MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2021; 27: e928676
DOI: 10.12659/MSM.928676

Received: 2020.09.19
Accepted: 2020.11.29
Available online: 2020.12.23
Published: 2021.02.23

Different Responses to Identical Trauma Between BALB/C and C57BL/6 Mice

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

CDEF 1 Xiuying Chen*
BCD 1 Chang Cheng*
EF 2 Wenchao Cheng
EF 1 Yuhan Wang
B 1 Xuzheng Zuo
B 1 Weiju Tang
CD 3 Zhanyang Yu
B 4 Zhihuan Yang
AD 4 Zhengguo Wang
AG 4 Peifang Zhu
ADG 1 Wen Huang

1 Department of Neurology, Second Affiliated Hospital of Army Medical University, Chongqing, P.R. China
2 Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, P.R. China
3 Neuroprotection Research Laboratory, Departments of Radiology and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, U.S.A.
4 Department 4, Institute of Surgery Research, Daping Hospital, Army Medical University, Chongqing, P.R. China

* Xiuying Chen and Chang Cheng contributed equally to this work

Corresponding Authors: Wen Huang, e-mail: huangwen@tmmu.edu.cn, Peifang Zhu, e-mail: ozaka00806@163.com
Source of support: Departmental sources

Background: Different responses to identical trauma may be related to the genetic background of individuals, but the molecular mechanism is unclear. In this study we investigated the heterogeneity of trauma in mice and the potential biological explanations for the differences.

Material/Methods: Compared with other organs, the pathological response of the lung after injury is the earliest and most serious. We used C57BL/6 and BALB/C mice to explore the genetic background of different responses to trauma in the lung. We measured mortality rate, pulmonary microvascular permeability, and *Cxcl15* gene expression in BALB/C and C57BL/6 mice before and after blast-wave injury. Microvascular permeability was measured using a fluorescent tracer, and *Cxcl15* gene expression level and expression distribution were measured using fluorogenic probe quantitative polymerase chain reaction and northern blot.

Results: C57BL/6 mice showed lower mortality rates and pulmonary microvascular permeability than BALB/C mice after blast-wave injury; there was no significant difference in the permeability before blast-wave injury. The *Cxcl15* gene was expressed specifically in the lung tissue of mice. The level of *Cxcl15* expression in BALB/C mice was higher than in C57BL/6 mice before and after injury, and the variation trend of *Cxcl15* expression level after injury was significantly different between BALB/C and C57BL/6 mice.

Conclusions: Our results indicated that BALB/C and C57BL/6 mice had significant heterogeneity in posttraumatic response in terms of mortality and degree of lung damage. The differences in genetic factors such as *Cxcl15* may have played a role in this heterogeneity.

Keywords: Genetic Heterogeneity • Lung Injury • Mice, Inbred BALB C • Mice, Inbred C57BL

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/928676

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Background

Individuals have differential responses to similar traumatic events, a phenomenon known as the heterogeneity of traumatic responses. Most studies have used differences in nutritional, physical, or mental state to explain this phenomenon; however, the molecular mechanism remains unclear.

Increasing evidence [1-3] suggests that individual differences in genetic background may be an important reason for the heterogeneity of traumatic responses. Previous investigation in our laboratory [4] revealed that the pathological response in the lung, compared with other organs, is the earliest and most serious after injury. In our study, we randomly anatomized surviving mice 72 h after injury. Upon visual examination, we found that the degree of lung injury in BALB/c mice appeared more severe than in C57BL/6, and this finding was confirmed by microscopic imaging of pathological sections. In addition, our previous gene chip results suggested that the expression of the *Cxcll5* gene in the lung of C57BL/6 mice was upregulated after injury compared with the control group. In contrast, the *Cxcl15* gene expression was downregulated in the lung of BALB/c mice.

Given the previous findings, in addition to acute lung injury accounting for high rates of patient morbidity and mortality after physically traumatic events [5] and pulmonary microvascular permeability being an early impairment event after trauma that triggers further inflammatory responses, we speculated that anatomical structures associated with the microvascular permeability (such as vascular diameter, vascular compliance, and cell conjunction), among other factors, were associated with the trauma response differences. In order to explore the genetic background of different responses to trauma in the lung, we tested pulmonary vascular permeability before and after injury in C57BL/6 and BALB/C mice. We additionally detected *Cxcl15* gene expression at the same time.

