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Mechanisms of decreased intestinal epithelial proliferation and increased apoptosis in murine acute lung injury

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Abstract

Objectives—The aim of this study was to determine the effects of acute lung injury (ALI) on the gut epithelium and examine mechanisms underlying changes in crypt proliferation and apoptosis. The relationship between severity and timing of lung injury to intestinal pathology was also examined.

Design—Randomized, controlled study.

Setting—University research laboratory.

Subjects—Genetically inbred mice.

Interventions—Following induction of ALI, gut epithelial proliferation and apoptosis was assessed in a) C3H/HeN wild type and C3H/HeJ mice, that lack functional toll-like receptor 4 (TLR4, n=17), b) C57Bl/6 mice that received monoclonal anti-tumor necrosis factor- α (TNF α) or control antibody (n=22) and c) C57Bl/6 wild type and transgenic mice that overexpress Bcl-2 in their gut epithelium (n=21). Intestinal epithelial proliferation and death were also examined in animals with differing degrees of lung inflammation (n=24) as well as in a timecourse analysis following a fixed injury (n=18).

Measurements and Main Results—ALI caused decreased proliferation and increased apoptosis in crypt epithelial cells in all animals studied. C3H/HeJ mice had higher levels of proliferation than C3H/HeN animals without additional changes in apoptosis. Anti-TNF α antibody had no effect on gut epithelial proliferation or death. Overexpression of Bcl-2 did not change proliferation despite decreasing gut apoptosis. Proliferation and apoptosis were not correlated to severity of lung injury, as gut alterations were lost in mice with more severe ALI. Changes in both gut epithelial proliferation and death were apparent within 12 hours, but proliferation was decreased 36 hours following ALI while apoptosis returned to normal.

Conclusions—ALI causes disparate effects on crypt proliferation and apoptosis, which occur, at least in part, through differing mechanisms involving TLR4 and Bcl-2. Severity of lung injury does not correlate with perturbations in proliferation or death in the gut epithelium, and ALI-induced changes in intestinal epithelial proliferation persist longer than those in apoptosis.

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Keywords

Acute lung injury; gut; proliferation; apoptosis; TLR4; TNF- α

INTRODUCTION

Proliferation in the gut epithelium is restricted to the crypts of Lieberkuhn, and this production of new intestinal epithelial cells is balanced under basal conditions with cell loss through apoptosis and/or exfoliation (1,2). The relationship between proliferation and elimination is substantially altered in animal models of critical illness, although results vary depending upon model. Sepsis from murine *Pseudomonas aeruginosa* pneumonia causes a time-dependent decrease in gut epithelial proliferation measured by S-phase cells labeled with 5-bromo-2' deoxyuridine (BrdU) with a simultaneous increase in crypt apoptosis (3). Acute inflammation caused by intraperitoneal or intravenous injection of LPS also causes decreased gut epithelial proliferation in rats (4) and increased apoptosis in mice, rats or cats (5–8). In contrast, proliferation increases in rat cecal ligation and puncture, a model of ruptured appendicitis, when measured by incorporation of ^3H thymidine (9) although this is also associated with an increase in gut epithelial apoptosis in mice (10,11).

The mechanisms that mediate gut epithelial proliferation and apoptosis in systemic inflammatory states are incompletely understood. Under basal conditions, the gut is host to a large number of resident bacteria and bacterial products such as LPS, but this does not result in derangements of gut physiology (12,13). This may be because the gut is typically hyporesponsive to LPS, and intestinal epithelial cells downregulate expression of TLR4 (14–16). However, in a murine model of colitis, TLR4 is upregulated in the same tissue distribution as inflammation which suggests that this LPS receptor may have a role in the inflammatory response (17).

The effect LPS has on modulating normal intestinal epithelial proliferation via TLR4 signaling is, in turn, mediated by TNF α produced by enterocytes *in vitro* (18). Although there is little evidence to support a role for TNF α in modulating intestinal epithelial proliferation in critical illness, it has been shown to modulate gut epithelial apoptosis in both thermal injury and infection. Mice subjected to a 30% body surface area burn typically have a three-fold increase in intestinal cell death, but this can be abrogated by giving anti-TNF α antibody immediately following thermal injury (19). Apoptosis induced by invasion of intestinal cells *in vitro* with either *Escherichia coli* or *Salmonella dublin* can also be partially blocked by giving anti-TNF α antibody (20).

