Bcl-2 mediates sex-specific differences in recovery of mice from LPS-induced signs of sickness independent of IL-6

YOHANNES TESFAIGZI,1 KARIN RUDOLPH,1 MARK J. FISCHER,1 AND CAROLE A. CONN1,2
1Lovelace Respiratory Research Institute, Albuquerque 87185; and 2Department of Nutrition, University of New Mexico, Albuquerque, New Mexico 87131

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Tesfaigzi, Yohannes, Karin Rudolph, Mark J. Fischer, and Carole A. Conn. Bcl-2 mediates sex-specific differences in recovery of mice from LPS-induced signs of sickness independent of IL-6. J Appl Physiol 91: 2182–2189, 2001.—Chronic pulmonary diseases are more common in boys than in girls. Therefore, we investigated the differences in signs of sickness in male and female mice that were exposed to lipopolysaccharide (LPS) by intranasal instillation. Because apoptosis is important in the resolution of inflammation, we tested the hypothesis that reduced levels of Bcl-2, a regulator of apoptosis, may play a role in gender-specific differences in response to inflammation. Bcl-2 wild-type (+/+ ) female mice recovered from an LPS-induced drop in body temperature and loss in body weight significantly faster than male (+/+ ) mice. Female heterozygous (+/-) mice showed reduced Bcl-2 levels and exhibited a slower recovery than female (+/+ ) mice that was similar to the recovery pattern in male (+/+ ) and (+/-) mice. Interleukin-6 (IL-6) activity levels in the bronchoalveolar lavage fluid were higher in male than in female mice but were not different between (+/+ ) and (+/-) mice. We conclude that Bcl-2 plays a role in mediating the faster recovery of female (+/+ ) mice from LPS-induced signs of sickness independent of IL-6. These studies indicate that apoptotic mechanisms may be involved in gender-specific differences in chronic pulmonary diseases.

apoptosis; hypothermia; cytokines; inflammation; mucus

Asthma and childhood wheeze are more common and more severe in boys than in girls (29). Between 2 and 14 yr of age, boys are four times more likely to develop chronic asthma than girls (29) and are twice as likely to be hospitalized for asthma (35). Similarly, longitudinal studies in children with cystic fibrosis revealed that pulmonary function is decreased significantly more in boys than in girls (45). These studies suggest a vulnerability of boys to chronic pulmonary inflammation and identify the importance of investigating the cause of gender differences for these diseases.

Airborne endotoxins, lipopolysaccharides (LPS) from the cell walls of gram-negative bacteria, are potent proinflammatory substances that can induce airway obstruction and potentiate the obstructive response to subsequent stimuli (25). Exposure to LPS occurs by inhalation of endotoxin-contaminated water or certain types of organic dusts (2, 11, 32, 34). Exposure of mice to LPS by intranasal or intratracheal instillations has been used as models for inflammatory diseases, such as cystic fibrosis, chronic bronchitis, and pneumonia (10, 40, 41).

Mice injected with LPS, generally used as a model of systemic inflammatory response syndrome, develop an acute phase response accompanied by sickness symptoms such as hypothermia, fever, anorexia (i.e., decreased food intake), and cachexia (i.e., decreased body weight) (23). Several lines of evidence support the hypothesis that interleukin (IL)-6 and tumor necrosis factor (TNF)-α play a role in this syndrome (4, 22, 24). Furthermore, LPS injection causes oligonucleosomal and random DNA fragmentation in several organs, including the lung (3). Inhibition of caspase activity in these mice prevents the LPS-induced apoptosis and acute lung injury (14). Decreased or suppressed apoptosis of immune effector cells in inflamed tissues is crucial for the evolution of an inflammatory process in different organs (43). Furthermore, apoptotic cell death plays a critical role in the clearance of inflamed tissue and recovery from the inflammatory response (6, 7). It is also involved in the resolution of LPS-induced alveolar type II cell hyperplasia (39). The Bcl-2 protein enhances cell survival by inhibiting apoptosis induced under a wide variety of circumstances in leukocytes and in several epithelial tissues (30), suggesting that this protein acts at a central control point in the pathway to apoptotic cell death (1).

