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Accelerated intestinal transit in inbred mice with an increased number of interstitial cells of Cajal

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Bellier, Sylvain, Nelly R. Da Silva, Geneviève Aubin-Houzelstein, Colette Elbaz, Jean-Marie Vanderwinden, and Jean-Jacques Panthier. Accelerated intestinal transit in inbred mice with an increased number of interstitial cells of Cajal. *Am J Physiol Gastrointest Liver Physiol* 288: G151–G158, 2005. First published August 5, 2004; doi:10.1152/ajpgi.00048.2004.—The interstitial cells of Cajal (ICC) play an important role in coordinating intestinal motility, and structural alterations in ICC are found in several human digestive diseases. Mouse models with defects in ICC allow a better understanding of their functions. We investigated the pattern of intestinal motility and the distribution of ICC in the PRM/Alf inbred mouse strain, characterized by a selective intestinal lengthening. In PRM/Alf mice, the digestive transit time, evaluated by using thermophilic *Bacillus subtilis* spores, was normal, indicating accelerated transit. The contractility and slow-wave frequency, recorded on isolated segments from the proximal small intestine, were significantly increased. The number of ICC was also significantly higher along the small intestine and the colon. The concomitant increase of the contractility, the slow-wave frequency, and the number of ICC is consistent with the proposal of a role of ICC number increase in the higher intestinal transit speed. The PRM/Alf model should be useful to further investigate the roles of ICC in the control of digestive motility.

gut length; *kit* receptor; electrical slow waves; gastrointestinal motility; genetic model

INTRINSIC AND EXTRINSIC NEUROHUMORAL signals control intestinal motility. An essential part of the system is the electrical pacemaker activity that originates in interstitial cells of Cajal (ICC). These cells are present in close association with smooth muscle cells and neurons of the gastrointestinal tract. In the mouse, ICC are fully differentiated at weaning, when the animals are given adult diet (13). The possible role of ICC in coordinating the contractile activity of the intestine has come to light in recent years (17, 38). Alterations of ICC were reported in a variety of gastrointestinal disorders, including hypertrophic pyloric stenosis (24, 35, 41), Hirschsprung's disease (37, 42, 43), and intestinal pseudo-obstructions (19, 21, 44). Study of mouse models has proven to be a valuable strategy for studying the cause-and-effect relationship between ICC and motility problems, and *Kit*/*W* and *SCF*/*Steel* mutant mice have been instrumental in the study of the physiological roles of ICC (reviewed in Ref. 30). The *Kit* signaling pathway is essential for the development and maintenance of ICC (26). Both *Kit* and *Steel* mutant mice exhibit absence of electrical rhythmicity

(i.e., slow waves) associated with underdevelopment of certain classes of ICC, including ICC of the small intestine that lie in the plane of the myenteric plexus (ICC-MP) between the circular and longitudinal muscle layers (17, 38, 39). The absence of ICC and slow waves in the small intestine of *Kit* mutant mice was paralleled with altered peristaltic movements of intestinal contents (10). However, it remains difficult to predict how abnormalities in ICC could influence digestive motor activity in vivo, because peristalsis is controlled by multiple, simultaneously operating mechanisms, in particular within the enteric nervous system (34). Indeed, migrating motor complexes can be recorded in the small intestine even when the ICC-MP are largely missing and slow waves are consequently absent (32). Further studies are required to determine the exact contribution of ICC to the digestive motor activity.

We describe here a new mouse model that should prove useful for studying digestive motor mechanisms. The PRM/Alf strain was derived from mice of unknown genetic background, and it carries the recessive coat color mutation patchwork (2, 4). PRM/Alf mice were recently shown to exhibit a hereditary increase in intestine length (3). Here we have investigated the distribution of ICC and the pattern of digestive motility in the PRM/Alf strain, compared with two classical, inbred strains, DBA/2J and 129S2/SvPa (hereafter referred to as 129S2).

