Structural differences in the enteric neural network in murine colon: impact on electrophysiology

Andrei Sibaev,1 Hartmut Franck,1 Jean-Marie Vanderwinden,2 Hans-Dieter Allescher,1 and Martin Storr1

1Department of Internal Medicine II, Gastrointestinal Physiology (GAP), Technical University of Munich, 81675 Munich, Germany; and 2Laboratoire de Neurophysiologie, Faculte de Medicine, Universite Libre de Bruxelles, B-1070 Brussels, Belgium.

Submitted 27 November 2002; accepted in final form 15 July 2003.

Sibaev, Andrei, Hartmut Franck, Jean-Marie Vanderwinden, Hans-Dieter Allescher, and Martin Storr. Structural differences in the enteric neural network in murine colon: impact on electrophysiology. Am J Physiol Gastrointest Liver Physiol 285: G1325–G1334, 2003. First published July 24, 2003; 10.1152/ajpgi.00506.2002.—The enteric neural network in the proximal murine colon shows a regularly occurring hypoganglionic region, which is here characterized by using anatomical and electrophysiological techniques. Staining with NADPH diaphorase, methylene blue, and cuprolinic blue in standard whole mounts and three-dimensional gut preparations of the murine proximal colon consistently revealed two hypoganglionic areas surrounded by a dense clustering of enteric neurons. This irregularity in the ganglionic plexus was found to be present in mice of three different genetic backgrounds, as well as in rats. The lack of myenteric ganglia in these regions was associated with an absence of the longitudinal muscle layer, as shown in cross sections. Histochemical identification of interstitial cells of Cajal in KitW-lacZ/H11021 transgenic mice showed Kit-positive cells oriented parallel to both muscle layers of the colon. Kit-positive cells oriented parallel to the longitudinal muscle layers were absent in the hypoganglionic area described. Electrical field stimulation elicited TTX-sensitive inhibitory junction potentials (IJPs), which showed region-specific characteristics. The initial partly apamin-sensitive hyperpolarization was present in all parts of the murine colon, whereas a second sustained Nω-nitro-L-arginine-sensitive hyperpolarization was absent in the cecum and decreased from the proximal to the distal colon. Dissecting the hypoganglionic area from the surrounding tissue abolished the otherwise normal inhibitory neurotransmission to the circular muscle (1.6 ± 1.4 and 2.6 ± 1.7 mV for the fast and slow component of IJP amplitude in the hypoganglionic area vs. 16.5 ± 1.9 and 23.7 ± 2.7 mV for the fast and slow component of IJP amplitude in the neuron-rich area, respectively, P < 0.01, n = 6), whereas dissection of an area of identical size with an intact myenteric network showed normal inhibitory neurotransmission, indicating that the hypoganglionic area receives essential functional neural input from the neuron-rich surrounding tissue. In summary, in the murine and rat proximal colon, a constant and distinct hypoganglionic region is described with important concomitant changes in local electrophysiology.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
derestimation of the neuronal population, although for the colon, there seems to be a one-to-one correlation (2, 32). Stimulation of intrinsic NO-dependent nerves induced mechanical relaxation of murine cecum longitudinal muscle (31) and murine proximal colon circular muscle (24). Intracellular electrical recordings of the circular muscle cells of murine colon have demonstrated functional neural input of inhibitory neurotransmitters such as NO (14, 24).

On the basis of our own observations that inhibitory junction potentials (IJPs) sometimes differ in the murine proximal colon, we performed a circumference-preserving three-dimensional (3D) preparation for NADPH diaphorase and macroscopically discovered a ganglion-free area, which was of interest because it might be the explanation for the above-mentioned observations. The aim of the present study was therefore to characterize the observed hypoganglionic areas by using morphological and electrophysiological methods.

MATERIALS AND METHODS

Tissue preparation for electrophysiological experiments. Mice of three different genetic backgrounds (BALBc, C III H, and C57/black 6) or Wistar rats of either sex were anesthetized by phenobarbital sodium and killed by cervical dislocation in accordance with the recommendations of the animal ethics committee at the Technical University of Munich. The colon was exposed through an abdominal midline incision. The complete large bowel was removed and placed in oxygenated Krebs solution. The size of the large bowel, and hence the size of the segments of the large bowel, varied according to the weight of the animal, the temperature of the surrounding media, and the volume of the bowel contents. The need to cut short gut segments for the electrophysiological examination made it necessary to reliably identify locations along the large bowel by using fixed topographic points. The following fixed points, which did not change during the tissue preparation, were selected: the ileocecal junction, the ascending colon characterized by haustra (Fig. 1), and the aboral distal ileum. The tissue was pinned out while being stretched to normal size.

