium* XIVa group), Bac (*Bacteroides* group), Ebac (*Enterobacteriaceae* group), and Bif (*Bifidobacteriaceae* group) FISH probes. The concentrations of single bacterial groups are listed in Table 2a.

In healthy persons, three groups of bacteria: *F. prausnitzii* group (Fig. 1), *Roseburia*/*Clostridium* XIVa group, and *Bacteroides* group composed each 15%–50% of the microbiota and together 60%–90% of all fecal bacteria (Table 2a). Bacteria of these habitual groups were homogeneously distributed all over the cylinder, with similar fluorescence and concentrations at the center and periphery of feces.

Faecalibacterium prausnitzii was significantly reduced in all patients with diarrhea and all NET patients compared to controls (Table 2a). However, the reduction of Fprau was more pronounced in midgut NET and CD (foregut/midgut NET and midgut/hindgut NET and healthy controls/CD: $P < 0.00001$; Table 2b). A depletion of Fprau throughout the fecal cylinder was found in the patients with CD and patients suffering from midgut NET (Fig. 2a) ($P = 0.22$). Similar depletion of Fprau was not observed in patients with foregut and hindgut NET. There was a striking similarity between midgut NET and CD, suggesting a similarity in the pathogenesis or host response (Table 2b).

A marked reduction of *Bacteroides* was found in both groups with CD, patients with diarrhea, and with NET (Table 2a).

The reduction of the main habitual bacterial groups, represented by *F. prausnitzii, Bacteroides*, and *Roseburia*, was similar for all disease groups and statistically different from healthy controls in chronic diarrhea, all NETs, and partially in CD (Fprau and Bac only) (Table 2a).

The two other investigated bacterial groups were found only in some samples from the same person or they were absent in all samples. The distribution of these occasional bacterial groups was only homogeneous in single cases. In most cases a patchy distribution was seen with islands of highly concentrated bacteria in some regions, with practically no bacteria between these islands. Fecal *Enterobacteriaceae* were significantly increased in all NET groups, patients with diarrhea, and patients with CD compared to healthy controls ($P < 0.04$; Table 2a). There were no significant differences between patients with hindgut and foregut NETs and patients with chronic idiopathic diarrhea (Table 2b).

Increases in occasional bacterial groups, represented by *Enterobacteriaceae* and *Bifidobacteriaceae*, were similar

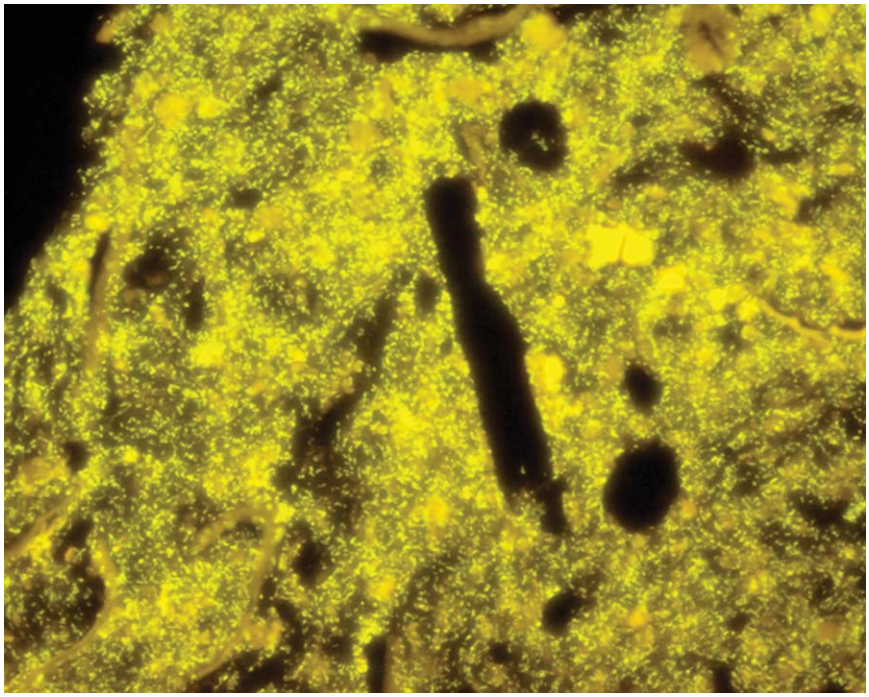

FIGURE 1. Hybridization with the Fprau Cy3 probe (orange fluorescence) of feces from a healthy control at magnification ×400.

for all disease groups and statistically different from healthy controls in chronic diarrhea, all NETs, and partially in CD (Ebac only) (Table 2a).