MATERIALS AND METHODS

Animals. Mice from the DBA/2J and 129S2 inbred strains were obtained from Charles River Laboratories (Saint-Aubin-Les-Elbeuf, France) and Institut Pasteur (Paris, France), respectively. Mice homozygous for the patchwork mutation (2, 4) were obtained from Karen Moore (National Cancer Institute, Frederick, MD) and an inbred strain, called PRM/Alf, was derived by >30 successive brother-sister matings (3). Transgenic 129S2-*Kit*^{W-lacZ}/+ mice were generated in our laboratory. These mice carry the *Escherichia coli lacZ* gene inserted in place of the first exon of the *Kit* gene (5). The presence of a wild-type (+) *Kit* allele allows *Kit*^{W-lacZ}/+ heterozygous mice to develop normally, whereas the *lacZ* reporter gene allows identification of the *Kit*-expressing cells by the β -galactosidase (X-gal) histochemical reaction. The pattern of *Kit*-expressing cells in 129S2-*Kit*^{W-lacZ}/+ heterozygous mice is identical to that of *Kit*-immunoreactive cells in 129S2 wild-type (*Kit*^{+/+}) mice (36). PRM/Alf mice heterozygous for the *Kit*^{W-lacZ}/+ allele were generated by mating PRM/Alf mice and 129S2-*Kit*^{W-lacZ}/+ mice. The progeny were repeatedly back-crossed onto the PRM/Alf background and selected for the *Kit*^{W-lacZ}/+ allele.

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At the 10th generation, i.e., after nine backcrosses, PRM/Alf·129S2-*Kit^{W-lacZ}/+* mice could be estimated to be identical to PRM/Alf mice for >99.8% of their genome. These mice will hereafter be referred to as congenic PRM/Alf-*Kit^{W-lacZ}/+*.

Animals were fed ad libitum with standardized mouse pellet food (formula A03; Usine d'Alimentation Rationnelle, Rennes, France). They were housed and bred under identical conditions and euthanized by CO₂ inhalation, in accordance with the principles and guidelines of the Ecole Nationale Vétérinaire d'Alfort on animal welfare. Three-month-old male mice were used.

Histology of the intestine. The intestines of PRM/Alf and DBA/2J mice ($n = 3$ and $n = 4$, respectively) were removed, and 1-cm-long samples were harvested from the proximal small intestine (2 cm after the pylorus), the distal small intestine (2 cm before the ileocecal junction), the proximal colon (1 cm after the ileocecal junction), and the distal colon (1 cm from the anus). Samples were fixed for 48 h in 10% formalin in PBS, embedded in paraffin, and sectioned at 4 μ m. Sections were deparaffinized in toluene, rehydrated, and stained with hematoxylin-eosin-saffron. Sections were viewed under a Leica (Rueil-Malmaison, France) bright-field microscope. The Leica imaging system (QWin software) was used for morphometric analysis. For each sample, at least four measurements per section were made on a minimum of five nonadjacent sections.

Quantification of Kit-expressing ICC in congenic PRM/Alf-*Kit^{W-lacZ}/+* and 129S2-*Kit^{W-lacZ}/+* mice. Intestine samples were harvested as above from four congenic PRM/Alf-*Kit^{W-lacZ}/+* and four 129S2-*Kit^{W-lacZ}/+* mice and fixed for 60 min in 4% (vol/vol) paraformaldehyde in PBS. X-gal histochemistry was performed as previously reported (5). Sections (4 μ m thick) were then counterstained with hematoxylin and eosin and mounted with Eukitt. For each sample, X-gal-positive cells in the entire circumference were counted on five nonadjacent sections, using a $\times 20$ objective. In the small intestine, ICC lie in the myenteric region between the circular and longitudinal muscle layers (ICC-MP) and at the inner surface of the deep muscular plexus (ICC-DMP). In the colon, ICC are found at the subserosa (ICC-SS), around the myenteric plexus (ICC-MP), in the circular muscle layer (ICC-CM), and at the border between the submucosa and the circular muscle layer (ICC-SMP). Their relative distribution in each location (proximal and distal small intestine, proximal and distal colon) was calculated as their mean numbers on two nonadjacent entire transverse sections divided by the mean number of total X-gal-positive cells, i.e., ICC-MP+ICC-DMP in the small intestine or ICC-SS+ICC-MP+ICC-CM+ICC-SMP in the colon.