Material and Methods

Animal Model And Injury Condition

In this study, we used inbred 8- to 9-week-old specific pathogen-free BALB/C and C57BL/6 mice (Shanghai Laboratory Animal Centre of the Chinese Academy of Sciences, Beijing, China). The average weight of the mice was 20.78±1.25 g, and the weight difference in the same batch of experimental animals was <1.00 g. We used pentobarbital anesthesia immediately before the blast injury procedure at a dose of 40 mg/kg. The animals were humanely sacrificed by cervical dislocation 72 h after blast injury.

The systemic blast injury (SBI) model was designed with a BST type-1 biological shock tube (Research Institute of Field Surgery, Army Medical University, Beijing, China) with a driving pressure of 6.0 MPa. Overpressure peak value and positive pressure action time of the vertical plane was measured by a baroceptor. Data were analyzed with DASYlab 5.0 software (Data Acquisition System Laboratory).

Mice were kept at room temperature (22±2°C), with ad libitum access to food and water. The Institutional Animal Care and Use Committee of the Army Medical University approved all animal care and experimentation (SYXK-PLA-2007035). Efforts were made to minimize the suffering of the mice. Due to the experimental intervention, no mice died without euthanasia.

Observation Indices

Mortality rate was measured at 1, 6, 24, and 72 h after injury (60 BALB/C mice and 59 C57BL/6 mice). Pulmonary tissue sections were extracted from euthanized animals and prepared according to standard procedures. Pathomorphological examination of the sections by light microscopy, using routine hematoxylin and eosin staining, revealed the hemorrhagic and edemic state of pulmonary tissues.

Comparison of Microvascular Permeability

Microvascular permeability was measured by the fluorescence tracer method at 0.5, 1, and 4 h after injury in 20 BALB/C mice and 20 C57BL/6 mice. Prior to trauma, 5 BALB/C mice and 5 C57BL/6 mice were injected with 0.2 mL of 0.5% fluorescein sodium (FINa) (with saline water as a blank control) through the caudal vein for 10 min, and 0.2 g of pulmonary tissue was collected, homogenized, and centrifuged (4000 rpm, 20 min). The supernatant was measured by fluorescence spectrophotometer (437-nm excitation, 518 nm emitted, and 5-mm slit width). Microvascular permeability was indirectly measured by the quantity of FINa per gram of tissue using a fluorescence intensity standard curve. The standard curve was delineated according to a standard intensity. The regression equation was y=551.3x+3.2491 (y, fluorescence intensity; x, FINa in milligrams; γ, coefficient correlation, 0.999). After trauma, the microvascular permeability measurement was repeated.

RNA Extraction and Fluorescence Quantitative Polymerase Chain Reaction

Primer sequences were synthesized from Shanghai Shenyou Company as follows:
Cxcl15 sense strand: 5'-TTA GCTCA CAACA GT G-ATAGG-3',
antisense strand: 5'-GTC ACC TGTGAA CAT TCA CT-3',
and probe: FAM5'-AAG TCAGCTTGA CAG GCAGTGATG C-3'TAMRA;
GAPDH sense strand: 5'-TGC ACC ACC AAC TGC TTAG-3',

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antisense strand: 5'-GGA TGC AGG GATGATGTTC-3',
and GAPDH probe: FAM 5'-CAG AAG ACTGTG GATGGC CCC TC-3' TAMRA.
A previously described procedure was used for RNA extraction [6], and RNA was stored at 4°C for future use. An extracted RNA sample volume of 20 µL was made up by 3 µL of template, 10×2 µL buffer, 4 µL of $MgCl_2$ (25 mmol/L), 2 µL of dNTP (10 mmol/L), 0.5 µL of enzyme inhibitor (40 U/µL), 1 µL of AWV reverse transcriptase (5 U/µL), 1 µL of Oligo dT-Adaptor primer (2.5 pmol/µL), and 6.5 µL of enzyme-free water. Water was added to the template for denaturation at 72°C, and then the rest of the sample volume was added for the reaction. The reaction conditions were 72°C for 2.5 min, 42°C for 40 min, and 95°C for 5 min, respectively. The sample volume for fluorescence quantitative polymerase chain reaction (PCR) included 1 µL of template, 10×5 µL of buffer, 5 µL of $MgCl_2$ (25 mmol/L), and 1 µL of dNTP (10 mmol/L). The following were then added to the reaction system: 0.8 µL of sense chain (20 pmol/µL), 0.8 µL of antisense strand (20 pmol/µL), 0.4 µL of probe (20 pmol/µL), 0.5 µL of *Taq* polymerase (5 U/µL), and 50 µL of H_2O. The efficiency of the PCR was evaluated for each of the amplification conditions. Reaction conditions were as follows: 94°C for 2 min, 94°C for 10 s, 53°C for 30 s, and 72°C for 40 s, repeating for 45 cycles, followed by 72°C for 10 min.