Stress frequently alters the relationship between the cell cycle and cell death (21–23). We have previously studied this relationship in the crypt epithelium in a high-mortality model of overwhelming infection and found that decreasing sepsis-induced crypt apoptosis can prevent decreases in intestinal epithelial proliferation. Mice that overexpress the anti-apoptotic protein Bcl-2 in their gut epithelium have decreased crypt apoptosis compared to wild type (WT) mice subjected to *Pseudomonas aeruginosa* pneumonia and this is associated with a partial restitution in the proliferative capacity of the gut epithelium (3).

It is well-established that pulmonary injury can induce distant pathology, including abnormalities in the intestine. Injurious mechanical ventilation superimposed on a model of acid aspiration in rabbits induces villus apoptosis (24). In addition, intratracheal injection of high-dose LPS induces both ALI and gut epithelial apoptosis in mice (25). In order to study mechanisms of gut epithelial proliferation and apoptosis without the confounding factor of mortality, we gave mice a nonlethal lung injury to determine whether a) crypt proliferation is

mediated by TLR4 or TNF α , b) increased apoptosis causes a compensatory change in gut epithelial proliferation that can be abrogated by decreasing cell death, c) severity of lung injury correlates with severity of abnormalities in the gut's proliferative capacity and d) temporal changes in intestinal epithelial proliferation are linked to changes in apoptosis.

MATERIALS AND METHODS

Animals

Six to twelve week-old C57Bl/6, C3H/HeN and C3H/HeJ mice were purchased from Charles River Laboratories (Wilmington, MA), Harlan International (Indianapolis, IN), and Jackson Laboratories (Bar Harbor, ME) respectively. Transgenic mice that overexpress Bcl-2 in their crypt epithelium (*Fabpl*-Bcl-2 mice) were generated on an FVB/N background (26) and were then backcrossed to C57Bl/6 mice for 11 generations. Mice were maintained on a 12 hour light-dark schedule in a specific pathogen-free environment and received standard laboratory mouse chow *ad libitum*. Details on all mice used in this study are contained in table 1. All animals examined were sacrificed by cervical dislocation under halothane anesthesia at predetermined timepoints. All studies complied with National Institutes of Health guidelines for the use of laboratory animals and were approved by the Washington University Animal Studies Committee.

ALI model

Intratracheal injection of LPS is a well-accepted model of ALI (27–30). Under halothane anesthesia, mice underwent a midline tracheal incision (25,31,32) and received an intratracheal injection of LPS, (from *E. coli* 055:B5; Sigma, St. Louis, MO) diluted in 50 μ l 0.9% NaCl. Commercially obtained LPS was not purified prior to intratracheal injection. Animals were held upright vertically for 10 seconds to enhance delivery into the lungs. All doses of LPS were diluted in the same volume of 0.9% NaCl. Sham animals were handled identically, but received an injection of 50 μ l 0.9% NaCl alone. Following incision closure, mice were injected subcutaneously with 1 ml 0.9% NaCl to compensate for fluid and insensible losses.

Antibody treatment

A subset of C57Bl/6 mice received a single 250 μ g intraperitoneal injection of hamster monoclonal anti-TNF α IgG or irrelevant hamster monoclonal IgG (TN3-19 and L2-3D9 respectively, generously provided by Dr. Robert D Schreiber, Washington University School of Medicine (33)) immediately after induction of ALI. The timing of antibody injection was chosen because anti-TNF α IgG has been shown to prevent burn-induced gut epithelial apoptosis when given immediately following thermal injury (19).

Endotoxin determination

LAL Pyrotell get-clot kit (Associates of Cape Cod, Woods Hole, MA) was used according to manufacturer specifications to determine if LPS was present in the bloodstream. A different lot of commercially available LPS was used for these experiments than for all others described in this manuscript secondary to the availability of the reagent.