Extracellular slow-wave recording on isolated proximal small intestine segments. Ten PRM/Alf and seven DBA/2J mice were used. A 5-cm segment of proximal small intestine with the pylorus attached was excised and pinned without tension on the bottom of a 5-ml water-jacket bath coated with Sylgard (Dow Corning, Midland, MI) at $37 \pm 0.5^\circ\text{C}$ and bathed at 2 ml/min with warmed modified Krebs solution (in mM: 125 NaCl, 5 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 4 NaHCO₃, 11 glucose, and 10 HEPES) aerated with 95% O₂-5% CO₂. Slow-wave activity was recorded with a serosal Ag/AgCl suction electrode (6, 10, 16, 33) 2 cm distal to the pylorus for a minimum of 15 min. The electric signal was amplified on a Duo 773 electrometer (World Precision Instruments, Sarasota, FL) and digitalized on a PC with an Acknowledge-MP100 Biopac system (World Precision Instruments). Slow waves were counted on trace printouts, and their average frequency was determined over the recording period.

Mechanical activity recording on isolated proximal small intestine segments. Three PRM/Alf and three DBA/2J mice were used. A segment of the proximal small intestine (1 cm in length) was excised from each animal and placed vertically in a 20-ml water-jacket bath (LO1 compact multichamber organ bath; Bioseb, Chaville, France). Tissues were bathed with modified Krebs solution aerated with 95% O₂-5% CO₂ and maintained at $37 \pm 0.5^\circ\text{C}$. The intestines were

allowed to equilibrate for at least 30 min, during which time they were subjected to slight tension just sufficient to remove slack in the apparatus. Tension generated along the longitudinal axis of the intestine was recorded with a force-displacement transducer (Biopac, Santa Barbara, CA), amplified with DA100B amplifier, and visualized with the Acknowledge-MP100 system.

Estimation of the gastrointestinal transit time. Four PRM/Alf and four DBA/2J fasted mice were force fed with 0.4 ml of a water solution containing 22×10^6 spores of a strictly aerobic and thermophilic *Bacillus subtilis* strain as an inert transit marker (8, 12, 29). All feces were collected after 2, 3, 4, 5, 6, 7, 8, 9, 10, and 24 h. Aqueous fecal slurries were prepared, diluted appropriately, spread on nutrient agar plates, and incubated at 65°C for 24 h. Thermophilic *Bacillus subtilis* spores germinated at 65°C to produce small, white colonies. No other thermophiles could be consistently cultured from feces under these conditions. From *Bacillus subtilis* concentrations, the mean transit time (in hours) through the whole intestine was calculated as $\sum(m_i t_i) / \sum m_i$, where m_i is the number of spores present in the feces passed at time interval t_i (27). The recovery of the transit marker (range = 50–97%) was satisfactory, considering the partial regurgitation that occasionally occurred during force feeding.

Measurement of gastric emptying and transit. Ten PRM/Alf and nine DBA/2J fasted mice were force fed with 0.1 ml of a semiliquid solution containing Evans blue (5% Evans blue in 0.9% NaCl with 0.5% methylcellulose). Fifteen minutes later, they were anesthetized and a laparotomy was performed. The stomach was rapidly clamped above the lower esophageal sphincter and beneath the pylorus to prevent leakage of Evans blue. The stomach was then cut just beneath the clamps. Gastric emptying was determined spectrophotometrically (11). Briefly, the stomach and its contents were put in 15 ml 0.1 N NaOH, minced, and homogenized. After dilution to a volume of 20 ml with 0.1 N NaOH, samples were kept at room temperature for 1 h. Then 5 ml supernatant was collected and centrifuged at 2,500 rpm during 20 min at 4°C . Samples were further diluted, and the absorbance was read at a wavelength of 565 nm (A565). The stomach and its contents obtained from a mouse killed immediately after orogastric administration of Evans blue served as standard (reference stomach). The percentage of gastric emptying was calculated by the formula $[(A565 \text{ reference} - A565 \text{ sample}) / A565 \text{ reference}] \times 100$.