Although the effects of LPS administered by injection have been studied extensively, the effects of LPS exposure through the respiratory tract on body temperature and other inflammatory responses have not been well characterized. We wanted to establish whether there are differences in clinical symptoms between young male and female mice that were exposed to LPS through the respiratory system. Furthermore, we hypothesized that Bcl-2 as a regulator of apoptosis may be involved in the gender-specific differences in LPS-induced inflammatory disease. Therefore, we examined the effects of reduced Bcl-2 levels on LPS-induced...
inflammatory response and symptoms of illness. We describe differences in the clinical outcomes in male and female mice after intranasal instillation with LPS and demonstrate that even reduced levels of Bcl-2 affect the gender-specific differences in the recovery from LPS-induced physiological and behavioral signs of sickness.

MATERIALS AND METHODS

Animals. Breeder mice heterozygous for the Bcl-2 gene (Bcl-2(+/−)) were obtained from Dr. Stanley Korsmeyer (Washington University School of Medicine, St. Louis, MO) and were bred in our barrier facility. The generation of Bcl-2 heterozygous mice by gene targeting and the genetic background of these mice are described elsewhere (42). Bcl-2 knockout (−/−) mice complete embryonic development but display early mortality postnatally (42) because of involution of the thymus and spleen and abnormal morphogenesis of the kidney and renal failure due to extensive apoptosis (27). Heterozygous (+/−) mice express less Bcl-2 protein than wild-type Bcl-2 animals; however, (+/+) and (+/−) mice show no significant differences in their development and life span (28, 36). Therefore, Bcl-2 heterozygous mice were used for the following experiments.

Offsprings were genotyped by polymerase chain reaction using the primer pair TCTCTGTGCTTTACAGGTATC and TAAGTCTGAGCTCAGAGACC to amplify across the Bcl-2 and the neo genes to identify (+/−) mice. The primer pair CTTGTGGAACGTACGGCCCGCAGCATCG and ACAGCCTGACGTTGGTACATC was used to amplify the Bcl-2 gene across the area where the neo gene was inserted for identification of (+/+ ) mice. Age- and gender-matched control and Bcl-2 (+/−) littersmates were used for each experiment.

Animal care. The Lovelace Respiratory Research Institute’s Animal Care and Use Committee approved all care and treatment of the mice. All mice were housed in individual plastic cages and maintained in a temperature-, humidity-, and light-controlled chamber set at 30 ± 1°C, with a 12:12-h light-dark cycle with lights on at 6 AM. Rodent laboratory chow and drinking water were provided ad libitum. Once the mice reached 6–8 wk of age, biotelemetry devices to monitor body temperature and motor activity were implanted under sterile conditions.

Body temperature measurement and locomotor activity. One week before the start of the experiment, mice were implanted intraperitoneally with battery-operated biotelemetry models (model VMPH, Mini-Mitter, Sunriver, OR) as described previously (17). Each transmitter was calibrated to ±0.1°C before implantation. Signals from the telemeters were collected by receivers (model RA1010, Mini-Mitter) placed beneath the floor of each cage. The frequency emitted by the transmitters is proportional to the abdominal temperature of the mice. Experiments were started after a regular rhythm of body temperature and activity in freely moving mice had been monitored for ≥3 days. Motor activity of the mice was measured with the biotelemetry system described above. Briefly, in this system, changes in activity are detected by changes in position of the implanted transmitter over the receiver board. This results in a change of the signal strength that is detected by the receiver and recorded as a "pulse" or "count" of activity. As the animal moved freely in the cage, an activity count was generated whenever the signal strength received by the antennas was altered more than the previously set limit for change. These counts were stored per unit time and provided an index of general locomotor activity. Recordings were made at 5-min intervals through a peripheral processor (Datacol III System) connected to an IBM personal computer.

Body weight and intake of food and water. Body weight and food and water intake were monitored daily by weighing each mouse on a top-loading balance accurate to ±0.1 g (Sartorius model MP 1206, Brinkman Instruments, Westbury, NY) between 8 and 10 AM.

LPS instillations. Mice were intranasally instilled with 180 μg of LPS once only, with 60 μg of LPS in 50 μl of saline on 3 consecutive days, or with 50 μl of saline only as a control during a short period of anesthesia. To avoid any circadian variation in body temperature, all instillations were made between 8 and 10 AM.