Bacteriologic Analysis

Blood for culture was obtained at the time of sacrifice from the vena cava. 100 μ l was diluted 1:10 in sterile phosphate-buffered saline, and serial dilutions were cultured on blood agar and MacConkey plates. Plates were examined 24 hours following incubation at 37°C

Determination of proliferation and division

Mice were injected intraperitoneally with 200 μ l of BrdU (5 mg/ml diluted in 0.9% NaCl; Sigma) 2 hours prior to sacrifice to label cells in S-phase. Following sacrifice, the small intestine was immediately removed, opened along the length of its cephalocaudal axis and washed to remove luminal contents. Intestines were immediately placed in 10% buffered formalin where they were fixed for 24 hours and then were rolled distal to proximal and placed in 70% ethanol. Immunohistochemical detection of BrdU was performed using a commercially available kit (BD PharMingen, San Diego, CA). M-phase cells were identified by the characteristic morphology of the mitotic spindle on sections stained with hematoxylin alone. S- and M-phase cells were quantified in the distal small intestine in 100 contiguous crypts from well-oriented crypt-villus units by an investigator blinded to sample identity.

Determination of apoptosis

Apoptotic cells were identified using active caspase-3 staining and morphological analysis of hematoxylin and eosin (H&E)-stained sections. Active caspase-3 staining was performed as previously described (3,34). Briefly, paraffin-embedded tissues were heated and rehydrated. Slides were incubated at 23° C in 3% H₂O₂ in methanol for 10 minutes and then microwaved in citrate buffer (pH 6.0) for 9 minutes at 89° C. Sections were then incubated with polyclonal rabbit anti-active caspase-3 (1:100; Cell Signaling, Beverly, MA) for one hour at 23° C. This was followed by another incubation at 23° C with secondary biotinylated goat anti-rabbit antibody (1:200; Vector Laboratories, Burlingame, CA) and then VECTASTAIN ABC (Vector), both at 23° C for 30 minutes. Slides were developed with metal-enhanced DAB solution and counterstained with hematoxylin and dehydrated. Apoptotic cells were identified on H&E stained sections by characteristic nuclear fragmentation and cell shrinkage with condensed nuclei.

Lung inflammation

Lungs were qualitatively assessed in H&E-stained sections for the presence of an inflammatory infiltrate and integrity of the alveolar architecture.

Statistical analysis

Data from three or more groups were compared using one-way ANOVA followed by the Newman-Keuls multiple comparison test. Pairwise comparisons were made with the Student's t-test. Statistical analysis was performed using Prism (GraphPad Software, San Diego, CA). P values <0.05 were considered to be statistically significant.

RESULTS

ALI decreases crypt proliferation and increases apoptosis independent of severity of injury

C57Bl/6 mice received an intratracheal injection of 150, 300, or 500 μ g LPS or 0.9% NaCl (n=3/group) and were sacrificed 12 hours later. Histologic analysis of the lungs of sham animals demonstrated normal alveolar architecture in the typical lacy pattern with clear airspaces (Figure 1A). In contrast, all animals that received LPS had dose-dependent evidence of lung injury. Animals injected with 150 or 300 μ g LPS had moderate consolidation and fibrin deposition while animals receiving 500 μ g had a substantial increase in inflammatory cells as well as hemorrhage (Figure 1 B, C, D).

C57Bl/6 mice given 150, 300, or 500 μ g LPS or 0.9% NaCl (n=6/group) were also assayed for crypt proliferation and death 12 hours later. Animals receiving lower doses of LPS had a statistically significant decrease in S-phase cells (p<0.01 and p<0.05 respectively) while those receiving the highest dose were statistically similar to sham animals (Figure 2A, 3A, B). The

number of M-phase cells also decreased 50% in animals receiving lower doses of LPS compared to sham, without an accompanying fall in animals receiving the highest dose (data not shown). The lack of correlation between lung injury severity and gut epithelial production was also noted with crypt apoptosis, where cell death was significantly increased in mice receiving 300 µg but was unchanged in the animals with the highest degree of ALI ($p < 0.05$, Figure 2B, 3C, D). Based upon these results, ALI was induced in all subsequent experiments with 300 µg LPS.