Influence of Treatment (Table 3)

Somatostatin analogs had no influence on the concentration of habitual or occasional bacterial groups in patients with

TABLE 2B. Bacterial Groups (Mean ± SD Concentrations x10^{10}/mL) and Percent of Patients with Depletion of Fprau

	Healthy Controls	Foregut NET	Hindgut NET	Midgut NET	Crohn's Disease Prior to Therapy	Crohn's Disease on Azathioprine Treatment	
	$n = 25$	$n = 25$	$n = 11$	$n = 30$	$n = 25$	$n = 25$	P
Fprau	16.4 ± 8.72	12.6 ± 5.26	11.13 ± 5.49	2.11 ± 4.37	0.63 ± 1.60	1.65 ± 2.54	F/M = 0.0001 M/H = 0.0001 Hc/M = 0.001
$\leq 0.5 \times 10^{10}$/mL[a]	0	0.04	0.09	0.666	0.84	0.56	
Erec	25.6 ± 9.70	17.23 ± 8.28	11.14 ± 7.47	8.95 ± 9.86	13.15 ± 7.00	20.72 ± 11.98	F/M = 0.009 Hc/M = 0.002
Bac	20.6 ± 8.45	15.65 ± 7.00	14.70 ± 5.79	13.77 ± 7.79	9.3 ± 8.12	13.88 ± 5.19	Hc/F = 0.033 Hc/M = 0.004
Ebac	0.07 ± 0.18	2.44 ± 4.3	0.95 ± 1.24	1.06 ± 1.75	0.91 ± 1.53	2.02 ± 4.22	Hc/F = 0.008 Hc/M = 0.006 Hc/H = 0.001
Bif	0.68 ± 0.97	3.27 ± 3.7	3.05 ± 3.7	2.04 ± 2.71	1.00 ± 1.55	0.77 ± 1.16	Hc/F = 0.001 Hc/M = 0.02 Hc/H = 0.005

Hc, healthy controls; F, foregut NET; M, midgut NET; H, hindgut NET.
Nonsignificant differences are not mentioned in the table.
[a]Percent of patients with a mean Fprau concentration of $\leq 0.5 \times 10^{10}$/mL.