Necropsy and tissue preparation. Thoracic contents were exposed, and the lungs were perfused through the pulmonary artery with phosphate-buffered saline (Life Technologies, Grand Island, NY). The trachea and lungs were isolated, and each was lavaged three times with 1.5 ml of ice-cold medium 199. The lavage fluid was collected. The lung was expanded to determine lung volume by intratracheal instillation of 10% zinc formalin (Stephens Scientific, Riverdale, NJ) at 25 cmH2O constant pressure for 3–4 h, as described elsewhere (37). Then the lung was immersed in the same fixative for 3–4 days.

Western blot analysis. Protein extracts were prepared from the entire right lung or entire spleen of (+/+ ) and (+/−) mice by homogenization in RIPA buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, and 5 mM EDTA) supplemented with the protease inhibitors phenylmethylsulfonyl fluoride (1 mM), pepstatin (10 μg/ml), aprotinin (2 μg/ml), and benzamidine (2 μg/ml). Protein concentration was determined using the bicinchoninic acid assay kit (Pierce, Rockford, IL); 120 μg of protein from each sample were loaded on each lane. Western blotting was carried out as described earlier (38), and filters were incubated with antibodies to actin (Santa Cruz Biotechnology, Santa Cruz, CA) to confirm that equivalent amounts of protein had been loaded on each lane. The antibodies to Bcl-2 (Pharmingen, San Diego, CA) and actin were used at 1:1,000 dilution.

Histopathology. The fixed lung was cut into slices, each ∼4 mm thick. Three to four slices were prepared, depending on the size of the lung, and slices were embedded in paraffin. Each slice was placed down so that the tissue sections represent the lung sequentially from cranial to caudal when 5-κm sections were prepared from the embedded tissues for staining with alcin blue to detect mucous cells. The number of mucous cells in all airways of the tissues sections was determined by counting all alcin blue-positive cells in the airways of the tissue sections prepared from each lung.

Quantification of neutrophils and macrophages. Cells recovered by lavage of the lungs were enumerated using a hemocytometer. Cytological specimens were prepared and stained with Wright-Giemsa (Fisher Scientific, Pittsburgh, PA) to determine the different types of cells present in the bronchoalveolar lavage fluid (BALF). Four hundred cells were counted from each slide to determine a percent distribution of the different cell types. The total numbers of each cell type were then calculated by multiplying the percentage distribution of the respective cell types by the total cell numbers obtained by lavage.

Bioassays for IL-6 and TNF-α. IL-6 and TNF bioactivity was measured in the BALF using the IL-6-dependent B-9 hybridoma cell line and the TNF-sensitive WEHI-164 subclone 13 cell line, essentially as described previously (5, 20). Briefly, the basis for the IL-6 bioassay is that the hybridoma cells are IL-6 dependent and replicate in direct proportion to

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the quantity of IL-6 present (21). The basis of the bioassay for TNF is that this cytokine is toxic to the fibroblast cell line (5). Cells were resuspended in medium before addition of the lavage samples or known amounts of recombinant cytokines. Triplicate standards and triplicate test samples were incubated with cells; cell growth was assayed by the addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and estimated by colorimetric assay. Because we did not neutralize the activities in the BALF with antibodies to IL-6 or TNF, the measured activity must be considered "IL-6-or TNF-like."

Data analysis. Three separate experiments were conducted, and combined data are presented as means ± SE. A three-factor ANOVA was used to test for differences among groups for changes in cytokine levels and mucous and inflammatory cell numbers in the BALF. For samples in which the activity of a cytokine was below the level of detection of the appropriate assay, the value zero was used in calculations of group means. Temperature and activity data, collected at 5-to 15-min intervals, were averaged over 12 h and analyzed.

RESULTS

Changes in body temperature after one high-dose and three low-dose instillations with LPS. Although most organisms are primarily exposed to LPS through the respiratory tract, our understanding of physiological and behavioral changes after intranasal instillation of LPS in mice is limited. Undisturbed C57Bl/6 mice kept at an ambient temperature of 30°C on a 12:12-h light-dark cycle revealed a rhythm in body temperature having two phases: a nighttime rise, then a daytime fall. Intranasal instillation of 50 µl of saline one or three times on consecutive days did not disturb the rhythm in body temperature, but the rhythm was disturbed after a single instillation of 180 µg of LPS in 50 µl of saline (Fig. 1). The body temperature did not rise during the night and stayed at daytime temperatures during the ensuing 3-day period. However, three consecutive daily instillations of 60 µg of LPS each caused the body temperature to decrease drastically (Fig. 1B). After the first inoculation, decreases to levels observed after a one-time instillation of 180 µg of LPS were observed. The second instillation caused a further decrease of ~0.5°C, and the body temperatures fell by ~2–3°C after the third LPS instillation. At 3 days after the final instillation, the body temperature recovered to values comparable to those of mice that were instilled only once. To maximize clinical symptoms, all further experiments were carried out by instilling mice on 3 consecutive days with 60 µg of LPS.