Northern Blot Analyses

Tissue samples from the heart, brain, spleen, lungs, liver, skeletal muscles, kidneys, and testicles were used for northern blot analyses. The expression of *Cxcl15* was detected by northern blot using isotope labeled probes. Random primers were prepared by reverse-transcription PCR (RT-PCR). The mouse housekeeping gene *Gapdh* was used as an internal reference. Probes and primers were synthesized by Shanghai Shenyou Company:
sense strand, 5'-TTA GCT CAC AAC AGT GAT AGG-3';
antisense strand, 5'-GTC ACC TGT GAA CAT TCA CT-3'.
An established protocol [6] was used for construction of the random primers and labeled cDNA probes and for purification.

In Situ Hybridization

Paraffin sections were prepared from pulmonary tissue of mice. The sections were placed on RNAse-free glass slides coated with adhesive. The slides were air-dried for 2 h, washed in diethyl pyrocarbonate (DEPC) plus phosphate-buffered saline (PBS), and then washed 3 times in DEPC-water, before being stored at -20°C.

Immunostaining for Digoxigenin

Pretreatment of the sections and washing were done according to a previously reported method [7]. Slides were incubated with pre-absorbed antidigoxigenin antibody (coupled to alkaline phosphatase) diluted to a concentration of 1: 2000 in 20% sheep serum in PBS with Tween (PBT) overnight at 4°C. Slides were then washed 3 times for 30 min each in PBT at room temperature. Next, they were washed in alkaline phosphatase buffer without levamisole for 5 min, followed by levamisole to block endogenous alkaline phosphatase at room temperature for 5 min. Subsequently, 4.5 µL of nitroblue tetrazolium (NBT) and 3.5 µL of 5-bromo- 4-chloro-3-indolyl phosphate (BCIP) were added to each milliliter of alkaline phosphatase buffer, and the slides were immersed in this solution. Slides were then developed in the dark for up to 16 h, depending on the abundance of RNA. The slides were washed with PBS after the process was completed. Alkaline phosphatase buffer was used to wash out the NBT/BCIP; because of the stability of alkaline phosphatase, the reaction could be repeated at a later time. Slides were fixed MEMFA [composed of 0.1 M 3-morpholinopropanesulfoinc acid, 2 mM ethylenebis(oxyethylenenitrilo) tetraacetic acid,1 mM magnesium sulfate, and 3.7% formaldehyde] at room temperature for at least 15 min. Mounting of slides, hybridization, processing, and color development were based on previous methods [7].

Statistical Analyses

Data were analyzed using SPSS18.0 software (SPSS, Chicago, IL, USA). The mortality rate between the 2 mouse lines and between male and female mice in the same group were compared by χ^2 test. Microvascular permeability was compared by independent sample *t* test. All data are expressed as mean±standard deviation (mean±SD).

Results

Differences in Mortality Between BALB/C and C57BL/6 Mice After Blast Injury

The mortality rates at 1, 6, 24, and 72 h after injury were measured (**Table 1**). Our data show a significant difference in total mortality between BALB/C and C57BL/6 mice after blast injury ($P<0.01$). C57BL/6 mice showed greater resistance to blast injury and a higher survival rate compared with BALB/C mice. Most of the animal deaths due to blast injury occurred within 1 h. No significant difference in mortality was found between male and female mice of the same strain (**Table 2**).

Comparison of Pathological Changes Before and After Lung Injury

We evaluated the histology of the mouse lungs after injury, using tissue sections stained with hematoxylin and eosin. We noted pathological manifestations of acute lung injury within 72 h after blast-wave injury. Our findings showed a large number of

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Table 1. Mortality in BALB/C and C57BL/6 mice after blast injury.

Time after injury, h	BALB/C mice (n=60)		C57BL/6 mice (n=59)	
	Deaths	Mortality rate	Deaths	Mortality rate
1	12	20.00	3	5.08
6	5	8.33	0	0.00
24	1	1.67	0	0.00
72	0	0.00	0	0.00
Total	**18**	**30.00***	**3**	**5.08**

* Compared with C57BL/6 mice, $P<0.01$.

Table 2. Mortality among male and female mice.