Statistics. Statistical analyses were performed with the StatView F-4.51.3.PPC software (Abacus Concepts, Berkeley, CA). Data are expressed as means $\pm$ SE. Distribution normality was tested by comparing the observed distribution to a normal distribution with the same mean and standard deviation and use of a Kolmogorov-Smirnov test. Variances were compared with an *F*-test. Statistical differences were tested using a Student's *t*-test or a multivariate analysis of variance (MANOVA). For not normally distributed values, or values with unequal variances, a Mann-Whitney *U*-test was used. *P* values <0.05 were considered statistically significant.

RESULTS

Morphometric analysis of the PRM/Alf mouse intestine. PRM/Alf mice exhibited an obvious abdominal distention (Fig. 1). This feature is a consequence of increased intestinal length in PRM/Alf mice (3). The intestine of PRM/Alf adult mice of both sexes showed, on average, a 30% increase in length when compared with 129S2 adult mice (74.8 ± 0.8 cm in 42 PRM/Alf mice compared with 57.6 ± 0.5 cm in 43 129S2 mice) and DBA/2J (3) adult mice. The difference in intestine length being much more pronounced between PRM/Alf and DBA/2J mice, we used the latter strain in a morphometric analysis. To check whether the intestine lengthening is associated with an enlargement, we measured the small and large intestine circumference in both the proximal and distal parts (Fig. 2). Proximal and distal small intestine circumferences

Fig. 1. External morphology of 3-mo-old male mice from DBA/2J, PRM/Alf, and 129S2 inbred strains.

were not significantly different between PRM/Alf and DBA/2J. Proximal colon circumference was significantly increased in PRM/Alf mice compared with DBA/2J mice (14.8 ± 1.2 and 10.9 ± 0.6 mm, respectively; $P = 0.01$, Student's *t*-test). The distal colon circumference was also significantly increased in PRM/Alf mice compared with DBA/2J mice (14.7 ± 0.7 and 10.0 ± 0.7 mm, respectively; $P = 0.002$, Student's *t*-test).

To search for a possible alteration of the intestinal thickness in PRM/Alf mice, we established a histological picture of the PRM/Alf and DBA/2J intestinal wall. The crypt height was significantly increased in the distal small intestine of PRM/Alf mice compared with DBA/2J mice (90 ± 3 and 74 ± 4 μ m, respectively; $P < 0.0001$, Student's *t*-test) but comparable in the proximal small intestine (Fig. 3A). The height of villi in the proximal and distal small intestine was similar in both strains (Fig. 3B). The thickness of the mucosa and the submucosa in the proximal and distal parts of the colon showed no significant difference between PRM/Alf and DBA/2J mice (Fig. 3C). The thickness of the tunica muscularis in the proximal and distal parts of the small intestine and the colon was not significantly different between PRM/Alf and DBA/2J mice (Fig. 3D).

Quantitative analysis of ICC distribution. Could the ICC distribution be modified in the lengthened intestine of PRM/Alf mice? To address this question, we made a quantitative analysis of X-gal-positive ICC distribution in the intestine of congenic PRM/Alf-*Kit*^{W-lacZ/+} mice. The *Kit* *lacZ* knock-in allele has been valuable in the study of ICC (5, 36). We used the 129S2-*Kit*^{W-lacZ/+} transgenic mouse line as a control. As shown in Fig. 4A, the ICC number was significantly higher in transverse sections of the proximal part of the small intestine in congenic PRM/Alf-*Kit*^{W-lacZ/+} mice compared with 129S2-*Kit*^{W-lacZ/+} mice (111 ± 22 and 94 ± 14 , respectively; $P = 0.02$, Student's *t*-test). The ICC number was also significantly higher in the distal part of the small intestine in congenic PRM/Alf-*Kit*^{W-lacZ/+} mice compared with 129S2-*Kit*^{W-lacZ/+} mice (111 ± 26 and 92 ± 14 , respectively; $P = 0.02$, Student's *t*-test). The difference was striking in transverse sections of the proximal and distal parts of the colon (Fig. 4, B and C). Indeed,

