

Adhesion formation and interanimal variability in a laparoscopic mouse model varies with strains

Adhesion formation after laparoscopic surgery was evaluated in mice of different strains. More adhesions were observed in Swiss, NMRI, and BALB/c mice, with less interanimal variability in BALB/c mice. These data point to genetics effects on adhesion formation, which open new insights in its pathogenesis and indicate the importance of a careful strain selection for animal studies. (*Fertil Steril*® 2005;83:1871–4. ©2005 by American Society for Reproductive Medicine.)

During the past years we developed a laparoscopic mouse model for the study of postoperative adhesion formation and reported that the CO₂ pneumoperitoneum is a cofactor in adhesion formation (1). Because adhesion formation increases with the duration of the pneumoperitoneum and with the insufflation pressure, without differences between CO₂ and helium pneumoperitoneum, our data indicate that this pneumoperitoneum-enhanced adhesion formation is mediated to a large extent by peritoneal hypoxia (1) and to a lesser extent by acidosis (2). This hypothesis of pneumoperitoneum-induced peritoneal hypoxia as a driving mechanism in pneumoperitoneum-enhanced adhesion formation was confirmed in transgenic mice that underexpress/overexpress genes encoding for factors regulated by hypoxia, such as hypoxia-inducible factors (3), members of the vascular endothelial growth factor family (4, 5), and of the plasminogen system (6). These transgenic mice and their controls were from different strains than the previously used NMRI mice (1, 7). The results from these consecutive experiments, evaluated retrospectively for strain differences, strongly suggested important strain effects on postoperative adhesion formation. Therefore, a prospective randomized study was performed to ascertain and document these strain-related differences in adhesion formation.

The studies were approved by the Institutional Review Animal Care Committee and performed in 10- to 12-week-old female mice. Animals were anesthetized with pentobarbital (IM, 0.07 mg/g), intubated and ventilated with room air with a tidal volume of 500 μ L at 80 strokes/min (Small Animal Ventilator, model 683; Harvard Apparatus Inc., Holliston, MA) as described (1–6).

Experiments were carried out at room temperature and laparoscopic surgery for induction of intraperitoneal adhe-

sions was performed as described (1–6). Briefly, a 2-mm endoscope with a 3.3-mm sheath was introduced into the abdominal cavity caudal to the xyphoides. Heated (37°C, Optitherm; Karl Storz, Tuttlingen, Germany) and humidified (100% relative humidity, Aquapor; Dräger, Lübeck, Germany) CO₂ at 20 cm H₂O (~14 mm Hg) of insufflation pressure was used for the pneumoperitoneum.

Standardized lesions in uterine horns and pelvic sidewalls were performed with monopolar and bipolar coagulation. The time required to establish the pneumoperitoneum and to perform the lesions was 5–6 minutes but the pneumoperitoneum was maintained for a longer period (60 minutes) to evaluate pneumoperitoneum-enhanced adhesion formation (2–6). After 7 days, adhesions were quantitatively (proportion) and qualitatively (extent, type, tenacity, and total) scored during laparotomy, as described (1–6).

Statistical analyses were performed with the GraphPad Prism 4 (GraphPad Prism Software Inc., San Diego, CA) using Kruskal-Wallis with Dunn's multiple comparisons tests for comparisons of nonparametric variables (adhesion scores) and one-way ANOVA with Bonferroni test for comparisons of parametric variables (body weights). Spearman test was used for correlation of strain and adhesion formation and of body weight and adhesion formation. Two-tailed *P* values <.05 were considered significant. Because the same trend was observed with both scoring systems, only the mean of the proportion of adhesions formed at the four individual sites are presented (means \pm SD).