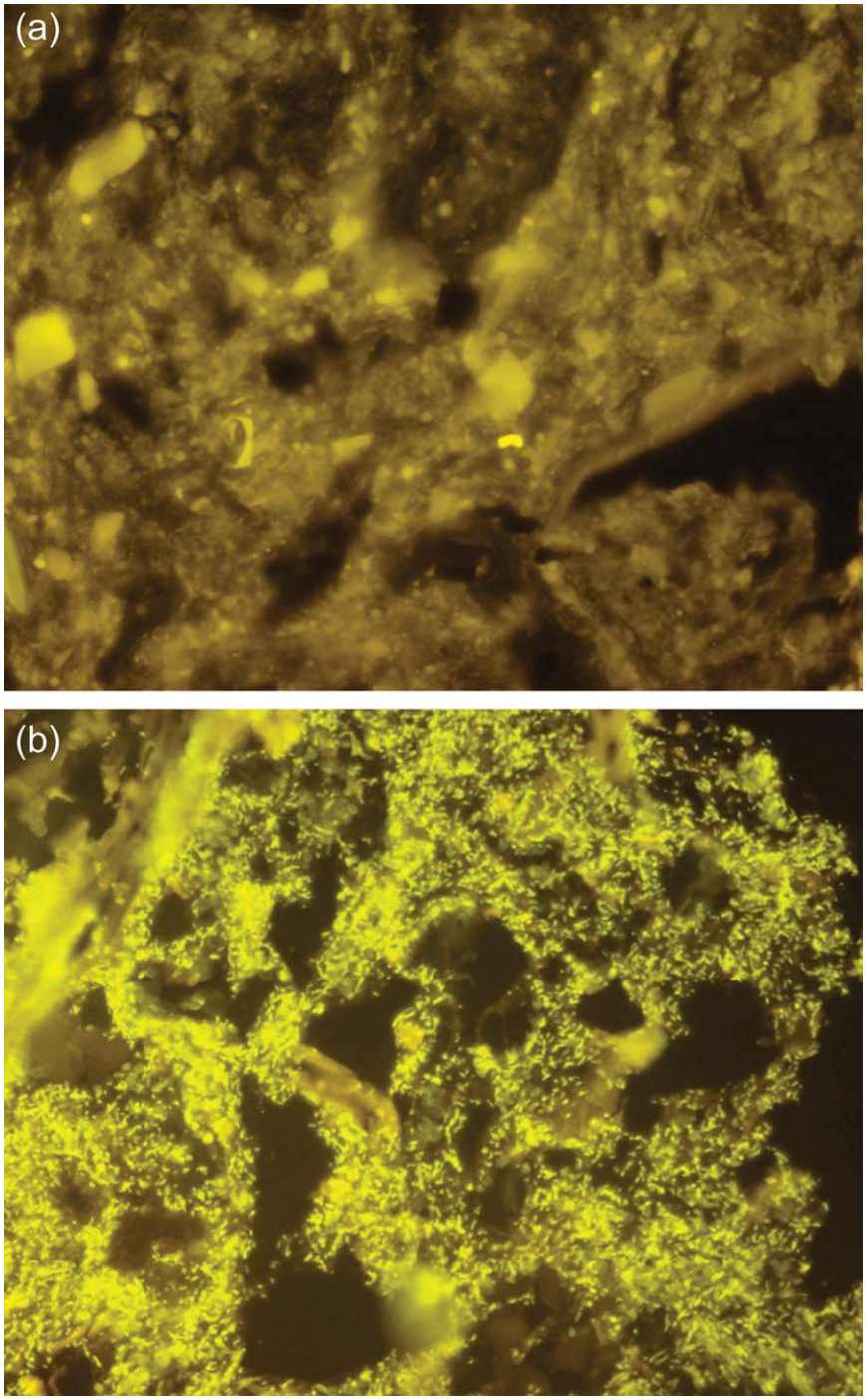

FIGURE 2. (a) Hybridization with the Fprau Cy3 probe shows total depletion of Fprau in diarrheal feces from a patient with a NET of the midgut prior to therapy (×400). (b) Diffusely distributed Fprau in diarrheal feces from the same patient as in 2a with a NET of the midgut during interferon alpha-2b therapy (×400; Cy3 orange fluorescence).

NET. Interferon alpha-2b massively increased the concentrations of Fprau in patients with midgut NET (compare Fig. 2a, 2b). Foregut and hindgut NETs were not treated with interferon.

In patients with midgut NET, the systemic chemotherapy led to a massive increase in the concentration of Fprau, leading to nearly complete normalization of counts in single patients. The systemic chemotherapy had no effect on Fprau counts in patients with foregut and hindgut NETs.

The concentrations of Erec and Bac were increased in CD patients during azathioprine treatment (Table 2b).

TABLE 3. Influence of Therapy on the Fprau Count (Mean + SD x10^{10}/mL) in NET Patients

Fprau Before/During Single Treatment Regimen	Foregut NET	Midgut NET	Hindgut NET
Before somatostatin analogs	14.55 ± 3.21 ($n = 7$)	2.79 ± 2.98 ($n = 18$)	10.55 ± 6.33 ($n = 2$)
During somatostatin analogs	14.10 ± 4.82 ($n = 7$)	2.97 ± 3.02 ($n = 18$)	12.03 ± 5.81 ($n = 2$)
Before interferon alpha-2b		1.38 ± 0.79 ($n = 11$)	
During interferon alpha-2b		11.79 ± 7.46 ($n = 11$)[a]	
Before systemic chemotherapy	13.10 ± 2.89 ($n = 6$)	0.98 ± 2.72 ($n = 5$)	12.23 ± 5.31 ($n = 2$)
During systemic chemotherapy	11.21 ± 3.11 ($n = 6$)	9.52 ± 5.17 ($n = 5$)[b]	11.52 ± 4.88 ($n = 2$)

n = number of patients.
[a]$P < 0.002$ as compared to the data before interferon alpha-2b therapy.
[b]$P < 0.001$ as compared to the data before systemic chemotherapy.