IJPs, elicited by stimulation of intrinsic inhibitory neurons, usually contained a transient (fast) and, if present, a sustained (slow) component. The amplitudes of the fast and slow components were measured in millivolts compared with the resting membrane potential (RMP) before application of the electrical stimulus.

Whole mount preparation for NADPH diaphorase, methylene blue, and cuprolinic blue staining. The large bowel was removed as described in the electrophysiological experiments. Segments of cecum and of the proximal and distal colon were opened along the mesenteric border, washed, and pinned in a dissecting dish containing Krebs solution with nifedipine, atropine, and guanethidine (all 1 μM). The mucosa and submucosa were removed, and the muscle layers were pinned out while being stretched to ~125% of the normal size.

NADPH diaphorase staining was performed as follows. The tissue was fixed for 2 h in 4% paraformaldehyde at room temperature. NADPH diaphorase activity was rendered visible by incubating the tissues in 0.05 M Tris-HCl buffer (pH 8.0) containing 0.1 mM β-NADPH (reduced form), 0.05 mM nitroblue tetrazolium, and 0.3% Triton X-100, at 37°C for 2 h. Washing of the whole mount preparations in 0.05 M Tris-HCl buffer terminated the reaction. After several washings with 0.05 M Tris-HCl, the whole mounts were placed on...
glass slides, air-dried, and coverslipped with DePex (Serva, Heidelberg, Germany).

Methylene blue staining is a nonspecific but effective and duration-dependent staining method for interstitial cells of Cajal (ICCs) and neural structures (21). After the removal of the mucosa, tissues were washed with fresh oxygenated Krebs solution and then incubated in Krebs solution containing 0.15 M sodium acetate blue at 37°C bubbled with 95% O₂-5% CO₂ (vol/vol) for 30–180 min in the dark with intermittent visual checking of the staining intensity. After staining, the tissues were immediately placed in 8% ammonium molybdate for 2–3 h, as described by Ehrlich (20). Then the tissues were placed on glass slides, air-dried, and coverslipped with DePex.

Cuprolinic blue staining is used as a marker for total neuron counting (12). The tissue was fixed for 1 h in Carnoy solution. The staining solution [0.3% cuprolinic acid in 0.025 M sodium acetate buffer (pH 5.6) with 1 M MgCl₂] was applied for 60 min at room temperature, and the tissue was then rinsed in distilled water and placed in a sodium acetate buffer (pH 5.6) containing 1 M MgCl₂. Finally, the tissue was washed in distilled water, placed in ethanol and then xylene, and mounted in DePex.

Circumference-preserving 3D preparation for NADPH diaphorase, methylene blue, and cuprolinic blue staining. Mice of different genetic backgrounds (BALBc, C III H, or C57/black 6), Wistar rats, or guinea pigs of either sex were anesthetized and killed as described in the electrophysiology section. The whole large bowel with adjacent terminal ileum were removed through an abdominal midline incision and placed in Krebs buffer (37°C) containing nifedipine, atropine, guanethidine, and 4 mM magnesium chloride. The preparation was rinsed in Krebs-Ringer buffer and then in distilled water and was mounted in an aqueous mounting medium.

Drugs. Ammonium molybdate, atropine, guanethidine, N⁰-nitro-L-arginine (L-NNA), nifedipine, Triton X-100, TTX, Tris (base), the Krebs-Ringer buffer salts, and potassium ferricyanide were obtained from Sigma. β-NADPH (reduced form), nitroblue tetrazolium, and methylene blue were obtained from Neopharma (Aschau, Germany). Paraformaldehyde and glutaraldehyde were obtained from Agar Scientific (Sansted, Essex, UK), and X-gal from Boehringer-Mannheim (Mannheim, Germany).

Data presentation and statistical analysis. All data are expressed as means ± SE. The significance of differences among groups was determined by using the paired Student’s t-test. For multiple comparisons, adequate Bonferroni correction was performed. A P value < 0.05 was considered significant. Values given with n refer to the number of experiments performed in tissues from different animals.