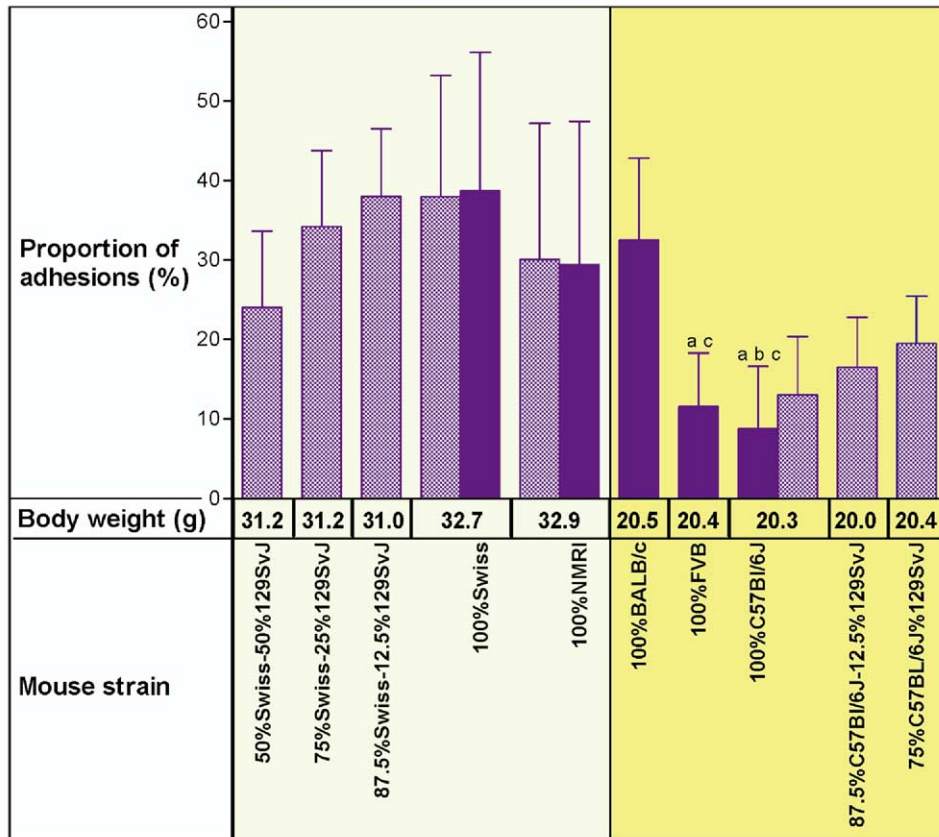
In the retrospective study (Fig. 1), wild-type mice of the following strains were evaluated: 100% Swiss (n = 20; 32.8 \pm 1.9 g) (4, 5), 87.5% Swiss-12.5% 129SvJ (n = 5; 31.0 \pm 1.0 g) (3), 75% Swiss-25% 129SvJ (n = 5; 31.2 \pm 1.3 g) (4), 50% Swiss-50% 129SvJ (n = 10; 31.2 \pm 1.2 g) (3, 4), 100% NMRI (n = 10; 32.8 \pm 1.8 g) (2), 100% C57BL/6J (n = 5; 20.2 \pm 0.8 g) (4), 87.5% C57BL/6J-12.5% 129SvJ (n = 5; 20.0 \pm 0.7 g) (6), and 75% C57BL/6J-25% 129SvJ (n = 5; 20.4 \pm 1.1 g) (6). In mice with a mixture of Swiss and 129SvJ background, more adhesions were observed with more Swiss background (*r* = 0.5,

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FIGURE 1

Postoperative adhesion formation was evaluated in inbred and outbred mice of different strains and with different body weights (means are indicated). Animals, body weight clustered into two groups, one of ~32 g (*yellow background*) and another of ~20 g (*orange background*). Pneumoperitoneum-enhanced adhesions were induced with standardized lesions during laparoscopy (CO₂ pneumoperitoneum for 60 minutes at 20 cm H₂O) and scored after 7 days during laparotomy in a retrospective study (*grid bars*) and in a prospective randomized study (*closed bars*). The proportion of adhesions (means ± SD) with differences statistically significant vs. Swiss (a), NMRI (b), and BALB/c (c) mice are indicated.



Molinas. Mouse strain and adhesion formation. *Fertil Steril* 2005.

$P = .0002$, Spearman correlation). In mice with a mixture of C57BL/6J and 129SvJ background, more adhesions were observed with less C57BL/6J background ($r = -0.5$, $P = .01$, Spearman correlation).

In the prospective randomized study (Fig. 1), wild-type mice of the following strains were used: 100% Swiss ($n = 10$; 32.6 ± 1.7 g) a 100% NMRI ($n = 10$; 33.1 ± 1.5 g), 100% BALB/c ($n = 10$; 20.6 ± 1.3 g), 100% FVB ($n = 10$; 20.4 ± 1.3 g), and 100% C57BL/6J ($n = 10$; 20.4 ± 0.8 g). The choice of these strains was determined by the availability at the Katholieke Universiteit Leuven. The study was carried out using block randomization by days (i.e., one block comprising one animal of each strain was operated on the same day), and within a block animals were operated on in a random order. In this study intergroup

differences in adhesion formation were evaluated (Kruskal-Wallis with Dunn's multiple comparisons tests). No differences in adhesion formation was observed between Swiss, NMRI, and BALB/c mice ($P =$ not significant [NS] for all comparisons), but in all these strains adhesion formation was higher than in FVB ($P < .01$, $P =$ NS, and $P < .01$) and in C57BL/6J ($P < .001$, $P < .05$, and $P < .01$) mice. No differences in adhesion formation were observed between FVB and C57BL/6J mice ($P =$ NS).