in these regions, the numbers of ICC in congenic PRM/Alf-*Kit*^{W-lacZ/+} mice were almost twice the numbers of ICC seen in 129S2-*Kit*^{W-lacZ/+} mice (569 ± 95 vs. 324 ± 29 and 219 ± 47 vs. 114 ± 33 in the proximal and distal colon, respectively; $P < 0.0001$, Student's *t*-test). Normalization of ICC number to the intestine circumference showed that an increased circumference did not significantly account for the higher number of ICC in each of the regions (data not shown). Thus the number of ICC in the intestine of congenic PRM/Alf-*Kit*^{W-lacZ/+} mice was increased compared with 129S2-*Kit*^{W-lacZ/+} mice.

Four types of ICC are found in the colon, named by their location (reviewed in Refs. 15 and 30): at the subserosa (ICC-SS), around the myenteric plexus (ICC-MP), in the circular muscle layer (ICC-CM), and at the border between the submucosa and the circular muscle layer, in association with the submuscular plexus (ICC-SMP). In the small intestine, ICC-MP and ICC-DMP are present in the myenteric region and along the deep muscular plexus, respectively. Is the number of ICC uniformly increased among these ICC types in PRM/Alf intestine? To answer this question, the relative distribution of the distinct ICC types was calculated on transverse sections through the wall of specific regions (Fig. 5). In the small intestine of congenic PRM/Alf-*Kit*^{W-lacZ/+} and 129S2-*Kit*^{W-lacZ/+} mice, the percentage of ICC-MP decreased from the proximal to the distal regions: from 93 to 61% in 129S2-*Kit*^{W-lacZ/+} mice and from 93 to 67% in congenic PRM/Alf-*Kit*^{W-lacZ/+} mice. The relative percentages of the ICC types were not significantly different between congenic PRM/Alf-*Kit*^{W-lacZ/+} and 129S2-*Kit*^{W-lacZ/+} mice in the proximal and distal small intestine and in the distal colon [MANOVA, *F* values (Wilks' lambda) (2, 5) = 0.163 and 0.726 in proximal and distal small intestine, respectively, and $F(4, 3) = 4.168$ in the distal colon, $P > 0.05$ in the 3 regions]. In the proximal colon, the relative percentages of the four types of ICC were significantly different between congenic PRM/Alf-*Kit*^{W-lacZ/+} and 129S2-*Kit*^{W-lacZ/+} mice [MANOVA, $F(4, 3) = 28.795$, $P = 0.01$]. Indeed, significantly different percentages of ICC-SS and ICC-MP were seen in the proximal colon of congenic PRM/Alf-*Kit*^{W-lacZ/+} mice compared with 129S2-*Kit*^{W-lacZ/+} mice (univariate *F*-tests, $P < 0.05$). By contrast,

Fig. 2. Comparison of intestine circumference between DBA/2J ($n = 3$) and PRM/Alf mice ($n = 4$). Values are means $\pm$ SE for the given populations. Asterisks indicate the statistical significance of the Student's *t*-test between DBA/2J and PRM/Alf mice: * $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$. NS, not significant.

Fig. 3. Morphometric comparison of the layers of the intestinal tract between DBA/2J ($n = 3$) and PRM/Alf ($n = 4$) mice. *A*: comparison of crypt height in the small intestine epithelium. *B*: comparison of villi height in the small intestine epithelium. *C*: comparison of mucosa and submucosa thickness in the colon. *D*: comparison of tunica muscularis thickness in the small intestine and in the colon. Values are means $\pm$ SE for the given populations. *** $P < 0.001$ between DBA/2J and PRM/Alf mice (Student's t -test).

the percentages of ICC-SMP and ICC-CM in the proximal colon did not differ significantly between congenic PRM/Alf-*Kit^{W-lacZ}/+* and 129S2-*Kit^{W-lacZ}/+* mice (univariate F -tests, $P > 0.05$). Importantly, the absolute number of ICC-MP in this region was significantly higher in congenic PRM/Alf-*Kit^{W-lacZ}/+* mice compared with 129S2-*Kit^{W-lacZ}/+* mice (216 ± 26 vs. 155 ± 35 , respectively; $P < 0.05$, Student's t -test).