We observed a similar recovery of the microbiota in cases of successfully treated NET and CD despite different therapies used.

DISCUSSION

The most striking finding of our study was the similar depletion of Fprau observed in patients with untreated midgut NET and CD but not in the controls or patients with chronic idiopathic diarrhea, suggesting a relationship in the pathomechanisms of midgut NET and CD. The second even more astonishing finding is the observation of a similar recovery of the microbiota in cases of successfully treated NET and CD, despite different therapies.

In patients with midgut NET, interferon alpha-2b and also systemic chemotherapy led to a massive increase in the concentration of Fprau and even complete normalization in single patients. A similar increase was observed in CD patients in remission. Sokol et al[14] described several antiinflammatory effects of Fprau or its supernatant in vivo and in vitro.

Somatostatin analogs had no influence on the concentration of habitual or occasional bacterial groups in patients with NET, indicating that the positive effects of the octreotide therapy on gastrointestinal symptoms of NET did not parallel improvement of the disturbed colonic biofermentation.

With longer-lasting disturbances of the colonic biofermentation, the occurrence and concentrations of occasionally bacterial groups such as *Bifidobacteriaceae* and *Enterobacteriaceae* increased relative to the habitual bacteria and in absolute numbers of each occasional group, indicating a temporarily imperfect restocking. Exactly these changes, characteristic for noninflammatory dysfunction of colonic fermentation, were observed in patients with hindgut and foregut NET and in patients with chronic diarrhea. We observed a significant reduction of the habitual bacterial groups, without their elimination, increase in concentrations of occasional bacterial groups, and absence of leukocyte migration into the mucus of stool.

It appears that Fprau is the most vulnerable of the three habitual bacterial groups. Massive reduction of Fprau in the stool is a specific sign of active CD.[6,8] Since habitual bacterial groups are always present in healthy subjects and in patients with non-inflammatory bowel disease (IBD), a complete depletion of Fprau is a finding that cannot be overlooked.

In the present study the depletion of Fprau was observed in 66.6% of patients with midgut NET, in 84% of patients with untreated CD, and 56% of treated CD patients, indicating a similarity between midgut NET and CD. The depletion of Fprau was observed in only 0%–9% of the controls, patients with chronic idiopathic diarrhea, foregut NET, and hindgut NET. The lack of detection of these bacteria within a section of the stool cylinder does not mean that they were completely absent in feces. Sometimes the concentrations of bacterial groups in single samples were too low to be detected by FISH.

From a clinical point of view, these results are also of diagnostic relevance since they may help to identify NET of unknown primary tumor location. Primary tumors may be very small in size (<5 mm) even if liver metastases are present. Even with the use of modern imaging methods, 15%–30% of NET primary tumor sites are not identified. Since therapeutic strategies differ considerably for foregut and midgut NET, this is of major clinical importance. Analysis of microbiota in fecal sample represents an approach for improving tumor localization.

There is evidence that Ki67 is of importance for the prediction of the prognosis in NET.[15,16] In our patients the Ki67 indices were typically higher in foregut and hindgut NET as compared to midgut NET, in whom the tumor is general slowly progressive even if metastatic.

It has recently been shown that serotonin may be a growth-promoting factor, especially in neuroendocrine tumor cell lines from the small intestine.[17] Serotonin

activates the immune cells to produce proinflammatory mediators and by manipulating the serotonin system it is possible to modulate gut inflammation.[18] There is also increasing evidence that a close interaction exists between bacteria, epithelial cells, and dendritic cells to maintain intestinal immune homeostasis.[19] In contrast, hindgut tumors very rarely produce serotonin, thus a carcinoid syndrome is uncommon. The shared preferential anatomical site of CD and small intestinal carcinoids in the terminal ileum with its specific features in this part of the intestine (enterochromaffine cells as the major cell type, amine secretion pattern, and different structure of the enteric immune system compared to rectal region) might explain the differences with respect to microbiota observed here between midgut NET and NETs of other primary tumor origin. This might be due to the location of endocrine cells in the gut mucosa.