Bcl-2 levels in the spleens and lungs from (+/+ ) and (+/− ) mice. The abundance of Bcl-2 was determined in (+/+ ) and (+/− ) mice by Western blot analysis. In the spleen, Bcl-2 was detected in the (+/+ ) and (+/− ) mice; however, as previously shown by others (42), Bcl-2 levels in (+/− ) mice were about half those in the wild-type mice, confirming that mice were correctly allelotyped as Bcl-2 (+/+ ) and (+/− ) mice (Fig. 2). The actin levels demonstrate that the same amounts of protein from (+/+ ) and (+/− ) mice were analyzed. Similar results were obtained when Bcl-2 levels were examined in lung extracts (data not shown).

Female (+/+) mice recover faster than female (+/−) mice from an LPS-induced decrease in body temperature and motor activity. Male and female Bcl-2 (+/+ ) and (+/− ) mice housed at 30°C on a 12:12-h light-dark cycle displayed a regular rhythm in body temperature. Female mice, regardless of their genotype, had significantly higher body temperatures before LPS instillation (Fig. 3) and were more active during dark periods (data not shown) than male mice. Because maximum changes were observed with three consecutive LPS instillations, this protocol was used for determining the role of reduced Bcl-2 levels on LPS-induced...
Interestingly, the gender difference in the recovery from LPS-induced reduction in body temperature was not observed in Bcl-2 (+/–) mice (Fig. 3C). Male and female (+/–) mice did not differ, and their rates of recovery were similar to that observed in male (+/+ ) mice. Furthermore, the female (+/+ ) mice recovered significantly faster than the female (+/–) mice. The difference between male (+/+ ) and (+/–) mice was not significant. Similar results were obtained for the activity measurements in these mice (data not shown).

Female (+/+ ) mice recover faster than female (+/–) mice from LPS-induced decrease in body weight. The weight of the mice in the different groups was not significantly different from each other 2 days before saline instillation (21.4 to 23.7 g). At 3 days after saline instillation, male mice showed a larger increase in body weight than female mice, which is consistent with the faster growth of male mice (Fig. 4A). LPS instillation induced a significant loss of body weight in all groups (Fig. 4, B and C). The female (+/+ ) mice lost significantly less body weight than the male (+/+ ) mice and recovered to the original body weight within 4 days (Fig. 4B). The difference in body weight between the male and female (+/–) mice was significantly different only at day 1, and both groups of mice did not recover their original body weight by day 4 after LPS instillation (Fig. 4C). Similar observations were made for the LPS-induced decrease in water and food intake, whereby the changes in water and food intake precede the changes observed in weight changes (data not shown). Therefore, the LPS-induced weight loss is likely to be primarily a result of decreased food intake.

LPS-induced changes in IL-6 and TNF-α activity in the BALF and plasma. IL-6 activity could not be detected in BALF from any of the saline-instilled mice. However, IL-6 activity in BALF from LPS-instilled mice was significantly elevated on days 1 and 2 (Fig. 5). Among the LPS-instilled mice, male mice had significantly higher IL-6 activity in the BALF than female mice on day 1 after inoculation. In male and female mice, IL-6 activity had returned to undetectable levels by day 4. On day 3 after LPS inoculation, male mice, but not female mice, had significantly elevated levels of IL-6 in the BALF. These results indicate that male mice had a more pronounced IL-6 response in the lung than female mice and were delayed in returning to the normal undetectable levels of IL-6 activity after inoculation compared with female mice. However, there were no significant differences between heterozygous and wild-type mice in BALF IL-6 activity for any day after inoculation.