To verify this was a model of sterile inflammation, blood cultures were performed on mice that received each of the three doses of LPS. All cultures were sterile (lower limit of detection 10 cfu/ml, $n = 27$ mice). In addition, all animals ($n = 4$ /group) were noted to have LPS in the bloodstream 12 hours following intratracheal injection, regardless of the dose examined.

ALI-induced decreases in cellular proliferation last longer than increases in apoptosis

To determine whether early changes in the gut epithelium persist following the induction of ALI, C57Bl/6 mice given 300 µg of LPS were sacrificed 12, 24, or 36 hours later. Sham animals injected with 0.9% NaCl were also sacrificed 12, 24, or 36 post-operatively ($n = 6$ all groups) to verify that intratracheal injection alone did not alter crypt proliferation or death. Statistically significant decreases in S-phase cells were noted at both early and late timepoints in mice subjected to ALI (Figure 4A). A 25–50% decrease was also seen in M-phase cells at all timepoints (p values between 0.053 and 0.09), and there was a smaller than two-fold increase in the ratio of S-phase to M-phase cells between animals with ALI and sham littermates at all timepoints (data not shown). In contrast, apoptosis rose early, peaked at 24 hours and returned to baseline by 36 hours (Figures 4B).

Mice that lack functional TLR4 have higher levels of gut epithelial proliferation following ALI

C3H/HeN and C3H/HeJ mice (that are genetically identical except for a missense mutation in the TLR4 gene in C3H/HeJ animals) (35) ($n = 8–9$ /group) were both given a single intratracheal injection of 300 µg commercially obtained unpurified LPS and were sacrificed 24 hours later. Of note, this timepoint is later than that used in the dose response curve in figure 2 and is based upon the brisk apoptotic response demonstrated at this timepoint in figure 4. C3H/HeJ mice had a statistically significant increase in S-phase cells compared to C3H/HeN mice ($p < 0.05$, Figure 5A, B, C). In contrast, apoptosis was similar in each group whether assayed by active caspase 3 (Figure 5D) or H&E (data not shown).

Since C3H/HeJ mice are hyporesponsive to LPS, we also verified that they have the ability to mount a response to ALI. Intratracheal injection of LPS causes similar lung injury in C3H/HeN and C3H/HeJ mice.

Gut epithelial proliferation and death are not altered by giving a single dose of anti- TNF α antibody immediately after ALI

C57Bl/6 mice were given either an intraperitoneal injection of anti-TNF α antibody or an irrelevant monoclonal IgG immediately after induction of ALI. Animals ($n = 11$ /group) had similar levels of proliferation and apoptosis regardless of whether they received anti-TNF α antibody (Figure 6) despite having similar levels of lung injury (data not shown).

Bcl-2 has no effect on proliferation despite decreasing apoptosis

Fabpl-Bcl-2 mice and their WT littermates on a C57Bl/6 genetic background ($n = 10–11$ /group) subjected to ALI were compared to sham operated ($n = 6$) animals. Overexpression of Bcl-2 completely prevents ALI-induced apoptosis (Figure 7A, B, C, of note, our lab has previously shown this in mice on an FVB/N genetic background using 800 lg LPS at 12 hours (25)).

Fabpl-Bcl-2 mice and WT mice had similar amount of proliferation following ALI (Figure 7D).

DISCUSSION

This study demonstrates that ALI decreases crypt proliferation and increases intestinal apoptosis. The proliferation decrease is at least partially TLR4 dependent since C3H/HeJ mice have more S-phase cells than C3H/HeN mice following the induction of lung injury. However, giving a single dose of monoclonal anti-TNF α antibody following ALI failed to alter the number of S-phase cells. Cell cycle and cell death are uncoupled in ALI as neither alterations in proliferation in C3H/HeJ mice nor decreases in apoptosis in *Fabpl*-Bcl-2 mice are accompanied by compensatory changes in cell death or production respectively. Surprisingly, animals with the greatest severity of lung injury were the only ones that had no changes in intestinal epithelial proliferation or death.

