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Innate Mucosal Immune System Response of BALB/c versus C57BL/6 Mice to Injury in Setting of Enteral and Parenteral Feeding

Rebecca A Busch, MD^b, Mark A Jonker, MD^b, Joseph F Pierre, PhD^{b,c}, Aaron F Heneghan, PhD^b, and Kenneth A Kudsk, MD^{a,b}

^aVeterans Administration Surgical Services, William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, United States of America

^bDepartment of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, United States of America

^cDepartment of Medicine- Gastroenterology, University of Chicago, Chicago, Illinois, United States of America

Abstract

Background—Outbred mice exhibit increased airway and intestinal immunoglobulin A (IgA) following injury when fed normal chow, consistent with humans. Parenteral nutrition (PN) eliminates IgA increases at both sites. Inbred mice are needed for detailed immunological studies; however, specific strains have not been evaluated for this purpose. BALB/c and C57BL/6 are common inbred mouse strains, but demonstrate divergent immune responses to analogous stress. This study addressed which inbred mouse strain best replicates the outbred mouse and human immune response to injury.

Methods—Intravenously cannulated mice received Chow or PN for 5 days and then underwent sacrifice at 0 or 8-hours following controlled surgical injury (BALB/c: n=16-21/group; C57BL/6: n=12-15/group). Bronchoalveolar lavage (BAL) was analyzed by ELISA for IgA, TNF- α , IL-1 β and IL-6 while small intestinal wash fluid (SIWF) was analyzed for IgA.

Results—No significant increase in BAL IgA occurred following injury in chow-or PN-fed BALB/c mice (Chow: $p=0.1$; PN: $p=0.7$) despite significant increases in BAL TNF- α and SIWF IgA (Chow: 264 ± 28 vs. 548 ± 37 , $p<0.0001$; PN: 150 ± 12 vs. 301 ± 17 , $p<0.0001$).

Injury significantly increased mucosal IgA in chow-fed C57BL/6 mice (BAL: 149 ± 33 vs. 342 ± 87 , $p=0.01$; SIWF: 236 ± 28 vs. 335 ± 32 , $p=0.006$) and BAL cytokines. After injury, PN-fed C57BL/6 mice exhibited no difference in BAL IgA ($p=0.9$), BAL cytokines or SIWF IgA ($p=0.1$).

Corresponding author: Kenneth A. Kudsk, MD, 600 Highland Ave, H4/736 CSC, Madison, WI 53792-7375, Tel: 608-263-1396, Fax: 608-263-7562, kudsk@surgery.wisc.edu.

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Conclusions—C57BL/6 mice exhibit similar airway responses to injury as outbred mice and humans, providing an appropriate model for studying mucosal responses to injury. The BALB/c mucosal immune system responds differently to injury and does not replicate the human injury response.

Background

Outbred Institute of Cancer Research (ICR) mice are frequently used as murine models of human responses. Our laboratory previously demonstrated similarities between humans and male ICR mice in airway mucosal immunoglobulin A (IgA) and cytokine response before and after injury.^{1, 2} Our work shows that route of feeding affects IgA levels within airway and intestinal secretions in both unstressed and stressed mice.^{3, 4} Under unstressed conditions, parenteral nutrition with decreased enteral stimulation (PN) reduces baseline levels of both airway and intestinal IgA.⁵ Normally, the Th2 type cytokines- interleukin-4 (IL-4), -6, -10, and -13 stimulate production and release of IgA from plasma cells at mucosal sites.⁶⁻⁹ Reductions in IL-4, IL-6 and IL-10 within the intestine and specifically within the small intestine lamina propria during PN implicate loss of Th2 cytokine stimulation as one cause of reduced IgA levels in uninjured mice.¹⁰