TNF-α activity was not elevated in BALF from LPS-instilled mice compared with saline-instilled mice at any time from day 1 through day 4 after inoculation (data not shown). Neither IL-6 nor TNF-α activity was significantly elevated in plasma from the saline- or LPS-instilled mice on any day after inoculation (data not shown).
LPS-induced changes in inflammatory cells from the BALF. Infiltration of the lung by inflammatory cells was analyzed to determine its association with the observed physiological changes. Mice instilled with saline had low cell counts in BALF. Only neutrophils and macrophages were found in significant numbers in BALF from the four LPS-instilled groups of mice. The numbers of neutrophils were highest at day 1 after LPS instillation and gradually decreased to background levels over 4 days (Fig. 6A). No significant differences could be observed on any day among the four groups treated with LPS. The numbers of macrophages were significantly elevated relative to saline groups by day 1, remained elevated over 3 days, and decreased to background levels at day 4 (Fig. 6B). The number of infiltrating lymphocytes was similar to that observed for macrophages and showed no significant differences among groups over the 4 days after instillation (data not shown). Mucous cells in airway epithelia were significantly increased after LPS, but not saline, instillation on days 3 and 4 after instillation. At days 2, 3, and 4 after LPS instillation, the numbers of mucous cells were not significantly different among the four groups of mice (data not shown).

Fig. 4. Change of body weight before and after 3 intranasal instillations with 50 μl of saline (A) and 60 μg of LPS in 50 μl of saline in female and male Bcl-2 wild-type (B) and heterozygous (C) mice. Mice were instilled at −2, −1, and 0 days (arrows). Sample sizes (n) shown in parentheses decrease as mice are killed daily after instillation. Error bars, SE. Three-way ANOVA showed that the gender × genotype interaction was significant at P = 0.038, and the gender × time interaction was significant at P = 0.005. Female wild-type mice had a significantly (P < 0.03) attenuated change in body weight compared with any other group. Female heterozygous mice lost significantly (P < 0.03) more weight than female wild-type mice but significantly (P < 0.03) less than both male groups. The male groups lost significantly (P < 0.003) more weight than all female mice.

Fig. 5. Interleukin-6 (IL-6) activity levels in bronchoalveolar lavage fluid (BALF) of male (A) and female (B) mice killed 1, 2, 3, and 4 days after instillation. Three-way ANOVA showed that the gender × time interaction was significant at P = 0.099. On day 1, IL-6 activity levels in BALF of male mice were significantly (P < 0.03) higher than in BALF of female mice. IL-6 activity levels in LPS-instilled mice were significantly (P < 0.03) different from zero on days 1 and 2 for female mice and on days 1, 2, and 3 for male mice; no saline-instilled mice showed IL-6 activity in the BALF. Numbers in parentheses indicate sample size. Error bars, SE.
The major conclusions from this study are that the faster recovery of female (+/+) than male (+/+) mice from LPS-induced signs of sickness is abrogated when Bcl-2 levels are reduced. This effect of Bcl-2 is independent of IL-6 levels and independent of the influx and clearance of inflammatory cells in the lungs.

A single intranasal instillation of 60 or 180 \( \mu \)g of LPS caused similar effects in the decrease in body temperature, indicating that at these high doses the body temperature is not affected in a dose-dependent manner. However, three consecutive instillations of 60 \( \mu \)g of LPS caused a drastic reduction in body temperature, confirming that repeated exposures exacerbate the detrimental effects of environmental toxins (13, 31, 33). The drop in body temperature was not followed by fever, as was observed for LPS injected intraperitoneally (15, 23), indicating a difference in the inflammatory response depending on the route of LPS administration. Intranasal inoculation of mice with influenza virus causes decreased body temperature, general locomotor activity, and food and water intake (5, 16). The similarity in the effects of LPS or viral DNA administered through the respiratory tract suggests that the localization of the inflammatory response may be critical in the observed physiological changes. Influenza pneumonitis in mice is associated with a dose-dependent decrease in blood oxygen saturation (16). Hypoxia itself can induce production of cytokines, including IL-6 (9), depress metabolism, and decrease body temperature. Therefore, one mechanism underlying the intranasal instillation of LPS-induced hypothermia may also involve pneumonitis-induced hypoxia.

All groups of mice showed increased neutrophils, macrophages, and lymphocytes in the BALF followed by increased mucous cell numbers over the 4 days after LPS instillation. However, the numbers of these inflammatory indicators were not statistically different among genders and/or genotypes, suggesting that Bcl-2 levels in (+/-) mice were sufficient for inflammatory cell migration and clearance and the development of mucous cell metaplasia in the lung airways.