RESULTS

Electrophysiology. In basal conditions in the presence of nifedipine, atropine, and guanethidine (all 1 μM), circular smooth muscle cells of murine large bowel displayed stable RMPs (~56.7 ± 6.9 mV, n = 13) in the cecum; ~46.1 ± 2.3 mV, n = 7 in the proximal colon; ~46.4 ± 3.8 mV, n = 11 in the distal colon; cecum RMP is significantly different from colon RMP, P < 0.05). Stimulation of enteric inhibitory neurons via electrical field stimulation (EFS) gave rise to TTX-sensitive IJPs varying in size and form along the longitudinal axis of the colon.

In the cecum (midway between the ileocecal junction and the apex of the cecum), EFS (single stimuli) elicited monophasic IJPs of short duration (918 ± 200 ms, n = 8) with an amplitude of 16.8 ± 3.6 mV. Varying stimulus parameters including 10 pulses at 5 Hz or 20-s long continuous stimulation at 5 Hz did not elicit additional components of IJPs in the cecum. In the oral, middle, and aboral proximal colon and in the distal colon, single stimuli of EFS produced IJPs that consisted of two components. The initial “fast component” was characterized by a rapid hyperpolarization and did not vary within the proximal colon [duration:
A second “slow” component (duration: 7.0 ± 1.4 s in the oral and 3.1 ± 0.6 s in the distal colon; P < 0.05; n = 10), elicited by EFS, showed major differences between the proximal and distal colon and varied even within short distances in the proximal colon. The slow component was characterized by a distinct and gradual recovery to RMP and had an amplitude of 13.6 ± 2.0 mV in the oral, 10.3 ± 3.0 mV in middle, 7.3 ± 1.7 mV in the aboral proximal colon, and 3.8 ± 1.5 mV in the distal colon. The size of the slow component differed significantly at all locations (P < 0.01, n = 10). Both components of the IJP were blocked by 3 μM TTX (n = 5). Larger, slow components of IJPs were obtained in the proximal and distal colon with multiple stimuli (10 pulses at 5 Hz). In these conditions, EFS revealed a clear, slow component in the distal colon, which was still significantly smaller than in the proximal colon (8.6 ± 1.6 mV in the distal colon vs. 15.3 ± 2.9 mV in the proximal colon; P < 0.01, n = 4).

The NOS inhibitor L-NNA (100 μM) produced a significant depolarization (7.3 ± 3.8 mV, n = 9, P < 0.01) of smooth muscle cells in the murine proximal colon, which was of permanent nature. Maximal effects of L-NNA occurred within 10 min of exposure. When single stimuli or 10 pulses (5 Hz, 0.3 ms, 15 V) were tested, L-NNA completely abolished the sustained hyperpolarization (slow component of IJPs) (16.3 ± 3.2 mV vs. 2.1 ± 1.7 and 8.5 ± 2.3 vs. 1.4 ± 0.6 mV; n = 6; P < 0.05 in the proximal and distal colon) (Fig. 2B). L-NNA did not change the form and size of IJPs in the murine cecum (16.8 ± 3.6 vs. 18.8 ± 4.5 mV fast component, NS; the slow component is absent in the cecum) (Fig. 2B), demonstrating an absence of functional NO-dependent neural input.

**NADPH diaphorase histochemistry.** Neuronal NOS (nNOS)-positive neurons were found throughout the large bowel. There were usually several positive cells in each myenteric ganglion. The results of NADPH diaphorase histochemistry were similar in whole mount preparations and in the circumference-preserving 3D preparation. The number of nNOS-positive cells per ganglion was highest in the proximal colon and decreased in the oral direction (to the cecum) and distally (Table 1). In the aboral third of the proximal colon, ~30 mm aboral from the ileocolonic junction, NADPH diaphorase staining reveals two areas that