Slow-wave frequency in PRM/Alf intestine. Generation of electrical slow waves is an intrinsic property of ICC (23, 25), and regular slow waves occur with the highest frequency in the duodenum (reviewed in Ref. 15). To test whether the increased ICC number in PRM/Alf mice was correlated with a higher slow-wave activity, we compared the spontaneous electrical activity of isolated small intestines from PRM/Alf and DBA/2J mice. Rhythmic variations of resting membrane potentials were recorded from isolated duodenum segments at 37°C. Slow-wave activity was normal in PRM/Alf isolated duodenum (Fig. 6A), and the slow waves were recorded at a frequency of 53 ± 3 cycles/min (Fig. 6B). In contrast, the frequency of slow waves in isolated duodena from DBA/2J mice was 45 ± 3 cycles/min. This 19% increase of the slow-wave frequency in PRM/Alf intestine was highly significant ($P < 0.0001$, Student's t -test).

Contractility of isolated small intestine segments from PRM/Alf mice. Intestinal smooth muscle cells contract phasically in response to electrical slow waves (reviewed in Ref. 18). To test the possibility that the intestine contractility could be modified in PRM/Alf mice, we isolated small intestine segments from PRM/Alf and DBA/2J mice and measured their contractility ex

vivo. Figure 7A illustrates plots of the phasic spontaneous longitudinal contractions displayed at 37°C by proximal small intestine segments without external stimulation. PRM/Alf proximal small intestines displayed phasic contractions at a regular rate. However, the frequency of proximal small intestine contractions was significantly higher in PRM/Alf mice compared with DBA/2J mice (46 and 39 sweeps/min, respectively; $P = 0.04$, Student's t -test) (Fig. 7B).

Gastrointestinal transit time in PRM/Alf mice. The increased contractility of isolated small intestine segments from PRM/Alf mice could result in an acceleration of the gastrointestinal transit. To test this possibility, we investigated the digestive transit time by using spores of *Bacillus subtilis* as a physically and chemically inert marker. The mean transit time of *Bacillus subtilis* spores in PRM/Alf mice was 10.6 ± 1.5 h. This duration was not statistically different from the mean transit time in DBA/2J mice (10.3 ± 1.0 h; $P > 0.05$, Student's t -test) (Fig. 8). An increased gastric emptying in PRM/Alf mice could explain the similar mean transit time despite the important lengthening of their intestine. Thus we determined gastric emptying in PRM/Alf mice. Gastric emptying of semiliquid bolus was not significantly different in 10 PRM/Alf and 9 DBA/2J mice ($55 \pm 11\%$ in PRM/Alf mice, $58 \pm 9\%$ in DBA/2J mice; $P > 0.05$, Student's t -test). Therefore, gastric emptying was not increased in PRM/Alf mice. Together, because PRM/Alf mice had a 30% longer gastrointestinal tract than DBA/2J mice (3) and an unchanged transit time, the speed of the gastrointestinal transit in PRM/Alf mice was increased compared with DBA/2J mice.

DISCUSSION

PRM/Alf mice show intestinal lengthening and fast digestive transit. The length of the intestine is genetically controlled in mammals. For instance, when European wild boars were crossed with domesticated Large White pigs, the length of the

Fig. 4. Interstitial cells of Cajal (ICC) distribution in the small intestine and colon from 129S2-*Kit^{W-lacZ}/+* ($n = 4$) and congenic PRM/Alf-*Kit^{W-lacZ}/+* ($n = 4$) mice. Quantification of ICC on 5 distant transverse sections per animal are shown. A: proximal and distal small intestine. B: proximal and distal colon. Numbers indicate the actual number of ICC; values are means $\pm$ SE for the given population in the given location. Asterisks indicate the statistical significance of the Student's *t*-test between 129S2-*Kit^{W-lacZ}/+* and PRM/Alf-*Kit^{W-lacZ}/+* mice: $*0.01 \leq P < 0.05$; $***P < 0.001$. C: β -galactosidase nuclear labeling of ICC on transverse sections of the proximal colon from PRM/Alf-*Kit^{W-lacZ}/+* (a) and 129S2-*Kit^{W-lacZ}/+* (c) mice and in the distal colon from PRM/Alf-*Kit^{W-lacZ}/+* (b) and 129S2-*Kit^{W-lacZ}/+* (d) mice. LM and CM, longitudinal and circular muscle layers, respectively. Scale bars = 100 μ m.