Time after injury, h	Male BALB/C (n=30)		Female BALB/C (n=30)		Male C57BL/6 (n=29)		Female C57BL/6 (n=30)	
	Deaths	Mortality Rate	Deaths	Mortality Rate	Deaths	Mortality Rate	Deaths	Mortality Rate
1	5	16.67	7	23.33	1	3.45	2	6.67
6	3	10.00	2	6.67	0	0.00	0	0.00
24	0	0.00	1	3.33	0	0.00	0	0.00
72	0	0.00	0	0.00	0	0.00	0	0.00
Total	**8**	**26.67**	**10**	**33.33**	**1**	**3.45**	**2**	**6.67**

red blood cells, increased alveolar edema fluid, a widened alveolar gap, inflammatory cell infiltration, shedding of bronchial epithelial cells, and disruption of normal lung tissue structure (**Figure 1**). Overall, the pathological changes in C57BL/6 mice were significantly milder compared with BALB/C mice.

Changes in Pulmonary Permeability

We calculated FINa levels as a measure of microvascular permeability (**Table 3**). At 1 and 4 h after injury, FINa levels in BALB/C mice were significantly higher than in C57BL/6 mice ($P<0.05$). A significant change in the level of FINa occurred earlier in BALB/C mice compared with C57BL/6 mice (0.5 vs 1 h, respectively; $P<0.05$). The pulmonary microvascular permeability in BALB/C mice was significantly higher than in C57BL/6 mice at 1 and 4 h after injury. Interestingly, the microvascular permeability of both strains continued to increase up to 4 h after injury.

Differences in *Cxcl15* Gene Expression

Cxcl15 gene expression was measured by fluorescence quantitative PCR. Under physiological conditions, the expression of *Cxcl15* in C57BL/6 mice was significantly lower than in BALB/C mice ($P<0.01$; **Table 4**).

Furthermore, we found *Cxcl15* gene expression in BALB/C mice was significantly higher than in C57BL/6 mice after injury ($P<0.05$). *Cxcl15* gene expression continuously increased until 6 h after injury in BALB/C mice, but it descended in C57BL/6 mice at 1 h after injury. Peak expression level of *Cxcl15* was observed at 6 h after injury and return to baseline after 24 h (**Table 5, Figures 2, 3A, 3B**).

Distribution of *Cxcl15* Gene Expression in Different Tissues of Mice

The *Cxcl15* gene was hybridized in 8 major tissue sources of mice on Poly A+RNA blots by the northern blot method (**Figure 3C**). Our results show *Cxcl15* gene expression, specifically in lung. Our data also suggest that the *Cxcl15* gene had 2 subtypes, one that was 1 kb and another that was 2.4 kb.

Distribution of *Cxcl15* in Mouse Lung Tissue

We investigated the distribution of the *Cxcl15* gene in lung tissues of mice with in situ hybridization. Anti-digoxigenin antibody labeled with alkaline phosphatase was applied, and a positive signal was the development of a purple color following addition of BCIP/NBT after labeling with a digoxin random primer. The results suggest that expression of the *Cxcl15* gene was located mainly in the cytoplasm of the bronchial epithelial cells and may also have been present in the nucleus, which needs to be further characterized (**Figure 3D, 3E**).