Table 1. Distribution of nNOS-positive neurons in the murine large bowel

<table>
<thead>
<tr>
<th></th>
<th>Cecum</th>
<th>Proximal Colon, Oral</th>
<th>Proximal Colon, Middle</th>
<th>Distal Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuron-rich</td>
<td>34.5</td>
<td>21.1</td>
<td>19.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Hypoganglionic</td>
<td>3</td>
<td>4.7 ± 1.5</td>
<td>4.7 ± 1.5</td>
<td>9.7 ± 1.4</td>
</tr>
<tr>
<td>Distal Colon</td>
<td>15.7</td>
<td>12.6 ± 3.1</td>
<td>12.6 ± 3.1</td>
<td>8.7 ± 0.9</td>
</tr>
</tbody>
</table>

Data shown are means ± SD for the numbers of ganglia and neurons counted in 6 preparations. Note that in the aboral proximal colon, the heterogeneous neural distribution made it necessary to distinguish between neuron-rich and hypoganglionic areas, each of which made up different parts of the extent of the gut circumference. Neurons were calculated per gut segment 1 mm in length (last row). nNOS, neuronal nitric oxide synthase.
contain few neuronal ganglia with an average of just 4.4 ± 2.8 neurons per ganglion. One of these is located at the mesenteric attachment, 4 mm wide and 13–15 mm long, and the other antimesenterically, 3–5 mm wide and 10–14 mm long (see Figs. 1 and 3). These two hypoganglionic regions are surrounded by a dense clustering of enteric ganglia, each containing a high number (12.6 ± 1.1) of neurons per ganglion (Table 1 and Fig. 4). The nerve cell density in this surrounding clustering of ganglia was not significantly different from the cell density in the gut segment oral to the hypoganglionic regions, where the network is still regularly distributed around the circumference (NS, n = 6) (Fig. 3, Table 1). When estimating the total number of neurons per segment, the heterogeneous segment consisting of the two hypoganglionic areas within its circumference shows lower total numbers than the segment with a homogeneous neuronal network just oral of the heterogeneous segment. Hence, the number of nNOS-positive neurons was maximal in the oral proximal colon and decreased on both sides, orally and aborally. The number of nerve fibers within the circular muscle layer was measured in transected tissue of the hypoganglionic and the ganglionic sections. The nerve fibers were counted and are given in number per millimeter of tissue length. By counting the nerve fibers, we could not find a significant difference in this number comparing the ganglionic and the hypoganglionic area (ganglionic area: 57 ± 9.1; hypoganglionic area: 54 ± 14.6; n = 5; NS).

The finding of the hypoganglionic regions in the proximal colon was consistent in mice of different genetic backgrounds (BALBc, C III H, or C57 black 6) and was also demonstrated in rat large bowel (Wistar rats). In guinea pigs, however, only one hypoganglionic region is present at the mesenteric attachment.

**Cross sections of murine proximal colon.** Cross sections of murine proximal colon at the location of the hypoganglionic region demonstrated that the longitudinal muscle layer was lacking in the hypoganglionic region (Fig. 5).

**Methylene blue and cuprolinic blue histochemistry.** Methylene blue vital staining (Fig. 6) and cuprolinic blue staining of a 3D preparation and of standard whole mounts confirmed the location and size of the hypoganglionic regions, the maximum nerve cell density in the proximal colon, and the nerve cell density gradient decreasing on both sides in the oral and anal directions. Generally, additional neurons were stained in all regions of the large bowel, so that 51–54% of the total number of cuprolinic blue-stained neurons were NADPH-diaphorase positive (Tables 1 and 2).

**Identification of ICCs by using X-gal histochemistry in KitW-lacZ+/ mice.** Kit-expressing ICCs were oriented parallel to both muscle layers of the colon. The X-gal staining density was higher in the proximal...
surrounding ganglion-rich area (24.6 ± 6.6 vs. 22.6 ± 4.3 and 12.1 ± 2.1 vs. 13.1 ± 2.1 mV fast and slow component of IJP, respectively, n = 6, NS). After microscopic sharp separation of the hypoganglionic region from its surrounding tissue, however, the normal inhibitory neurotransmission to the hypoganglionic area was disrupted (Fig. 7) (1.6 ± 1.4 and 2.6 ± 1.7 mV for the fast and slow component of IJP amplitude in the hypoganglionic area vs. 16.5 ± 1.9 and 23.7 ± 2.7 mV for the fast and slow component of IJP amplitude in the neuron-rich area, respectively, P < 0.01, n = 6). In contrast, a similar microscopic separation of the neuron-rich surrounding tissue with identical sheet size had no influence on the inhibitory neurotransmission (22.6 ± 4.3 and 13.1 ± 2.1 mV fast and slow component of IJP, respectively, n = 6). These findings indicate that the hypoganglionic area receives functional neural input from the neuron-rich surrounding tissue in a radial pattern.