Fig. 5. Distribution of the types of ICC in intestine from 129S2-*Kit^{W-lacZ}/+* ($n = 4$) and congenic PRM/Alf-*Kit^{W-lacZ}/+* ($n = 4$) mice. 129, 129S2-*Kit^{W-lacZ}/+*; PRM, congenic PRM/Alf-*Kit^{W-lacZ}/+*; ICC-MP, ICC surrounding the myenteric plexus; ICC-DMP, ICC in the region of the deep muscular plexus; ICC-SS, subserosal ICC; ICC-CM, ICC in the circular muscle layer; ICC-SMP, submucosal ICC. Data are given as percentages, calculated as described in MATERIALS AND METHODS. Interpretation of the differences was made by MANOVA, with degrees of freedom involved with the test equal to 2 and 5 in the small intestine and to 4 and 3 in the colon. $*0.01 \leq P < 0.05$. NS, not significant.

small intestine in the F2 progeny was shown to be associated with a quantitative trait locus on chromosome 4 (1). The PRM/Alf strain was derived from a stock originally bred by mouse fanciers. The only selection applied during the inbreeding process was for homozygosity at the *patchwork* locus

Fig. 6. Slow-wave frequency of isolated duodena from DBA/2J ($n = 7$) and PRM/Alf ($n = 10$) mice. A: electrical activity of representative recordings from DBA/2J and PRM/Alf mouse duodena. The slow waves are sinusoidal. B: mean frequency of duodenal slow waves in DBA/2J mice and PRM/Alf mice. Values are means $\pm$ SE for the given population. $***P < 0.001$ between DBA/2J and PRM/Alf mice (Student's *t*-test).

Fig. 7. Representative tension recordings of isolated duodena from DBA/2J ($n = 3$) and PRM/Alf ($n = 3$) mice. **A**: patterns of intestine tension from a DBA/2J mouse and a PRM/Alf mouse. **B**: contraction frequency in DBA/2J mice ($n = 3$) and PRM/Alf mice ($n = 3$). $*0.01 \leq P < 0.05$ between DBA/2J and PRM/Alf mice (Student's t -test).

which, when mutated, is responsible for a singular coat color phenotype (2, 4). However, the patchwork mutation alone is not responsible for the intestinal phenotype of PRM/Alf inbred mice (3). Indeed, the genetic control of intestine length in PRM/Alf mice is polygenic and involves a maternal effect (3). The molecular etiology of the intestinal lengthening in PRM/Alf mice is still unknown; thus it is not possible to establish cause-and-effect relationships between the various features of PRM/Alf intestine described in this study.

PRM/Alf inbred mice exhibited a 30% increase in intestine length compared with control DBA/2J mice. The thickness of the intestinal wall was not modified despite a 40% increase in colonic circumference. Measurements of the tunica muscularis thickness were similar to those previously reported in BALB/c mice (7). By contrast, small intestine lengthening in transgenic mice overexpressing IGF-I locally in smooth muscle cells is associated with increased thickness of the muscular layers (40). Besides these anatomic and histological aspects, the intestinal motility of PRM/Alf mice showed striking alterations. The mean transit time was normal despite the intestine lengthening, indicating accelerated movement of chyme. Moreover, the frequency of ex vivo duodenal contractions at 37°C was higher in PRM/Alf mice ($46 \pm 2/\text{min}$) compared with DBA/2J mice ($39 \pm 3/\text{min}$). Duodenal contractions are known to occur in vivo at a frequency of $\sim 47/\text{min}$ in randomly bred mice (10). The lower frequency measured by our assay likely resulted from absence of extrinsic innervation. Indeed, without extrinsic innervation, the enteric nervous system together with the ICC controls smooth muscles coordinated activity. Double immunofluorescence with antibodies specific to ICC (Kit) and neurons (PGP9.5) were realized on whole mount intestines and showed a normal morphology of the myenteric plexuses in the PRM/Alf small intestine (unpublished results).