These results expand our understanding of the mechanisms involved in the gut's response to ALI and how they differ from those seen in other models of critical illness. First, while gut levels of TLR4 are different between basal states and chronic inflammation (17), the role of this receptor in the gut epithelium in acute illness was previously unknown. In order to examine this question, it was first important to show that C3H/HeJ mice would respond to lung injury induced by unpurified LPS since TLR4 is a receptor for this molecule (36,37). Our results demonstrate that ALI can be induced in the absence of TLR4 based upon the fact that lung pathology was similarly abnormal in C3H/HeJ and C3H/HeN mice. Since C3H/HeJ mice have more intestinal epithelial S-phase cells than C3H/HeN mice following ALI, we conclude that signaling through this receptor is at least partially responsible for alterations in crypt S-phase cells following pulmonary injury.

Our results do not support a definite role for TNF α in the ALI-induced changes in either gut epithelial proliferation or apoptosis. This was somewhat surprising since TNF α has been shown to modulate LPS-induced enterocyte proliferation under basal conditions (18) and induce gut apoptosis in both thermal injury and direct bacterial invasion (19,20). However, the fact that the number of S-phase cells was similar regardless of whether WT C57Bl/6 mice (with intact TLR4 signaling) received monoclonal anti-TNF α antibody suggests that TNF α is not a major mediator of gut cell production or death in ALI. These negative results may be important since they suggest that the host response to critical illness and the mechanisms underlying this response vary widely depending on the animal model used (38), an important consideration when developing therapeutic agents. However, our results must be interpreted with caution because we gave only a single dose of a single type of anti-TNF α antibody at a single timepoint, and there may actually be a TNF α effect that we failed to uncover using this strategy.

Our results also demonstrate that gut epithelial proliferation and apoptosis are uncoupled after ALI. While numerous insults have been reported to alter crypt proliferation and apoptosis, they have rarely been studied simultaneously. In thermal injury, gut epithelial proliferation and death appear to be linked. A 30% burn causes increased small bowel proliferation with a simultaneous increase in apoptosis (39). Gut epithelial proliferation and apoptosis also change in parallel after jejunoileal resection, with both responses being lost in Bax^{-/-} mice (21). In contrast, intestinal cell production and destruction are disparate in *Pseudomonas aeruginosa* pneumonia which causes a decrease in crypt proliferation with a simultaneous increase in apoptosis (3). While the results contained herein are grossly similar to those published in pneumonia, the kinetics are different. The ratio of S- to M-phase cells, a common method of studying cell cycle kinetics in the gut epithelium (40,41), increased less than two-fold between sham animals and mice with ALI while mice with pseudomonal sepsis have a 7-fold decrease in this ratio.

This absence of an association between proliferation and apoptosis is further demonstrated by our results demonstrating that changes in cell cycle do not alter cell death (and conversely changes in apoptosis do not alter proliferation). We have previously shown that preventing gut apoptosis in overwhelming infection via overexpression of intestinal Bcl-2 lessens the decrease in sepsis-induced proliferation. However, neither the increase in S-phase cells in mice without functional TLR4 signaling nor the decreased number of apoptotic cells by Bcl-2 overexpression resulted in any compensatory changes that would indicate “crosstalk” between the cell cycle and cell death.

The most unexpected finding of these studies was that intensity of lung injury does not correspond to the severity of dysregulation in crypt proliferation or death. Previous studies have demonstrated that mice with a 30% burn have increased gut epithelial proliferation (as measured by proliferating cell nuclear antigen) (39) while rats with a 60% burn have decreased gut epithelial proliferation (as measured by BrdU) (42). While it is difficult to compare data between different species, injuries and proliferation assays, we assumed based upon these results that mice receiving higher doses of intratracheal LPS would have further decreases in proliferation and increases in apoptosis compared to mice receiving lower doses. However, animals that received 500 µg (which had the highest degree of lung injury) were the only ones with no quantitative abnormalities in the number of S-phase, M-phase, or apoptotic cells. We do not have a good explanation why animals with the biggest insult were the only ones whose intestines had no abnormalities in the parameters measured.