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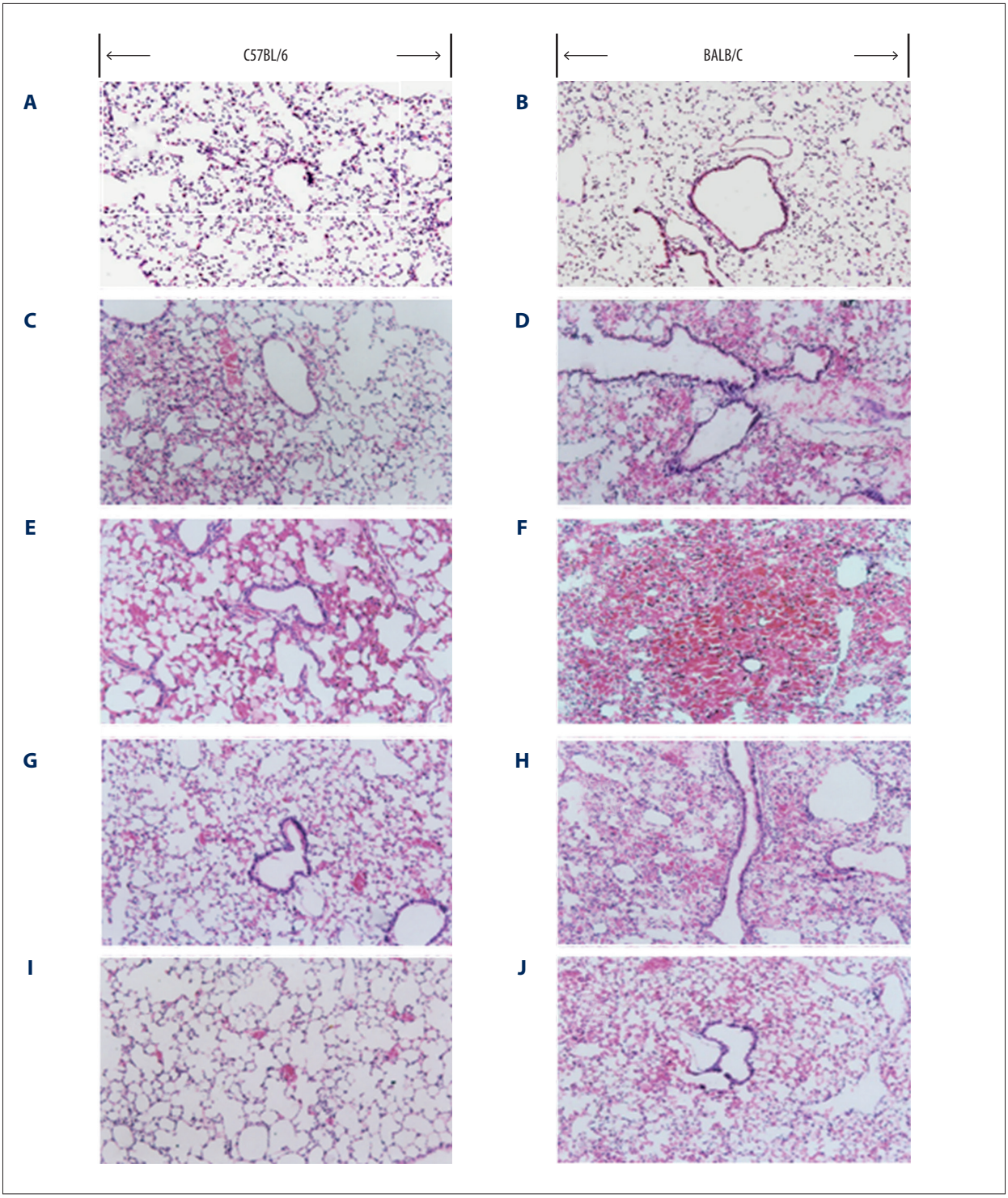

Figure 1. Histopathological changes in C57BL/6 and BALB/C mice before and after systemic blast injury (SBI). **Left panels:** C57BL/6; **right panels:** BALB/C. (**A, B**) before SBI; (**C, E, G, I**) 1, 6, 24, and 72 h post-SBI, respectively; (**D, F, H, J**) 1, 6, 24, and 72 h post-SBI respectively. All sections stained with hematoxylin and eosin; magnification, ×100.

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Table 3. Fluorescein sodium (FINa) intensity before and after injury (n=5, mean±SD).

Groups	Before Injury	After Injury, h		
		0.5	1	4
BG FINa, μg	1.026±0.251	1.846±0.361*	5.686±0.954*,#	11.974±5.782*,#
CG FINa, μg	0.800±0.504	1.658±0.682	2.742±1.205*	4.950±1.090*

BG – BALB/C mice; CG – C57BL/6 mice.* Compared with before injury, $P<0.05$; # compared with CG, $P<0.05$.

Table 4. *Cxcl15* expression under physiological conditions measured by fluorescence quantitative polymerase chain reaction (mean±standard deviation).

Groups	GAPDH		Cxcl15		Cxcl15/GAPDH
	CT	Copies	CT	Copies	
BG	29.024±1.646	493 362.380±345 203.905	28.836±1.137	484 599.980±268 622.314	1.163±0.424*
CG	26.628±3.181	420 7156.480±624 4036.264	28.448±2.454	97 823.386±108 9432.324	0.394±0.145

BG – BALB/C mice; CG – C57BL/6 mice. * Compared with CG, $P<0.05$.

Table 5. Expression of *Cxcl15* after trauma measured by northern blot.