Fig. 8. Comparison of the mean transit times of a gastrointestinal transit marker between DBA/2J ($n = 4$) and PRM/Alf ($n = 4$) mice. The difference observed between DBA/2J and PRM/Alf was not significant.

Slow-wave frequency and the number of ICC are increased in PRM/Alf mice. The slow-wave frequency is species specific and characteristic for each location within the intestine. In C57BL/6 mice, the slow-wave frequency reaches 56 cycles/min at 0.5 cm distal from the pylorus and decreases to 45 cycles/min at 5–8 cm from the pylorus (9). In our experiments, the slow-wave activity was recorded 2 cm distal to the pylorus and the slow-wave frequency was found to be in the same range of values. As for contractions, the slow-wave frequency in PRM/Alf-isolated duodenums showed an almost 20% increase compared with DBA/2J (53 and 45 cycles/min, respectively). The slow waves govern the maximal frequency of the muscle contraction in response to excitatory stimuli from the enteric nervous system. A linear relationship may exist between the slow waves and the frequency of the contractions.

It is established that the slow-wave frequency can be modulated. For example, *Trichinella spiralis* infection causes electrical uncoupling and focal increase in slow-wave frequency, leading to orally propagating contractions that slow the transit in the proximal small intestine (9). In PRM/Alf mice, in vivo video analyses of peristalsis after gavage with barium fluid revealed normal aborally propagating contractions and rapid progression of the barium fluid by comparison with a control strain (data not shown), suggesting that the general control of peristalsis is normal. The number of ICC was significantly higher both in the small intestine and in the colon of congenic PRM/Alf-*Kit*^{W-lacZ/+} mice compared with 129S2-*Kit*^{W-lacZ/+} mice. The enlargement of the colon in the PRM/Alf strain had only a minor contribution to this increase. Furthermore, all types of ICC were more abundant in the PRM/Alf intestine. To our knowledge, this is the first spontaneous animal model in which an ICC number increase has been observed. This result raises new questions about the relationship between the number of ICC and the frequency of the slow waves. Interestingly, in patients with severe slow-transit constipation, the number of ICC was significantly decreased in all layers of the sigmoid colon (14). The data presented in this study are consistent with the proposal that the rise in the slow-wave frequency in PRM/Alf mice could be related to the ICC number increase. Conversely, the ICC number increase could be an adaptive response to the faster transit. Indeed, adaptation of the ICC number to physiological modifications may occur; partial obstruction of the gastrointestinal tract induces the disruption of the ICC network, but ICC regenerate when the obstruction is removed (7). Of course, the other partners in the peristaltic contractions (the enteric nervous system and the smooth muscles) could also play a key role in the faster digestive transit. In particular, we cannot exclude that specific subpopulations of enteric neurons might be altered in PRM/Alf intestine.

Interestingly, hyperplasia of ICC, with 10–20 cell layers instead of 1 or 2 layers in the normal intestine, was observed in the small and large intestines in patients with familial and multiple gastrointestinal stromal tumors (GISTs) (reviewed in Ref. 22). A Val⁵⁵⁹ deletion mutation in the Kit receptor was identified in a human familial GIST case (28). This mutation was recently introduced into the mouse germline by using knock-in gene targeting technology. Mice carrying the engineered mutation showed patchy hyperplasia of Kit-positive cells similar to that of the disease observed in human patients (31). It will be of interest to study the slow-wave activity, contractility, and gastrointestinal transit time in the mutant mice.

The intestine is a single organ composed of cells of various embryological origin combined for performing a vital function: digestion of food. The PRM/Alf inbred strain shows that despite the huge constraints imposed on each of the different cell types, enormous lengthening may happen. However, the transit time in PRM/Alf mice remains unchanged compared with other mice. As François Jacob wrote, “[A]lthough the function brooks no fantasy, the organ preserves some slight degree of freedom” (20).

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