Route of nutrition also affects immune responses to injury in male ICR mice. Clinically, lower respiratory tract levels of IgA measured in bronchoalveolar lavage (BAL) specimens of severely injured trauma patients increase soon after injury.¹ Experimentally, normal chow fed mice generate a similar response which is lost if mice are pretreated with PN.¹¹ A similar response occurs within the intestine: IgA levels increase in injured mice.⁴ Within the lung, this appears to be related to the generation of stress cytokines. BAL levels of TNF- α , IL-1 β and IL-6 all demonstrate bimodal peaks at 3 and 8 hours after injury with a peak in BAL IgA at 8 hours.⁶ These cytokine increases do not correspond to serum increases, and antibodies which block TNF- α and IL-1 β activity eliminate increases in airway (but not intestinal), IgA levels.^{2, 4, 11} Intraperitoneal administration of TNF- α , IL-1 β and IL-6 also induces the airway IgA increase *in vivo*. While TNF- α and IL-1 β do not fall under the definition of Th1 or Th2 type cytokines, they are capable of generating stress responses in a wide variety of conditions.¹² These IgA responses to injury may represent innate protective mechanisms designed to prevent infection at mucosal surfaces after injury, especially the human respiratory tract. Once secreted, IgA binds to pathogens and preventing mucosal attachment and subsequent invasion.^{13, 14} Experimentally, PN also eliminates this protective mechanism in ICR mice.^{4, 11}

While these immunological responses correlate between outbred mice and humans, developments in molecular genetics over the past three decades have allowed for increasingly complex genomic manipulation of murine models. Inbred mouse models allow more precise definition of molecular events while controlling for genetic diversity. They also allow cell transfer between animals.^{15, 16} However, different inbred strains expressing distinctly different genetic backgrounds can exhibit critically different phenotypic responses. The importance of inbred strain background on phenotypic assessment has been well reviewed in the fields of neurobiology and behavior and is expanding into the field of metabolism and endocrine disorders.¹⁷⁻²⁰ These observations mandate investigation to

determine which mouse strain should be employed in experiments modeling human conditions. This requires a critical evaluation of the genetic backgrounds and relevant phenotypic differences present among inbred mouse strains used in the research to increase the clinical relevance of experimental results.

BALB/c and C57BL/6 mice are two common inbred strains used frequently in research. Several studies highlight differences in immunological response of these two strains resulting in the designation of BALB/c mice as Th2 type (e.g. IL-4, IL-6, IL-10 and IL-13) responders and C57BL/6 mice as Th1 type (TNF- β and IFN- γ) responders based on their respective cytokine profiles and responses to infectious challenges and immunizations.²¹⁻²⁸ In general, BALB/c mice are more susceptible to viral infections while exhibiting decreased cytotoxic responses but increased humoral or immunoglobulin responses to allergens and infections consistent with its cytokine profile. In contrast, C57BL/6 mice are more resistant to viral infection exhibiting increased cytotoxic responses and decreased humoral and allergic responses.

Since Th2 cytokines favor IgA production, and PN results in a Th2 cytokine dependent reduction in respiratory and intestinal IgA,^{10, 29-31} it made intuitive sense that animals such as BALB/c mice, who genetically favor Th2 cytokine production and hence IgA production would provide the most relevant model for replicating our outbred mouse work. Our primary aim is therefore to determine which inbred mouse strain (BALB/c or C57BL/6) mimics the immune responses of the outbred mouse. The present study tests the hypothesis that BALB/c mice assimilate the ICR mouse IgA response to injury and nutrition due to its preferential Th2 cytokine profile.

Materials and Methods

Animals

All protocols were approved by the Animal Care and Use Committee of the William S. Middleton Memorial Veterans Hospital-Madison and the University of Wisconsin-Madison. Male ICR mice were purchased from Harlan (Indianapolis, IN) and housed 5 per covered/filtered box under controlled temperature and humidity conditions in an American Association for Accreditation of Laboratory Animal Care-accredited facility. Animals were fed standard mouse chow (Rodent Diet 5001; LabDiet, PMI Nutrition International, St. Louis, MO) and water *ad libitum* for an acclimation period of 1 week prior to initiation of study protocol. After entering study protocol mice were housed individually in metal cages with wire grid floors to eliminate coprophagia.