Groups	Before injury (n=2)	After injury			
		1 h (n=3)	6 h (n=3)	24 h (n=3)	72 h (n=3)
BG FINa, μg	4.066±3.619	4.574±1.053*	11.233±6.214*	5.469±3.581	5.196±2.316
CG FINa, μg	1.551±0.539	1.217±0.708	4.663±1.973	1.417±0.277	1.250±0.629

BG – BALB/C mice; CG – C57BL/6 mice. * Compared with CG, $P<0.05$.

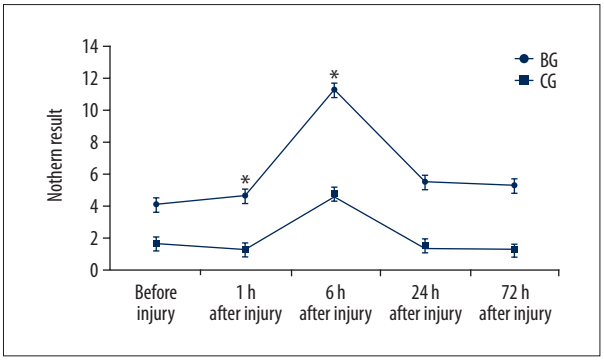

Figure 2. Results of Cxcl15 expression in C57BL/6 and BALB/C after injury (n=3, mean±SD). The abscissa is the time point of observation; the ordinate shows gene expression values for Cxcl15.

Discussion

A heterogeneous traumatic response has been observed among individuals, with different genetic backgrounds presumably explaining varying degrees of resistance to the same level of trauma. The majority of published studies offer preliminary data, but they lack sufficient evidence to form a useful clinical explanation. Therefore, in this study we sought to address some key discrepancies regarding which animal models are most appropriate and what experimental measures best represent relevant measurements to support human diagnostic and therapeutic advances.

The most relevant experimental animal model should satisfy several key conditions. First, it should offer a heterogeneous traumatic response. Second, its genetic background should be well defined. Therefore, we used BALB/C and C57BL/6 inbred mice as experimental animals in this study. They have similar body weights, and the inbreeding coefficient of homologous series mice reaches 98.6%, with hybrid genes representing 1.4% [8]. Some studies have indicated that BALB/C and C57BL/6 mice have different responses to some pathological factors, mainly due to differences in their genetic backgrounds [9-11]. Therefore, we believe that inbred animals are most suitable for studying heterogeneity of traumatic responses.

Regarding the choice of injury model, we believe that the most relevant experimental system should satisfy the following

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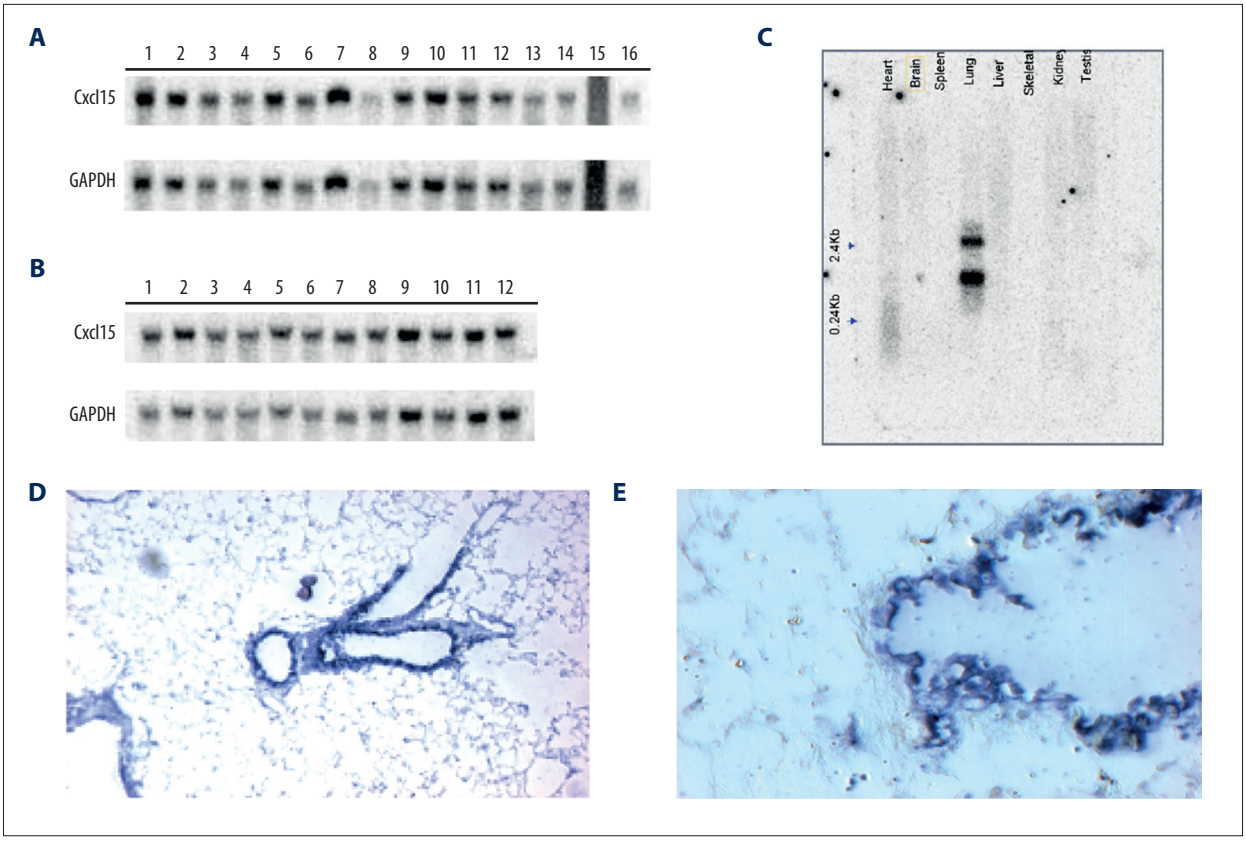

