Reproductive Sciences
https://doi.org/10.1007/s43032-022-00900-7

GENERAL GYNECOLOGY: SHORT COMMUNICATION

Genetic Predisposition of Postoperative Adhesions Varies in Substrains of BALB/c Mice

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Received: 21 October 2021 / Accepted: 20 February 2022

Abstract
Postoperative adhesions are a major clinical problem because of the associated infertility, chronic pain, bowel obstruction, and the associated costs. Variability in adhesion formation was suggested by clinical observations that apparently similar interventions can cause little to severe adhesions. This is supported by the presence of polymorphisms and genetic predisposition to develop adhesions in animal models and humans. We previously demonstrated differences in postoperative adhesions between different mouse strains. In this study, we aimed to investigate the variability in adhesion formation in inbred substrains of BALB/c mice. Since genetic differences in inbred substrains are minimal, they might be an opportunity to tackle the genetics of adhesion formation.

Keywords Postoperative adhesions · Laparoscopy · Strains · Substrains · BALB/c · Genetic predisposition

Introduction

Postoperative adhesions are a major clinical problem because of the associated infertility, chronic pain, bowel obstruction, and associated costs [1]. The pathophysiology begins to be understood, but prevention remains limited to meticulous surgery, barriers, and peritoneal conditioning. However, adhesion formation is variable and poorly predictable in the individual, thus preventing individualization of therapy. Moreover, standard statistical analyses cannot detect eventually hidden subgroups.

Variability in adhesion formation was suggested by clinical observations that apparently similar interventions can cause little to severe adhesions. This is supported by the presence of polymorphisms and genetic predisposition to develop adhesions [2]. In animal models, differences in adhesion formation between different strains of mice are well established, i.e. Swiss, NMRI, and BALB/c mice develop more adhesions than FVB and C57BL/6 J mice [3].Variability is less in inbred than in outbred strains. Although the mechanism of these differences is not yet understood, underlying genetic differences seem a reasonable assumption.

To this variability in strains, we want to add variability in three inbred substrains of BALB/c mice, i.e. BALB/cByJ, BALB/cOlaHsd, and BALB/cJRj. Variability in substrains was investigated, using our laparoscopic mouse model, with standardised and documented variables as manipulation, duration of surgery, pneumoperitoneum pressure, temperature, desiccation, irrigation fluid, mesothelial hypoxia and adhesion prevention by barriers, more than 5% N_2O, and numerous medications [4] [5] [6]. Since genetic differences in inbred substrains are minimal, they might be an opportunity to tackle the genetics of adhesion formation.

Materials and Methods

Three substrains of female 9–10-week-old BALB/c mice were investigated ($n = 36$, 6 groups, $n = 6$/group): BALB/cOlaHsd (Harlan Laboratories B.V., The Netherlands-Groups I and II), BALB/cByJ (Charles River Laboratories, France-Groups III and IV), and BALB/cJRj (Bio Services

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Published online: 08 March 2022