Experimental Design

Male BALB/c and C57BL/6 mice, ages 6 to 8 weeks, were randomized to Chow (BALB/c, n=33; C57BL/6, n=29) or PN (BALB/c, n=40; C57BL/6, n=26). All animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and acepromazine (10 mg/kg), weighed, and underwent surgical central line placement of a silicon rubber catheter (0.012-inch I.D./0.025-inch O.D.; Helix Medical, Inc., Carpinteria, CA) via the right external jugular vein. The distal end of the catheter was tunneled subcutaneously over the

back and exited at the midpoint of the tail. Animals were partially immobilized by tail restraint following the procedure to protect the catheter during infusion. This technique has proven to be an acceptable method of nutritional support and does not produce physical or biochemical evidence of stress.³²

Mouse catheters were connected to infusion pumps and the animals received 0.9% saline at a rate of 4 mL/day and *ad libitum* chow and water for 48 hours of recovery. This recovery period allows serum cytokines, corticosteroid levels, and IgA secretion induced by surgical stress from catheterization to return to baseline, with resumption of regular independent oral intake. After the recovery period, experimental diets were initiated. Animals in the Chow group continued to receive 0.9% saline at 4 mL/day as well as *ad libitum* chow and water. PN animals received *ad libitum* water and PN solution through their catheters at rates of 4 mL/day (day 1), 7 mL/day (day 2) and 10 mL/day (day 3 to 5), because a graded infusion period is necessary for the mice to adapt to the changes in glucose and fluid loads. The PN solution contained 6.0% amino acids, 35.6% dextrose, electrolytes, and multivitamins, containing 1440 kcal/L and a non-protein calories/nitrogen ratio of 128:1. These values were calculated to meet the nutrient requirements of mice weighting 25 to 30 and metabolically scaled to the weight of our animals.³³

After 5 days of experimental diet (7 days post-catheterization), mice were again anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and acepromazine (10 mg/kg). The mice were then randomized to two groups: 1) sacrifice without injury (0h) to determine baseline protein levels and 2) sacrifice 8 hours following injury (BALB/c Chow 0h, n = 16; Chow 8h, n = 17; PN 0h, n = 19; PN 8h, n = 21; C57BL/6, n = 14, 15, 14, and 12). Animals in the second group (8h) underwent controlled surgical injury consisting of two wounds. First, a 3.0 cm celiotomy incision was made, and the bowel was gently eviscerated before being returned to the abdominal cavity. The second wound consisted of a 1.5 cm left neck incision with blunt dissection carried to the pretracheal tissue plane. Both incisions were then immediately sutured closed and the mice returned to their cages with water *ad libitum*. They remained disconnected from feeding catheters and were not provided chow. Eight hours following the controlled injury, mice were again anesthetized and sacrificed. Sacrifice was performed in both groups by exsanguination via left axillary artery transection.

Sample Collection

Immediately following sacrifice, the mouse trachea was cannulated with an 18-g angiocatheter and 1 mL of 0.9% saline was injected distally. Approximately 1 mL of bronchoalveolar lavage (BAL) fluid was then gently aspirated from the lungs. Samples were placed on ice and stored at -80°C until assayed.

Additionally, the small intestine (pylorus to terminal ileum) was removed and the lumen flushed with 20 mL Hanks Balanced Saline Solution (Bio Whittaker, Walkersville, MD). This small intestinal wash fluid (SIWF) was collected and kept on ice until they were centrifuged at 2000 × g for 10 minutes at 4°C. Supernate was stored at -80°C until assayed.