Figure 3. Distribution and expression of Cxcl15. (**A**) Northern blot results of the *Cxcl15* gene and GAPDH gene (internal reference) expression in C57BL/6 and BALB/C mice before injury, and 1 and 6 h after injury. The odd numbers indicate C57BL/6 mouse samples, and the even numbers indicate BALB/C mouse samples: 1-4 are samples from mice pre-injury; 5-10 are samples from mice 1 h after injury; and 11-16 are samples from mice 6 h after injury. (**B**) Northern blot results of the *Cxcl15* gene and *GAPDH* gene (internal reference) expression in C57BL/6 and BALB/C mice 24 and 72 h after injury. The odd numbers indicate C57BL/6 mouse samples, and the even numbers indicate BALB/C mouse samples; 1-6 are samples from mice 24 h after injury; 7-12 are samples from mice 72 h after injury. (**C**) Distribution of Cxcl15 in 8 main tissues in mice. (**D**) Expression of Cxcl15 in bronchial epithelial cells (magnification, ×100). (**E**) Expression of Cxcl15 in lung tissue of mice with bronchial epithelial cell cytoplasm (magnification, ×400).

conditions: have a single-wound agent, the most appropriate being a physical one; follow a nonanesthetized animal protocol; be capable of processing a high number of animals quickly; and demonstrate reproducibility. Previous studies indicate that the shockwave generated by a biological shock tube offers a straightforward method to control injury pressure with consistent, systemic trauma to animals. Thus, this injury method has become the standard research protocol. The BST type-1 biological shock tube manufactured by the Research Institute of Field Surgery of the Third Military Medical University generates a stable vertical plane wave producing uniform injury to animals in a fixed posture and position. Our previous work [12] showed that the SBI model with BST type-1 is reliable, reproducible, and comparable among test subjects.

Regarding the choice of index to quantify the sensitivity to trauma of different animals, we believe that mortality rate is the preferred index. Considering that we chose a whole-body shock injury model and that the ultimate effect of trauma is a direct impact on the organism's survival, we believe that mortality rate is a reliable and easily quantifiable index. In addition to the mortality rate, we chose the pathological changes in pulmonary tissue to quantify the sensitivity to trauma. Previous studies in our laboratory have observed the following phenomena [4]. Compared with other organs, the pathological response in lungs is the earliest and most serious after injury, and it is primarily characterized by congestion and edema. Pulmonary edema is a common characteristic of pulmonary injury. It leads to changes in microvascular permeability, which is associated with vascular structure alteration. Important pathological characteristics of the response to injury include morphological and functional changes in blood vessels, typically their microvascular permeability. The endothelial cytoskeleton plays a critical role in maintaining vascular permeability [13]. We therefore chose microvascular permeability as an indirect index for anatomical structure correlated with the heterogeneity of traumatic responses.