This study has a number of limitations. First, it is possible that TNFα actually mediates LPS-induced changes in proliferation but we were unable to detect this. Although we were using a well-characterized antibody at a published dose, this does not exclude the possibility that a different antibody, repeated doses of the same antibody, or a different dose of the same antibody would have resulted in different effects. We also did not purify the LPS used in this study. It is well known that commercially available LPS may be contaminated with lipoproteins and peptidoglycan (and therefore signal via receptors other than TLR4). We therefore have not shown that ALI in this study is exclusively due to LPS injection since a contaminant may have caused the lung injury observed. Rather, we demonstrate that commercially available unpurified LPS causes ALI, and the degree of lung injury is similar regardless of whether TLR4 is present. While we do not know if this effect is secondary to a contaminant, the same LPS was used in both groups, resulting in similar levels of lung injury but different levels of intestinal epithelial proliferation in C3H/HeJ and C3H/HeN mice. It is possible that the reason lung injury was independent of TLR4 status was due to the fact that it was caused by a contaminant in unpurified LPS. While this does not negate the fact that LPS was clearly present in the bloodstream in all animals and could be responsible for the difference in intestinal proliferation in C3H/HeJ and C3H/HeN mice, our results must be interpreted with caution.

Interpreting a relationship (or lack thereof) between lung injury and intestinal pathology must be made with caution since we did not examine systemic inflammation in this study. Although we show that mice that receive 500 µg of LPS have more lung injury than mice that receive 150 µg, this does not inherently mean that local inflammatory changes in the lung are reflected systemically. While we believe it is unlikely that higher doses of LPS cause more severe lung injury but less systemic inflammation, it is possible that unknown anti-proliferative and/or pro-apoptotic factors are released at a higher level by the injured lungs in animals that received higher doses of LPS. Since LPS is present in the bloodstream of all animals, we also cannot rule out that the results obtained are secondary to endotoxemia alone independent of ALI. We believe this is unlikely since cytokines and lung injury differ substantially between mice given LPS via intratracheal or intraperitoneal routes (24,43). However even if our results reflect a combination of both ALI and endotoxemia, each represents a noninfectious inflammatory insult with relevance to critical illness, and our overall conclusions are minimally altered by

the relative contributions of each. Finally, we cannot know the biological significance of altered gut epithelial proliferation in this nonlethal model of ALI. We speculate that a decrease in production of new cells in the proliferating component of the intestinal epithelium may be detrimental to the host via altered permeability, translocation of toxic substances into the mesenteric lymph or via immunomodulation; however, additional studies are required to address the functional significance of our findings.

Despite these limitations, these results give new insights into how ALI decreases gut epithelial proliferation while increasing crypt apoptosis. They also demonstrate that even if pathology in one organ leads to a systemic response with resultant distant organ injury, the severity of the initial response does not predict what the secondary effect will be. Further studies of the gut's response to critical care models of increasing mortality should help determine if similar results are found in more lethal injuries.

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Figure 1.

Lung inflammation increases in C57Bl/6 mice injected with escalating doses of LPS. (A) H&E-stained sections of sham animals that received 0.9% NaCl demonstrates normal alveolar architecture with clear spaces. Mice injected with 150 (B) or 300 μ g (C) LPS have moderate consolidation and fibrin deposition while animals receiving 500 μ g (D) have a substantial increase in inflammatory cells as well as hemorrhage and airway obliteration. All photos are at 10 $\times$ magnification.

Figure 2.

Crypt proliferation and apoptosis in C57Bl/6 animals injected with escalating doses of intratracheal LPS. Number of S-phase cells (A) and apoptotic cells as assayed by active caspase 3 staining (B) following injection of 0.9% NaCl (sham) or 150, 300, or 500 µg of LPS. Asterisks represent p values <0.05 compared to sham mice. Neither intestinal epithelial proliferation nor cell death is altered in mice that receive 500 µg despite the fact that these animals have the highest amounts of lung injury.

Figure 3.