Sample Analysis

Airway and SIWF were analyzed for IgA secretion by enzyme-linked immunosorbent assay (ELISA). Proinflammatory cytokines TNF- α , IL-1 β and IL-6 were also evaluated by ELISA, but only the airway samples were assayed as previous studies indicated that respiratory IgA secretion was cytokine dependent, whereas gastrointestinal IgA secretion is cytokine independent.^{4, 6}

Briefly, respective solid phase sandwich ELISA kits (BD Biosciences, San Diego, CA) for IgA and TNF- α , IL-1 β and IL-6 were used according to manufacturer's instructions. Samples were diluted 1:5 BAL IgA quantitative analysis and 1:100 for SIWF IgA quantitative analysis. BAL samples for TNF- α , IL-1 β , and IL-6 were all run neat. The absorbance at 450 nm was determined using a Vmax Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA). Relative concentration of secreted proteins was determined by using a 4-parameter logistic fit standard curve (SOFTmax PRO software; Molecular Devices; Sunnyvale, CA) and normalized to total luminal protein content.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) and Fisher's protected least significance difference (PLSD) post hoc test corrected for multiple comparisons using Stat View (SAS Institute, Cary, NC). Differences of $p < 0.05$ were considered statistically significant. All results are presented as mean \pm standard error of the mean.

Results

Weight Assessment

BALB/c—There were no significant differences in pre-experiment body weight between groups of BALB/c mice ($p > 0.05$). Significantly more weight loss occurred in the PN-fed BALB/c mice than the Chow-fed BALB/c mice; however there was no difference in weight loss between Chow-fed mice with and without injury or PN-fed mice with and without injury. (Table 1)

C57BL/6—There was no significant difference in pre-experiment body weight between groups of C57BL/6 mice ($p > 0.05$). Significantly more weight loss occurred in the PN-fed groups than the Chow-fed groups; however there were no differences in weight loss between injured and uninjured animals in each experimental diet group. (Table 2)

Respiratory IgA Response to Injury

BALB/c—Neither Chow-fed BALB/c mice nor PN-fed BALB/c mice demonstrated a change in respiratory IgA in BAL samples 8 hours following injury compared to baseline (Chow-Injury: 190 ± 8 vs. Chow: 236 ± 24 , $p = 0.11$; PN-Injury: 215 ± 21 vs. PN: 203 ± 18 , $p = 0.65$). (Figure 1)

C57BL/6—Compared to uninjured controls, Chow-fed C57BL/6 mice significantly increased respiratory IgA in BAL specimens 8 hours following injury (Chow-Injury: $342 \pm$

87 vs. Chow: 149 ± 33 , $p = 0.01$). PN-fed C57BL/6 mice demonstrated no increase in respiratory IgA 8 hours following injury (PN-Injury 143 ± 24 vs. PN: 153 ± 25 , $p = 0.90$). (Figure 1)

Respiratory Cytokine Response to Injury

BALB/c—There were statistically significant increases in proinflammatory cytokines TNF- α , IL-1 β and IL-6 in respiratory secretions of Chow-fed BALB/c mice 8 hours following surgical stress when compared to baseline. In PN-fed BALB/c mice, there was a significant increase in TNF- α in BAL fluid 8 hours following injury ($p = 0.02$). Additionally there were increases in cytokines IL-1 β and IL-6 in BAL fluid of PN-fed BALB/c mice 8 hours following injury, but they did not reach statistical significance, (IL-1 β : $p = 0.08$; IL-6: $p = 0.05$). (Table 3)

C57BL/6—There were statistically significant increases in proinflammatory cytokines TNF- α , IL-1 β and IL-6 in respiratory secretions of Chow-fed C57BL/6 mice 8 hours following surgical stress when compared to baseline. PN-fed C57BL/6 mice did not display change in respiratory secretion of any of these proinflammatory cytokines 8 hours following surgical stress when compared to baseline. (Table 4)

Small Intestinal IgA Response to Injury

BALB/c—Compared to uninjured controls, both Chow-fed and PN-fed BALB/c mice significantly increased intestinal IgA secretion as measured by SIWF 8 hours following injury (Chow-Injury: $548 + 37$ vs. Chow: $264 + 28$, $p < 0.0001$; PN-Injury: $301 + 17$ vs. PN: $150 + 12$, $p < 0.0001$). (Figure 2)