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We chose to examine *Cxcl15* gene expression based on the following reasons. First, the lung is one of the organs with the earliest and most obvious response to trauma [14,15]. The ELR+ chemokine Cxcl15, which recruits neutrophils during pulmonary inflammation, is also known as lungkine due to its reported exclusive expression in the lung [16]. Although Cxcl15 has also been reported to be expressed in mucosal and endocrine organs, it is more highly expressed in the lung than in other organs [17]. Second, previous studies indicated that the *Cxcl15* gene plays a role in mediating inflammation [18], with other studies also showing that it is related to the formation and function of blood vessel walls, especially vascular permeability [19,20]. Most importantly, our previous gene chip results showed that the expression of the *Cxcl15* gene differed in C57BL/6 and BALB/C mice after injury [4].

Our current results revealed significant differences in the mortality and the severity of lung injury between C57BL/6 and BALB/C inbred mice within 72 h after blast injury. The C57BL/6 mice showed greater resistance to the blast injury and had milder lung injuries than the BALB/C mice. Furthermore, most of mouse deaths occurred within 1 h after blast injury. As previously mentioned, we aimed to reduce the impact of nonexperimental factors, and this heterogeneity in the same injury condition was very likely the result of differences in the genetic background between BALB/C and C57BL/6 mice. Furthermore, our results showed no significant differences in mortality and lung injury severity between the male and the female mice of the same strain, which is consistent with previous reports [9,11,21] showing that the differences in acute lung injury susceptibility between different strains of mice is an autosomal genetic characteristic.

We also observed significant death in both strains at 1 h after injury. Thus, we chose this time point to compare microvascular permeability between the 2 strains. Our hematoxylin-eosin staining results showed that the cell structure of BALB/C mice was significantly more damaged compared with the C57BL/6 mice. There was also a significant difference in vascular permeability between the C57BL/6 and BALB/C mice. The increase of pulmonary microvascular permeability occurred earlier in BALB/C mice than in the C57BL/6 mice, and the degree of changes was more dramatic. Our findings were consistent with those of Barone et al [22], who found significant differences in the sensitivity to cerebral ischemia injury in BDF, CFW, and BALB/C mice. Their further studies on cerebral angiotomy revealed that the differences in cerebral ischemia injury were related to the heterogeneity of blood vessels. With regard to mechanisms, previous studies showed that the glycocalyx, caveolae, and pericytes have functional importance for vascular permeability [23]. In a separate study, the phenotypes between C57BL/6 and BALB/c mice were different in the asthma model with different distributions of proinflammatory cytokines and inflammatory cells [24].

Our study also showed that the levels of *Cxcl15* gene expression were different in the 2 mouse strains before and after injury. This difference was consistent with the difference in mortality and pulmonary vascular permeability between the 2 groups. The results suggested that the *Cxcl15* gene was involved in the trauma response. In addition, our results showed that the variation trend of *Cxcl15* expression level after injury was significantly different between the 2 groups. The expression level of *Cxcl15* in the 2 groups changed in an opposite direction in the early stage after injury. The findings suggested that the *Cxcl15* gene was not only involved in the trauma response, but it also reflected the heterogeneity of post-trauma response between the 2 mouse strains. We deduced that the difference in the *Cxcl15* expression trend was likely due to differences in the upstream regulatory elements of the *Cxcl15* gene in the 2 mouse strains. In addition, our data also showed that the *Cxcl15* gene was specifically expressed in mouse lung and mainly in the cytoplasm of bronchial epithelial cells, observed as 1 kb and 2.4 kb subtypes. Gene expression profiling is an important clue to elucidate gene function, and there is an inevitable link between the structure and function. Specific to the *Cxcl15* gene, we believe that our results suggest that this gene may be associated with the function of lung, which is gas exchange and oxygen supply for the body. This involves 2 aspects: one is structural and based on gas exchange, and the other is modulation of structure foundation in various states. Cxcl15, as a chemokine, has been suggested [22,23] to play an important role in the generation and stability of blood vessels. It is possible that the Cxcl15 protein forms the congenital structural basis through the influence on angiogenesis and then adjusts the state of the blood vessel wall. Combined with our previous findings on pulmonary vasculature, we believe that this gene may play an important role in lung damage and even traumatic response.

Conclusions

In conclusion, our results indicate that a hereditary factor likely plays an important role in the response to trauma and the ultimate outcome. The *Cxcl15* gene is likely to be involved in the heterogeneity of trauma response through lung injury-related mechanisms. This underscores the assumption that genetic variations fundamentally affect the heterogeneity of traumatic responses. These experimental results will be beneficial for further studies on the heterogeneity of traumatic responses and the mechanisms related to trauma.

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Acknowledgments

We thank the laboratory support staff for their help in this study. We extend special thanks to Dr. Austin Cape for careful reading of our manuscript.

Conflicts of Interest

None.

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