Crypt proliferation and apoptosis in sham animals and C57Bl/6 mice injected with 300 µg LPS 12 hours earlier. (A) S-phase cells in sham animals. Cells staining positive for BrdU stain brown. (B) S-phase cells in animals subjected to ALI. The number of cells staining positive for BrdU is decreased. (C) Active caspase 3 stain in sham animal. (D) Active caspase 3 stain in animal subjected to ALI. Arrows point to apoptotic cells, which stain brown. Cell death is increased following ALI.

Figure 4.

Proliferation and death over time following ALI in C57Bl/6 mice. The number of S-phase cells (A) is decreased 12 hours in following LPS and remains low 36 hours following induction of inflammation. Sham values are included at each timepoint since it was previously unknown whether an intratracheal injection alone would alter crypt proliferation. In contrast, apoptosis (B) also increases early but returns to normal by 36 hours. Note that although the number of BrdU-positive and apoptotic cells is counted in 100 contiguous crypts, there is a 20-fold difference in values on the y-axis between the two groups.

Figure 5.

Effect of TLR4 on proliferation and death in ALI. (A) Quantification of S-phase cells in C3H/HeN WT mice and C3H/HeJ mice that lack functional TLR4. Asterisks represent p values <0.05 compared to WT mice. Qualitatively less BrdU staining is apparent comparing C3H/HeN mice (B) to C3H/HeJ mice (C). Quantification of apoptosis by active caspase 3 (D) reveals no difference in cell death between the groups. Similar results were obtained by H&E staining (data not shown).

Figure 6.

Effect of a single dose of anti-TNF α antibody immediately following ALI on gut epithelial proliferation and apoptosis. Quantification of C57Bl/6 mice given anti-TNF α antibody or irrelevant hamster monoclonal IgG (neutral IgG) demonstrates similar number of S-phase cells (A) and apoptotic cells whether assessed by active caspase 3 (B) or H&E staining (data not shown).

Figure 7.

Effect of Bcl-2 on intestinal apoptosis and proliferation in ALI. H&E stain of C57Bl/6 WT mouse (A) and transgenic animal of the same strain that overexpresses Bcl-2 in its gut epithelium (B) demonstrates that Bcl-2 prevents LPS-induced apoptosis (arrows point to apoptotic cells). (C) Quantitation of crypt cell death by active caspase 3 24 hours after intratracheal injection of LPS demonstrates that *Fabpl*-Bcl-2 mice have a three-fold decrease in apoptosis compared to WT animals and are similar to sham animals. Asterisk represents p values <0.05 compared to sham mice and number sign represents p value <0.02 compared to WT animals with ALI. Similar quantitative results were obtained with H&E. Quantitation of S-phase cells in these animals demonstrates that preventing apoptosis has no effect on the number of S-phase cells (D).

Table 1

Characteristics of mice studied.

Strain	LPS Dose	Genetic alteration or antibody treatment	Harvest Time (hours) *	Number of animals	Studying
C57Bl/6	150 µg	None	12	13	Dose response, LPS in bloodstream
C57Bl/6	300 µg	None	12	13	Dose response, Timecourse, LPS in bloodstream
C57Bl/6	500 µg	None	12	13	Dose response, LPS in bloodstream
C57Bl/6	0	None	12	6	Control for timecourse
C57Bl/6	300 µg	None	24	6	Timecourse
C57Bl/6	0	None	24	6	Control for timecourse
C57Bl/6	300 µg	None	36	6	Timecourse
C57Bl/6	0	None	36	6	Control for timecourse
C3H/HeJ	300 µg	Lack functional TLR4	24	9	TLR4 effect
C3H/HeN	300 µg	None	24	8	Control for
C57Bl/6	300 µg	Anti-TNF- α antibody	24	11	TNF- α effect
C57Bl/6	300 µg	Control antibody	24	11	Control for TNF- α effect
C57Bl/6	300 µg	Bcl-2 overexpression	24	10	Bcl-2 effect
C57Bl/6	300 µg	None	24	11	Control for Bcl-2 effect

* Interval between LPS administration and organ removal