DISCUSSION

On the basis of our own observations that IJPs sometimes differ in the murine proximal colon, we performed a circumference-preserving 3D preparation for NADPH diaphorase and macroscopically discovered a ganglion-free area. Starting from this initial observation, we now present a study in which we report regional differences in the network of the enteric nervous system in the murine large bowel with marked regional hypogangliosis. These anatomical differences were characterized by using different staining techniques, and the hypoganglionic region, which is also associated with functional differences in inhibitory NANC innervation, is characterized by electrophysiological methods.

The 3D preparation and the flat whole mount preparation both provide evidence that the murine proximal colon consistently contains two hypoganglionic regions. This finding was not limited to mice of a single genetic background and was also present in rats. The longitudinal muscle layer and the innervating structures, such as the associated ICCs and most neural ganglia, are polarized on two sides of the circumference, representing an exception to the tube-like structure of the gut. This hypoganglionic area is anatomically clearly distinct from whole aganglionic segments described in models for Hirschsprung’s disease, such as the lethal spotted mouse (22). Moreover, cross sections of the proximal colon and X-gal staining of ICCs in KitW−lacZ+/− transgenic mice revealed that the longitudinal muscle layer and the associated intramuscular ICCs (5, 28), which are closely associated with and oriented parallel to this muscle layer, are lacking in these hypoganglionic regions. The impact on function of these morphological findings is still unclear, but because the irregularly distributed longitudinal muscle results in flat pouches in the proximal colon, the morphology could be linked to a reservoir function, which could also decrease fluid propulsion.
Fig. 5. Overview (A), details (B and C) and further magnification (D and E) of a cross section of proximal murine colon, including the hypoganglionic regions in the myenteric plexus (MP) by using hematoxylin-eosin staining. D: lack of myenteric ganglia in positions located opposite to each other is associated with an absence of the longitudinal muscle layer (L). In these areas, the circular muscle (C) is covered only by the serosal layer. E: around these hypoganglionic areas, the gut wall contains normal circular (C) and longitudinal (L) muscle, as well as clustered ganglia in the myenteric plexus (MP). The asterisk marks the location of the mesenteric attachment.

Fig. 6. A and B: whole mount flat preparations of the proximal colon of \textit{W}^{\text{lac-Z/}} \textit{H11001} mice after X-gal staining. Kit-positive cells oriented parallel to the longitudinal muscle layers were lacking in the hypoganglionic area, thus confirming that the region lacking the longitudinal muscle layer also lacks the Kit-positive interstitial cells associated with this muscle layer. C and D: whole mount flat preparations of murine proximal colon with vital methylene-blue staining, showing the structural organization of the ICC network.
It is certainly surprising that to our knowledge these hypoganglionic areas have not been described previously. One possible explanation might be the preparation techniques used. For magnification of the gut wall using optical lenses, the general practice has been to cut the gut into pieces to mount the samples on glass. A serious limitation of this technique is that anatomical features at the edges of the preparation cuts are lost. A circumference-preserving 3D preparation technique in which the gut is stained and visualized without affecting the 3D anatomy provides a more complete visualization of the gut wall. The functional inhibitory innervation is organized on a segmental level and runs from the neuron-rich areas supposedly by using the tertiary plexus to reach the circular muscle layer of the hypoganglionic region.

In addition to the changes in electrophysiology in the hypoganglionic region, the present study characterizes regional differences in inhibitory neurotransmission from the cecum to the distal colon for the circular smooth muscle. Although there is no l-NNA-dependent slow IJP in the cecum or the distal colon, there is a prominent l-NNA dependent inhibitory neurotransmission in the proximal colon, and within the proximal colon there is a gradient in this l-NNA-dependent neurotransmission from oral to aboral. Interestingly, for the cecum, despite a few, but clearly present, nitricergic neurons, functional NO-dependent inhibitory input to the muscle was completely absent in our experiments. The cecum results partially contrast with