One of the main secretory products of well-differentiated NETs is chromogranin A. Circulating levels of chromogranin A are a sensitive marker for NETs; 82% of our NET cases were immunohistochemically positive for chromogranin A. Chromogranin A derived peptides may play a role in immune function and inflammation. These peptides exert antimicrobial effects. Various direct and indirect effects on the immune system are described; however, their exact role is unclear.[18,20] Recently, Sciola et al[21] found significantly higher plasma chromogranin A levels in patients with IBD compared to controls. The highest chromogranin A levels have been detected in patients with extensive disease and they correlated positively with serum tumor necrosis factor alpha (TNF-α) values.[21] It has been suggested that circulating chromogranin A could indicate the activation of the neuroendocrine system in chronic inflammatory disorders.[22]

We have now investigated over 10,000 fecal cylinders in healthy subjects and patients with different gastrointestinal diseases using FISH. We identified only two diseases, CD and NET, with a combination of depletion of Fprau, increased Ebac, and a marked reduction of Bac. Both CD and midgut NET have their preferential location in the terminal ileum. The interface between the enteroendocrine system, immune system, and microbiota may play an essential role in the development of inflammation and carcinoid.

West et al[23] reported that carcinoid tumors are 15 times more common in patients with CD (4/111) compared with controls (3/1199). Kortbeek et al[24] showed that by chance alone, one carcinoid tumor should only occur every 200 years in the population of patients with IBD. Since none of the carcinoid tumors developed in areas of CD, West et al[23] suggested that the development of carcinoid tumors may be secondary to distant proinflammatory mediators. Le Marc'hadour et al[25] suggest that hyperstimulation of enteroendocrine cells by inflammation occurs. Brown et al[26] speculated that the coexistence of carcinoid tumors and CD may be more frequent as suggested in the literature.

These data suggest that there is not just coexistence, but an association between the pathogenesis of NET of the midgut and CD.[27] If so, could the patients with CD profit from therapy used for NET and vice versa?

Our data demonstrate a clear recovery of the microbiota and specifically of Fprau in patients with midgut NET during interferon therapy and chemotherapy, but not with octreotide treatment, although the PROMID Study Group showed that octreotide LAR significantly lengthens the time to tumor progression in patients with well-differentiated metastatic midgut NETs.[28] Since the patients received various chemotherapeutic agents, it seems to be a general immunosuppressive effect and not caused by a specific substance. Chemotherapy is not usually used in CD, only in patients suffering from an adenocarcinoma secondary to CD or before stem-cell transplantation. In these rare cases a marked improvement of symptoms was noted with chemotherapy and no exacerbation of IBD was observed in any of the patients over months.[29–32] However, interferon alpha-2b, systemic chemotherapy, and azathioprine have a positive effect on clinical symptoms and colonic microbiota in midgut NET and/or CD.

Even more intriguing and promising than immunosuppressive and chemotherapeutic substances is interferon alpha-2b. Because interferon alpha-2b significantly improved the fecal biostructure in patients with midgut NET, we suggest that it may also contribute to the treatment of CD.

The safety and benefit of interferon administration was previously reported in patients with CD.[33–38] Others reported no effect of anti-interferon-gamma on the production of TNF in ileal CD, no difference was observed between patients receiving interferon beta-1a or placebo for the maintenance of remission in patients with CD, and no therapeutic clinical effect of interferon alpha in chronic active CD.[39–41] Most previous studies on the use of interferon in CD used interferon beta or gamma and did not differentiate between Crohn's colitis and ileocecal disease.

Interestingly, our patients with midgut NET who received interferon alpha therapy had after 4 weeks of therapy a nearly normal distribution of the *Clostridium* group XIVa/*Roseburia* group and Fprau in their feces. There was no longer a significant depletion of Fprau. We also saw a normal distribution of Fprau in feces of patients with ileal CD, but only after remission for several months.[6] We speculate that interferon administration could be of benefit to patients with active CD of the small intestine/cecum with respect to maintenance of remission on the one hand and prevention of occurrence of carcinoid tumors on the other. This speculation will need further research.

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