C57BL/6—Chow-fed C57BL/6 mice demonstrated a significant increase in SIWF IgA 8 hours following surgical stress compared to baseline (Chow-Injury: 335 ± 32 vs. Chow: 236 ± 28 , $p = 0.01$). PN-fed C57BL/6 animals had no difference in SIWF IgA levels 8 hours following surgical stress (PN-Injury: 198 ± 19 vs. PN: 138 ± 14 , $p = 0.10$). (Figure 2)

Discussion

Inbred mouse strains allow sophisticated immunologic studies such as cell transfer experiments as well as host transgenic and gene knockout studies. Such work is impossible in outbred mice due to antigenic incompatibilities between individual animals. However, different inbred mouse strains may demonstrate different phenotypic responses to the same independent variables.^{18-21, 34-40} This work shows that two commonly used strains of inbred mice, BALB/c and C57BL/6, display dramatically different mucosal immunologic stress responses to injury, highlighting the importance of choosing an appropriate background strain of mice for relevant study. In this work we examined whether BALB/c or C57BL/6 mice immunologically resemble the respiratory and intestinal mucosal immune responses to injury noted to occur in humans and outbred ICR mice.¹ The motives for this experiment were two-fold: firstly, many international laboratories studying mucosal immunity cannot obtain outbred mice and, secondly, outbred mice cannot be used for cell transfer

experiments needed to isolate the source and site of immunologic problems occurring in our studies of PN.

IgA is the primary specific immunologic antiviral and antibacterial defense protecting mucosal surfaces such as the respiratory, gastrointestinal, and urinary tracts. In the respiratory tract, reduced IgA levels in the BAL fluid correlate with increased mucosal bacterial adherence and increased rates in pneumonia.⁴¹⁻⁴³ Prior clinical work demonstrated that injury stimulates an innate respiratory IgA immune response in severely injured trauma patients within 30 hours of injury.¹ This response can be experimentally reproduced with a controlled injury in ICR mice. The airway IgA increase occurs within 8 hours with airway IgA levels returning to baseline by 24 hours after this limited, controlled injury. Experimentally, this ICR airway response is cytokine dependent (TNF- α , IL-1 β , and IL-6) in a manner consistent with localized airway increases in these cytokines in the human BAL samples.^{2, 6} Either pretreatment with PN or the administration of anti- TNF- α or anti- IL-1 β blocking antibodies to ICR mice eliminate the airway IgA increases after injury.¹¹ Experimentally, PN decreases BAL IgA levels and impairs both established anti-viral and anti-bacterial IgA mediated respiratory defenses, providing a cogent hypothesis for the increase in pneumonia documented in PN fed patients.⁴⁴⁻⁴⁹ The gastrointestinal tracts of mice also respond to injury with an innate IgA immune increase.⁴ The intestinal response is detectable within 2 hours of injury and continues to increase up to 8 hours following injury in mice. Unlike in the lung, gastrointestinal IgA production and secretion appears independent of the proinflammatory cytokines TNF- α , IL-1 β and IL-6, however. Like the airway, PN eliminates this intestinal IgA increase.

In this experiment we evaluated immune response to injury in BALB/c and C57BL/6 mice subjected to controlled injury after administering experimental diets of Chow or PN for 5 days. Since Th2 cytokine responses typically upregulate IgA production,^{29, 30, 50} we hypothesized that BALB/c mice would display findings similar to our outbred mouse and human data as BALB/c are considered to immunologically favor Th2 responses to infection, immunization, and stress.^{51, 52} Contrary to our hypothesis, C57BL/6 mice more aptly resembled the ICR and human immune responses. The C57BL/6 mice exhibited the same innate respiratory and gastrointestinal IgA responses to injury seen in ICR mice during Chow feeding which were eliminated with PN. PN also eliminated the respiratory cytokine response to injury in C57BL/6 animals. BALB/c mice, however, displayed no significant change in respiratory IgA levels in either Chow or PN-fed animals following injury despite increase in the respiratory cytokine TNF- α levels following injury as well as non-significant increases in IL-1 β and IL-6. Interestingly, the gastrointestinal IgA response remained intact in both the chow- and PN-fed groups.