In addition to the amount of adhesion formation, the variability of adhesion formation between animals was calculated. The coefficient of variation ($SD/\text{mean} \times 100$) was 45% for Swiss mice and 61% for NMRI mice, both outbred strains, but only 32% for the inbred BALB/c mice. For the inbred FVB and C57BL/6J mice, the coefficient of

variation were 59% and 90%, respectively, reflecting the very low adhesion formation potential.

Differences in body weight (one-way ANOVA with Bonferroni test) and the relationship between body weight and adhesion formation (Spearman correlation) were evaluated in detail because it seemed that most strains with low weight developed less adhesions than strains with high weight. Two clusters of body weight (yellow and orange background in Fig. 1) without overlap were found, that is, one cluster of mice weighing ~32 g (Swiss and NMRI mice) and another cluster of mice weighing ~20 g (BALB/c, FVB and C57BL/6J mice). Within each cluster no significant differences in body weight were observed ($P=NS$ for all comparisons), whereas both Swiss and NMRI mice weighed more than BALB/c ($P<.001$, $P<.001$), FVB ($P<.001$, $P<.001$), and C57BL/6J ($P<.001$, $P<.001$) mice. Within each cluster no correlation between body weight and adhesion formation was found. An overall correlation could, obviously, not be done because body weight clustered in two groups only. Moreover, BALB/c mice with a low body weight had more adhesions than all other strains with low body weight, whereas compared to mice with high body weight adhesion formation was not statistically different.

Strain differences have been reported for other processes involving fibrosis and healing responses such as hepatic, lung, and colorectal fibrosis (8–11), myocardial and ear wound healing (12, 13), and bone regeneration (14). To the best of our knowledge this is the first study indicating that genetic background also influences adhesion formation, at least after laparoscopic surgery. Among the strains evaluated we found that Swiss, NMRI, and BALB/c mice developed more adhesions compared to FVB and C57BL/6J mice, in which adhesion formation was minimal. About the mechanisms causing these interstrain differences, at present, we only can speculate. For none of the potential mechanisms, such as cellular interaction (e.g., macrophages, fibroblasts, mesothelial and endothelial cells) or molecular expression (e.g., plasminogen system, vascular endothelial growth factor, hypoxia-inducible factors, reactive oxygen species, matrix metalloproteases), modulating the processes of inflammation, fibrin deposition/degradation, extracellular matrix deposition/degradation, and angiogenesis (15–17), clear data about strain differences are available.

Our data also demonstrate that interanimal variability is less in the inbred BALB/c mice than in the outbred Swiss and NMRI mice. This is not surprising because inbred strains, maintained by sibling (brother \times sister) mating for 20 or more generations, are genetically almost identical, homozygous at virtually all loci, and with high phenotypic uniformity (18). This less interanimal variability in inbred strains has been reported for many processes such as sleeping time under anesthesia (19). The high variability in the

inbred FVB and C57BL/6J mice, with very low adhesion formation potential, is also not surprising because the absence of adhesions in many of these animals leads to artificially high coefficient of variation.

These observations on genetic influences contribute to the usefulness of the mouse model for adhesion formation studies. The mouse model has many advantages compared to other animal models because it is relatively cheap, easy to handle, and does not require strict sterile conditions for surgery. Furthermore, it is particularly useful for mechanistic studies because of the availability of animals with low genetic variability (i.e., inbred mice), underexpressing/overexpressing specific genes (i.e., transgenic mice), and immunodeficient by spontaneous mutation (i.e., nude mice [T-cell deficient] and SCID mice [T&B cell deficient]). In addition, many specific mouse assays and monoclonal antibodies are available.

Both observations (i.e., strain differences in adhesion formation potential and in interanimal variability) point to genetics effects on adhesion formation, which is not surprising and confirms clinical observations. The importance of these observations is twofold. First, to study the genetic involvement in detail, the use of two strains with high and low adhesion formation potential can be considered as an experiment of nature. Second, to study adhesion formation and prevention, it is preferable to use a strain with high adhesion formation potential and low interanimal variability, such as BALB/c mice. Furthermore, fewer inbred animals will be needed to achieve a given level of statistical precision than if outbred animals had been used (18). We, however, want to point out that inbred strains in general weigh less than outbred strains (average of 20 g vs. 32 g), which increases the technical skills required to do the experiments, especially those involving laparoscopic surgery.

In conclusion, this study demonstrates that some mouse strains develop more postoperative adhesions than others and that the interanimal variability in inbred strains is less. These data should not be underestimated for adhesion formation studies in animal models and, although very preliminary, can open new insights in the pathogenesis of adhesion formation in humans.

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