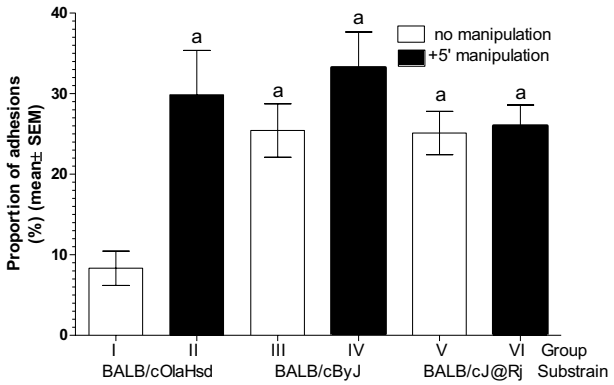
B.V., The Nertherlands which obtains mice from Janvier, France-Groups V and VI). The study was approved by the Institutional Review Animal Care Committee (P111/2011) of KU Leuven. Experimental conditions were described previously, i.e. housing. anaesthesia (intraperitoneal 0.08 mg/g pentobarbital, mechanical ventilation (Mouse Ventilator MiniVent, Type 845, Hugo Sachs Elektronik-Harvard Apparatus GmbH, Germany)), control of body temperature at 37 °C (Homeothermic Blanket, Harvard Apparatus LTD, UK), laparoscopy with a 2-mm endoscope without gaz leaks, pneumoperitoneum 15 mm Hg insufflation pressure for 60 min (Thermoflator Karl Storz, Germany) with humidified CO_2 (Storz Humidifier 204,320 33, Karl Storz, Tuttlingen, Germany), standardised 10 × 1.6-mm lesions in the antimesenteric border of both right and left uterine horns and pelvic sidewalls with bipolar coagulation (BICAP™, bipolar hemostasis probe, BP-5200A, 5 Fr; IMMED Benelux, Linkebeek, Belgium) at 20 watts (Autocon 200, Karl Storz, Tuttlingen, Germany). Besides CO_2 pneumoperitoneum–enhanced adhesions (Groups I, III, V), manipulation-enhanced adhesions were performed (Groups II, IV, and VI) by manipulating bowels in the upper abdomen with the plastic tip of a catheter for 5 min [6] (see experimental design at Fig. 1). Since scoring of adhesions was similar after 7 and 28 days [4], adhesions were blindly scored after 7 days. Scoring was done quantitatively (proportions: sum of the length of the individual attachments/length of the lesion) × 100) and qualitatively for extent (0: no adhesions; 1: 1–25%; 2: 26–50%; 3: 51–75%; 4: 76–100%), type (0: no adhesions; 1: filmy; 2: dense; 3: capillaries present), tenacity (0: no adhesions; 1: essentially fall apart, 2: require traction; 3: require sharp dissection), and total (extent + type + tenacity) during laparotomy using a stereomicrosope [5]. These experiments were performed as described [4, 6] using block randomisation by day and a factorial design with 6 animals per cell resulting in a power of 12 animals when comparing 2 subgroups (2 types of enhanced adhesions). Differences in body weight were evaluated using a one-way ANOVA and Bonferroni's Multiple Comparisons Test (Prism 5.0). Differences in adhesion formation were evaluated with two-way ANOVA and Bonferroni's post-tests. Multivariate analysis was performed using the Proc logistics procedure taking into account, the adhesion scores, the substrains, and the manipulation as variables (SAS System, SAS Institute, Cary, NC, USA). A significance level of 0.05 was used.

Results

BALB/cByJ substrain has a lower body weight (20.3 ± 0.3 gr) than BALB/cOlaHsd (21.8 ± 0.5 gr) ($p < 0.05$), and BALB/cJRj (22.6 ± 0.3 gr) ($p < 0.05$). Adhesion formation varies with substrains (Fig. 1). CO_2 pneumoperitoneum–enhanced adhesions were less in BALB/cOlaHsd than in BALB/cByJ (group I vs III: proportions: $p < 0.01$; extent: $p < 0.05$) or BALB/cJRj (group I vs V: proportions: $p < 0.05$; extent: $p < 0.03$; type: $p = 0.05$; total: $p < 0.03$) substrains. There are no differences between BALB/cByJ and BALB/cJRj substrains (group III vs V).

Manipulation-enhanced adhesions also vary with the substrain. Adhesions significantly increased in BALB/c OlaHsd (group I vs II: proportions: $p < 0.03$; extent: $p < 0.03$; type: $p < 0.035$; total: $p < 0.025$), but not in BALB/cByJ (group III vs IV) and BALB/cJRj (group V vs VI) substrains.

By multivariate analysis, adhesions vary simultaneously with the substrain of BALB/c mice (proportions: $p < 0.01$; extent: $p < 0.03$; total: $p < 0.04$) and with manipulation (proportions: $p < 0.003$; extent: $p < 0.01$; type: $p < 0.01$; total: $p < 0.02$).



Group	BALB/c Substrains	5 min Manipulation	Qualitative Scoring (Mean±SEM)			
			Extent	Type	Tenacity	Total
I	BALB/cOlaHsd	no	0.45±0.12	0.55±0.16	0.75±0.19	1.75±0.46
II		yes	1.33±0.25[a]	1.25±0.13[a]	1.33±0.17	3.91±0.54[a]
III	BALB/cByJ	no	1.25±0.18[a]	1.21±0.16	1.42±0.19	3.88±0.51
IV		yes	1.60±0.19[a]	1.30±0.12[a]	1.35±0.15	4.25±0.40[a]
V	BALB/cJ@Rj	no	1.25±0.11[a]	1.05±0.05[a]	1.10±0.10	3.40±0.23[a]
VI		yes	1.25±0.11[a]	1.25±0.11[a]	1.35±0.10[a]	3.85±0.26[a]