The multitude of inbred mouse strains permits a huge variety of genetic experiments. However, the investigator faced with this array of options must determine which strain provides the appropriate isogenic environment to study the intervention of interest. Rarely do experimental publications justify the experimental and clinical rationale for the inbred species used in an experiment. This oversight may lead to contradictory results depending on the mouse strain used in the work due to lack of appreciation of differences in inbred mouse phenotypes. Data in standardized collections such as the Mouse Phenome Database

on The Jackson Laboratory website⁵³ allows an educated experiment design and interpretation but it does not contain information on immune phenotypic responses to various stimuli. In our work investigating route of nutrition and respiratory and gastrointestinal responses to injury, C57BL/6 mice provide the appropriate model reflecting the experimental results found in our human and outbred mouse studies.

BALB/c and C57BL/6 mice infected with *Leishmania* major demonstrate classic differences in immunology between mouse strains with distinct genetic backgrounds. In these experiments, *L. major* infection assessed the role of CD4⁺ T-cell development in the host response to infectious disease.^{21, 54} The overarching conclusion from this model established that control of this infection and resolution of illness required an IFN- γ driven activation of macrophages, or Th1 response. C57BL/6 mice effectively mount this Th1 response to clear the infection, while BALB/c mice fail to mount a robust Th1 response and develop progressive disease. The weak Th1 response of BALB/c mice tips the immunologic balance in favor of a Th2 response, namely production of IL-4 and stimulation of immunoglobulin producing B cells, dampening cytolytic potential in addition to other defenses such as anti-tumor immunity.⁵⁵

Other works link differences in cytokine and chemokine expression to phenotypic variations in inflammatory and cancer responses of these two mouse strains.^{34, 36, 56-61} For instance, BALB/c mice prove more fibrosis resistant, radiosensitive, and cancer susceptible than their C57BL/6 counterparts, again highlighting the power of the dampened cytolytic potential seen in Th2 predominant mice, providing an attractive explanation for divergent phenotypes.^{38, 56, 62} Recent speculation also suggests that differences in gut microbial composition between inbred mouse strains contribute to differences in metabolic, inflammatory, and immune states independent of experimental variables.⁶³ However, directly linking genotypic variance to phenotypic differences has proved elusive. In our work the predominant Th2 response of BALB/c mice may allow them to propagate an IgA response to injury despite PN treatment. This response may be worth investigating further in the future.

A limitation of this study remains the inability to attribute differences in responses to specific genetic differences between these two mouse strains i.e., there is no cause and effect relationship between genetics and the outcome variables in our experiments. Other investigators struggle with the same issue, linking available cytokine milieu to effects without defining a root cause.^{40, 64-67} An additional limitation includes the lack of kinetic studies performed in these two inbred mouse strains. Instead, time points for sample collection were based on our findings in outbred ICR mice. The goal for this experiment was to determine if either inbred mouse strain assimilated the same response as our outbred mice and the data confirm that C57BL/6 mice exhibit the same response as outbred mice in a similar timeframe. BALB/c mice may display similar immunologic reactions to injury as outbred mice and humans but these immunologic responses would have to occur on a different time scale. Quantitative analysis of immune differences between different mouse strains was also not performed.

In conclusion, different inbred mouse strains exhibit differential phenotypic expression to stimuli which must be considered in experimental design. In this case, C57BL/6 inbred mice respond to injury in the same manner consistent with outbred ICR mouse and human data investigating respiratory and gastrointestinal tract IgA response to injury. Experimentally, PN eliminates this immune response in both male ICR and C57BL/6 mice, confirming C57BL/6 mice as a relevant model to study human mucosal immune responses after injury and nutrient manipulation. BALB/c mice respond differently than outbred ICR mice to stress and decreased enteral stimulation.