<table>
<thead>
<tr>
<th>Gut circumference, mm</th>
<th>Cecum</th>
<th>Proximal Col, Oral</th>
<th>Proximal Col, Middle</th>
<th>Proximal Col, Distal Third</th>
<th>Distal Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganglia per mm²</td>
<td>2.8 ± 1.5</td>
<td>9.3 ± 2.4</td>
<td>12.1 ± 1.9</td>
<td>16.2 ± 3.5</td>
<td>4.7 ± 1.5</td>
</tr>
<tr>
<td>Neurons per mm²</td>
<td>42.8 ± 19.4</td>
<td>405.1 ± 13.5</td>
<td>453.3 ± 44.1</td>
<td>715.2 ± 98.9</td>
<td>54.5 ± 12.1</td>
</tr>
<tr>
<td>Neurons per ganglion</td>
<td>10.7 ± 4.9</td>
<td>29.6 ± 3.6</td>
<td>29.2 ± 4.1</td>
<td>30.8 ± 4.3</td>
<td>8.1 ± 1.8</td>
</tr>
<tr>
<td>Neurons per segment</td>
<td>1,478.6 ± 671.5</td>
<td>8,524.7 ± 284.7</td>
<td>8,920.8 ± 866.0</td>
<td>10,132.2 ± 1427.9</td>
<td>4,254.7 ± 569.8</td>
</tr>
</tbody>
</table>

Data shown are means ± SD for the number of ganglia and neurons counted in 6 preparations. Note that in the aboral proximal colon, the heterogeneous neutral distribution made it necessary to distinguish between neuron-rich and hypoganglionic areas, each of which made up different parts of the extent of the gut circumference. The neurons were calculated per gut segments 1 mm in length (last row).

Fig. 7. Microscopic separation of the hypoganglionic region from its neuron-rich surrounding area reveals the difference in neuronal supply to the circular muscle layer in the area of the hypoganglionic region. As long as the circumference is intact, the circular muscle cells display IJPs that do not differ significantly from those in the neighboring neuron-rich area of the same segment. In contrast, cutting the bowel wall at the borders of the hypoganglionic region results in disruption of the IJPs in the hypoganglionic region (n = 6, P < 0.01). *Location of the mesenteric attachment.
the findings of Young et al. (31), who reported NO-dependent relaxation of cecum longitudinal muscle, although with a different experimental design by using a muscle strip-contraction setup. Although this difference might be due to the differences in the observed muscle (circular vs. longitudinal), it might also be due to stimulus differences. In the colon, the electrical recording proved to be sensitive enough to demonstrate clear differences in functional NO-dependent neuronal supply to the muscle. The NO-dependent functional innervation was prominent in the oral third of the proximal colon and decreased in the distal direction. This functional gradient was associated with a decrease in neural density, specifically in the density of NADPH diaphorase-positive neurons. In addition to the nerve cell density suggested here, other causes might contribute to the functional gradient reported here, such as increased sensitivity of the muscle cells to NO (16) or enhanced synthesis or release of NO from myenteric neurons (19, 25).

The hypoganglionic area in the proximal colon described here should not be mistaken for the aganglionic colon of lethal (ls/ls) mice described recently (29). Interestingly, in the lethal aganglionic mice, the IJPs were completely absent, whereas in the hypoganglionic region described here, electrophysiological responses remain unchanged until the hypoganglionic region is separated from the surrounding tissue. When the hypoganglionic region is separated, the electrophysiological responses are nearly absent and therefore comparable to the responses in lethal aganglionic mice (29).

In summary, the proximal colon displays clear morphological and functional irregularities. This part of the colon represents a clear exception to the generally accepted view that the bowel has a homogeneous hollow organ structure. The fact that the hypoganglionic region, when studied in isolation, does not display a functional inhibitory neural supply has implications for future examinations of the proximal colon, because any one spot on the gut circumference cannot necessarily be taken to represent either the anatomy or the function of the organ as a whole. The physiological impact of this newly described hypoganglionic region remains speculative at the present stage but merits further investigation.

The authors are grateful to Prof. V. Schusdziarra, Dr. H. Salmhofer, and Prof. W. Neuhuber for providing vital input for this article.

DISCLOSURES

A. Sibaev was supported by a grant from the Gastroenterology Foundation. Research was supported by Special Research Field Grant SFB 391-C5 and German Research Association Grant DFG Fr-1170.

REFERENCES