Fig. 1 Postoperative adhesions after laparoscopic surgery in BALB/c related substrains. Adhesions were induced during laparoscopy by a bipolar lesion, 60 min of CO_2 pneumoperitoneum (all the groups), and also 5 min of manipulation (groups II, IV, VI) in BALB/cOlaHsd, BALB/cByJ, and Balb/cJRj, respectively. Postoperative adhesions were scored after 1 week using a quantitative (*on the left*) and a qualitative scoring system (*on the right*). [a]Comparison vs group I: $p < 0.05$ (2-way ANOVA, Bonferroni post-test)

Discussion and Conclusion

These data demonstrate that adhesions vary with substrains of inbred mice. The results otherwise confirm that manipulation in the upper abdomen increases adhesion formation in the lower abdomen, an effect transmitted through peritoneal fluid [6]. Results also confirm the low variability in adhesion formation in inbred strains [7] because of isogenicity (all animals are genetically almost identical), and homozygosity (animals are homozygous at virtually all genetic *loci*).

Our data add adhesion formation to the known minor differences observed in substrains of similar inbred mice such as behavioral differences [8], fear response [9], tissue rejection [10], arthritis susceptibility [11], body and organs weight [12], and sperm abnormality [13]. These minor differences may result from residual heterozygosity, incomplete inbreeding, spontaneous mutations, genetic drift, and genetic contamination. Minor genetic differences have been demonstrated, such as a deletion of the *alpha-synuclein* locus in C57BL/6JOlaHsd7 [14] and mutations at the *Raf-1* locus in BALB/cJ, at the *Gdc-1* locus in BALB/cHeA, at the *Qa-2* locus in BALB/cByA [11]. Discriminatory single-nucleotide polymorphisms (SNPs) were found in ten C57BL/6-related substrains [15]. Thakur M *et al.* [16] found several SNPs, genetic mutations, and upregulation of messenger RNAs involved in postoperative adhesions, i.e. in genes for transforming growth factor beta, vascular endothelial growth factor, interferon-gamma, matrix metalloproteinase, plasminogen activator inhibitor-1, and interleukins. In addition, transgenerational transmissible epigenetic changes caused by environmental factors have to be considered. Atta *et al.* demonstrated a role of histone methyltransferase EZH2 in adhesion formation [17].

Inbred strains of mice and their substrains might be an opportunity to investigate the genetic and epigenetic mechanisms of adhesion formation. This could lead to oriented and targeted prevention in the human, especially if associated with the identification of those at risk. Adhesion formation is known to vary widely between strains of mice. The individual variability within each strain is much less in inbred strains probably since genetically very similar with transgenerational genetic immortality and long-term stability. Inbred mice strains therefore are ideally suited to identify which genes are important. First, the low variability of adhesions permits to limit the number of animals in experiments, and thus the cost of experiments. In addition, mice are well-known models for developing knock out or genetically modified strains. In addition to genetic changes, these inbred strains are an ideal model to investigate eventual epigenetic effects either by manipulation or by environmental effects, which might explain the differences in substrains bred at different locations. This model might in addition be an opportunity to investigate the poorly understood transgenerational transmission of epigenetic changes as suggested in the human for aspects as music. Genetic and epigenetic research will moreover be facilitated by the identifiability of inbred strains, having a unique set of genetic markers, which today are used for genetic quality control and maintenance of homozygosity within the colony. Adhesion formation could be considered a specific aspect of individuality (phenotypic characteristics that are important for specific research questions).

In conclusion, postoperative adhesion formation varies not only between different strains and inbred strains [3] but also between substrains of inbred BALB/c mice. Since these mice are genetically almost identical, they might be an opportunity to investigate which genes are important in postoperative adhesion formation. If differences in substrains bred at different locations would be due to epigenetic changes, these mice would be a unique opportunity to investigate transgenerational transmissible epigenetic changes.

Acknowledgements Karl Storz Endoscopy is acknowledged for the generous supply of the laparoscopic equipment.

Availability of Data and Material Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval The study was approved by the Institutional Review Animal Care Committee of the Katholieke Universiteit Leuven (P111/2011).

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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