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Clinical Relevancy Statement

Animal models permit detailed mechanistic study of clinical problems such as inflammation, infection etc., which provide insights applicable to humans. Choice of a relevant model which produces similar pathophysiology is paramount to assuring relevance to the clinical condition. While outbred mice assimilate human mucosal immune responses to injury, a variety of inbred mouse strains are available to allow more detailed immunological studies but their relevance has yet to be established. This study examines the mucosal immune responses of two inbred mouse strains to injury and parenteral nutrition to determine an appropriate model which replicates human immunologic responses.

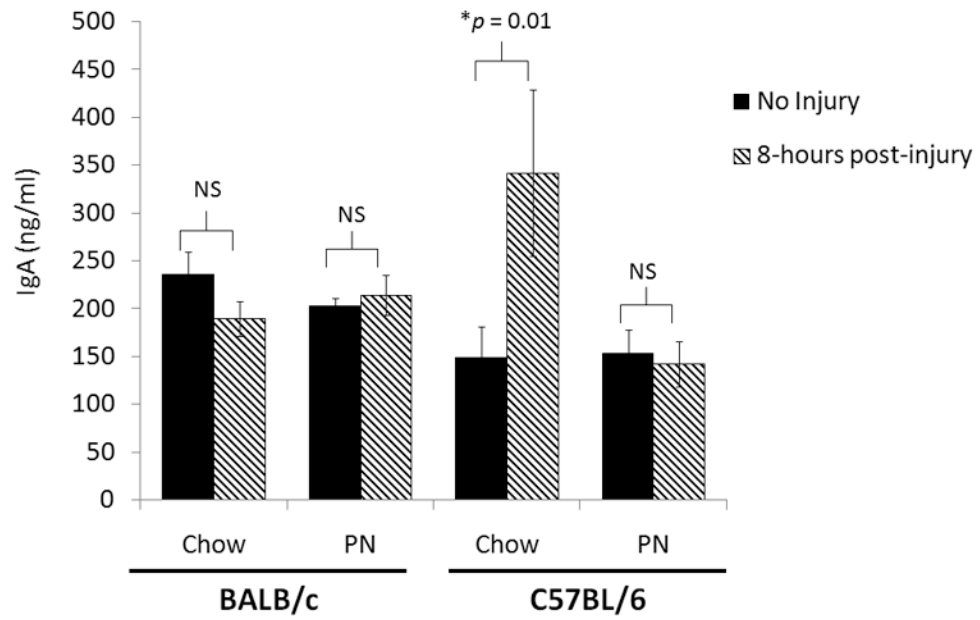


Figure 1. Bronchoalveolar Lavage IgA from BALB/c and C57BL/6 mice with and without Injury after Chow or Parenteral Nutrition. IgA, immunoglobulin A; PN, parenteral nutrition; NS, non-significant ($p > 0.05$).