The female (+/+) mice recovered significantly faster than the male (+/+) mice from hypothermia, cachexia, and anorexia after three intranasal instillations of LPS. Immune activation, which results in cytokine production, is modulated by circulating hormones, such as glucocorticoids and gonadal hormones (18). There is evidence that spleen cells from female mice have altered immune responses compared with those from male mice, which may be mediated by gender-specific hormones (18). Another study suggests that female mice eliminate the Coxsackie B-3 virus faster than male mice (12). Therefore, the faster recovery of female mice in the present study may be due to a faster development of tolerance to LPS or to an enhanced clearance of LPS by phagocytic cells. Immune responses change during the estrous cycle in rodents (19). Fever induced by IL-1Î² injection in rats was significantly higher and more prolonged in females at proestrus than at diestrus (26). In the present study, mice were not selected for the different stages of the estrous cycle; however, the faster recovery of female mice from the LPS-induced decrease in body temperature was consistent in three different experiments. The data presented in this study are combined from three independent experiments balanced for all experimental groups in which each replication consistently showed a difference in the female wild-type mice. These results suggest that female mice recover faster than male mice regardless of their stage in the estrous cycle or that the effect of the different stages was not diluted by female mice in the inactive stages of the cycle.

The female (+/+) mice recovered significantly faster than the female (+/-) and male mice from the LPS-induced signs of sickness. However, the rate of recovery of female and male (+/-) mice was statistically not significantly different, suggesting that mice with reduced levels of Bcl-2 do not show the gender-specific difference in recovery. Furthermore, this result implies that the immune system of female (+/+) mice responds in a manner similar to that of male (+/+) mice when Bcl-2 levels are reduced.

Our data and many other studies implicate IL-6 as one of the cytokines involved in the observed LPS-induced inflammatory responses. It is well established that IL-6 levels increase drastically after LPS exposure in several species (20, 44). In this study, male (+/+) mice had higher levels of IL-6 at day 1 after LPS instillation, indicating that at these high doses the body temperature is not affected in a dose-dependent manner.
instillation than female (+/+ ) mice. The inflammatory response comprises many aspects. Although male mice did not have a higher influx of neutrophils, one indicator of an inflammatory response, the increased IL-6 levels in male mice compared with female mice, indicates that male mice had a higher inflammatory response to LPS instillation. Similarly, significantly increased plasma IL-6 concentrations were also found in male but not in female mice that were subjected to hypoxemia (14a). By day 3 after LPS instillation, IL-6 activity in the BALF from female mice had already returned to undetectable levels, whereas IL-6 activity from male mice remained significantly elevated. This difference in cytokine activity levels in the BALF correlates with the faster recovery of female (+/+ ) mice from symptoms of illness. IL-6 appears to be involved in the LPS-induced clinical symptoms; however, no significant differences in IL-6 levels were observed between the female (+/+ ) and (+/−) mice at this or any time point analyzed. This observation suggests that the lower levels of Bcl-2 in heterozygotes are still sufficient to elicit a female IL-6 response and that Bcl-2 is not acting through IL-6 in its role to mediate the faster recovery of female (+/+ ) mice than the other groups of mice from LPS-induced inflammation. TNF-α levels are known to increase at early time points (2 h) post-LPS exposure and decrease to background levels after 6 h (13a). In this study, the measurements were done in the BALF obtained 24 h postinoculation, by which time TNF-α levels may have been reduced to undetectable levels.

Hormones modulate expression of apoptotic factors in lymphoid cell death, and Bcl-2 is upregulated by estrogen in several tissues (8). Inflammatory cell numbers in the BALF were not different among all LPS-treated groups, indicating that the clearance of inflammatory cells is not affected by decreased Bcl-2 levels. However, it is possible that reduced levels of Bcl-2 expression in heterozygous female mice inactivate the estrogen-dependent immune response of certain leukocytes to LPS. Because Bcl-2 is modulated by estradiol in some brain neurons (8), it is also possible that Bcl-2 levels have a direct effect on thermoregulatory or appetite centers in the brain. Further research is warranted to determine whether certain cells have reduced Bcl-2 levels in male compared with female mice and to identify the mechanisms by which Bcl-2 mediates the faster recovery in female mice. These studies are crucial to understand the molecular basis of sex-specific differences in chronic pulmonary diseases.

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