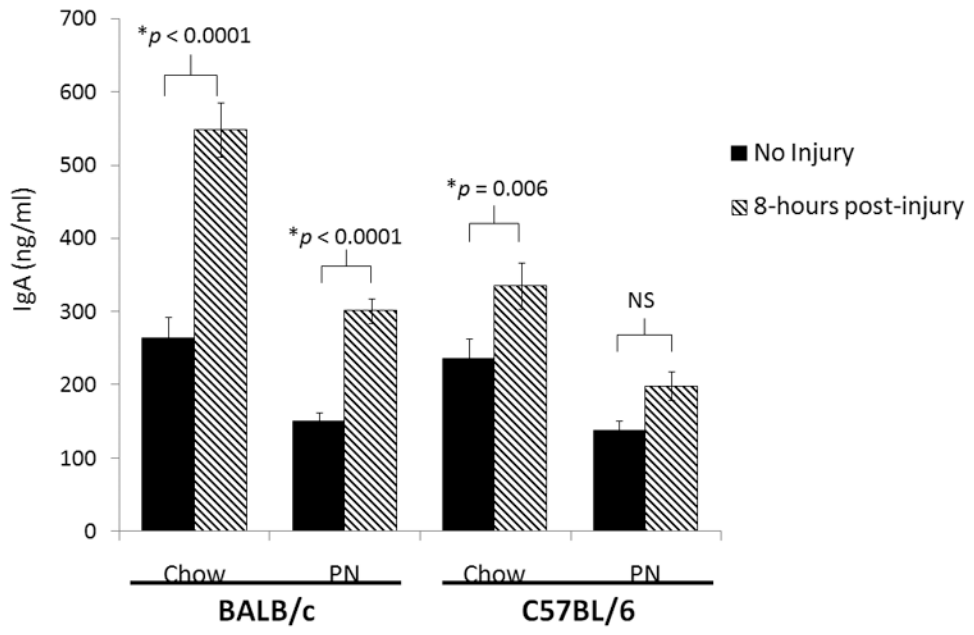


Figure 2. Small Intestinal Wash IgA from BALB/c and C57BL/6 mice with and without Injury after Chow or Parenteral Nutrition. IgA, immunoglobulin A; PN, parenteral nutrition; NS, non-significant ($p > 0.05$).

Table 1

BALB/c body weight and body weight change.

Group (BALB/c)	n	Body Weight (g)	Weight Change (g)
Chow	14	26.0 ± 0.4	-2.3 ± 0.4
Chow-Injury	17	26.1 ± 0.3	-1.6 ± 0.2
PN	20	25.8 ± 0.3	-4.6 ± 0.4*
PN-Injury	21	26.0 ± 0.3	-4.7 ± 0.3*

Values are means ± SEM. PN, parenteral nutrition.

* $p < 0.01$ versus Chow and Chow-Injury.

Table 2

C57BL/6 body weight and body weight change.

Group (C57BL/6)	n	Body Weight (g)	Weight Change (g)
Chow	13	24.7 ± 0.3	-1.2 ± 0.9
Chow-Injury	14	24.8 ± 0.3	-0.9 ± 0.4
PN	13	24.2 ± 0.2	-4.2 ± 0.3*
PN-Injury	17	24.1 ± 0.4	-4.1 ± 0.3*

Values are means ± SEM. PN, parenteral nutrition.

* $p < 0.01$ versus Chow and Chow-Injury.

Table 3

Cytokine response to injury in BAL fluid of BALB/c mice fed Chow or PN.

Lung Cytokine	Chow	Chow-Injury	PN	PN-Injury
TNF- α (pg/ml)	301 \pm 39	512 \pm 46*	345 \pm 26	481 \pm 53 [†]
IL-1 β (pg/ml)	875 \pm 103	1550 \pm 127*	1060 \pm 77	1349 \pm 156
IL-6 (pg/ml)	44.2 \pm 5.6	110 \pm 12*	67.7 \pm 9.8	99.1 \pm 15.8

Values are means \pm SEM. BAL, bronchoalveolar lavage; PN, parenteral nutrition.

* $p < 0.01$ versus Chow;

[†] $p < 0.05$ versus PN.

Table 4

Cytokine response to injury in BAL fluid of C57BL/6 mice fed Chow or PN.

C57BL/6	Chow	Chow-Injury	PN	PN-Injury
TNF- α (pg/ml)	39.8 \pm 6.0	101.6 \pm 19.6*	56.7 \pm 9.2	68.3 \pm 11.1
IL-1 β (pg/ml)	201 \pm 27	374 \pm 63*	264 \pm 39	316 \pm 46
IL-6 (pg/ml)	15.3 \pm 2.7	35.1 \pm 5.6*	26.8 \pm 6.5	27.2 \pm 4.0

Values are means \pm SEM. BAL, bronchoalveolar lavage; PN, parenteral nutrition.

* $p < 0.01$ versus Chow.