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Gastrointestinal Ischemia-Reperfusion Injury Is Lectin Complement Pathway Dependent without Involving C1q

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Complement activation plays an important role in local and remote tissue injury associated with gastrointestinal ischemia-reperfusion (GI/R). The role of the classical and lectin complement pathways in GI/R injury was evaluated using C1q-deficient (C1q KO), MBL-A/C-deficient (MBL-null), complement factor 2- and factor B-deficient (C2/fB KO), and wild-type (WT) mice. Gastrointestinal ischemia (20 min), followed by 3-h reperfusion, induced intestinal and lung injury in C1q KO and WT mice, but not in C2/fB KO mice. Addition of human C2 to C2/fB KO mice significantly restored GI/R injury, demonstrating that GI/R injury is mediated via the lectin and/or classical pathway. Tissue C3 deposition in C1q KO and WT, but not C2/fB KO, mice after GI/R demonstrated that complement was activated in C1q KO mice. GI/R significantly increased serum alanine aminotransferase, gastrointestinal barrier dysfunction, and neutrophil infiltration into the lung and gut in C1q KO and WT, but not C2/fB KO, mice. MBL-null mice displayed little gut injury after GI/R, but lung injury was present. Addition of recombinant human MBL (rhuMBL) to MBL-null mice significantly increased injury compared with MBL-null mice after GI/R and was reversed by anti-MBL mAb treatment. However, MBL-null mice were not protected from secondary lung injury after GI/R. These data demonstrate that C2 and MBL, but not C1q, are necessary for gut injury after GI/R. Lung injury in mice after GI/R is MBL and C1q independent, but C2 dependent, suggesting a potential role for ficolins in this model. The Journal of Immunology, 2005, 174: 6373–6380.

Gastrointestinal ischemia-reperfusion (GI/R)3 generally stems from interruption of blood flow within the superior mesenteric artery or vein and leads to small intestinal hypoperfusion and a mortality rate of ~70% (1). Clinically, GI/R is commonly associated with sepsis, hemorrhagic shock, vascular surgery, small bowel transplantation (2, 3), and multiple organ failure (4, 5). The complement system is a key component in GI/R, resulting in local intestinal and secondary tissue injury (6–11).

Research over the past 30 years has linked complement activation and consequent inflammation and tissue injury with I/R. Models of complement-dependent I/R injury include ischemia of the intestine, hindlimb, kidney, myocardium infarction, hemorrhagic shock, sepsis, and pulmonary injury (6–8, 12–15). Although the exact molecular mechanisms of complement activation after GI/R have not been fully elucidated, evidence from in vitro and in vivo models suggest that complement activation is an early event; therefore, inhibition of complement activation or its components may offer tissue protection after reperfusion. The specific contribution of each pathway to the inflammatory process and the pathways involved in initiation of complement activation after GI/R are still being investigated.

Recent studies targeting various levels of the complement cascade have demonstrated that GI/R injury is C3 and C5 mediated (6, 7, 16–18). We recently demonstrated that pulmonary injury and C3 deposition were not completely inhibited in factor D knockout (KO) mice after GI/R, suggesting initial activation by the lectin and/or classical complement pathway (8). Several studies have suggested that natural Abs and the classical pathway are primarily responsible for GI/R injury (13, 19–22). The premise for the role of classical pathway activation in I/R is based on C1q binding to natural Ab bound to neoepitopes present on ischemic tissue. However, the importance of the lectin complement pathway has been demonstrated in a number of models, including renal I/R (23), myocardial I/R (24, 25), and thoracoabdominal aortic aneurysm repair in humans (26).

To delineate the role of the classical and/or lectin pathway’s involvement in intestinal I/R injury, we investigated classical pathway contribution using C1q KO mice and lectin pathway contribution using MBL-null mice. We hypothesized that if the classical pathway is involved in I/R injury by binding to natural Abs, as suggested by others using the same animal model (13, 19, 20, 22), then C1q KO mice should be protected from GI/R injury. However, we demonstrate that GI/R injury is independent of C1q and classical pathway activation, and that MBL is required for intestinal, but not pulmonary, injury.

Materials and Methods

Intestinal I/R injury

All mice used in GI/R experiments were male; age, 8–12 wk old. C57BL/6 (WT), C1qa KO (C1qKO) (27), C2/factor B double-KO (C2/fB KO) (28),
and MBL-A/C KO (MBL-null) mice (29) were anesthetized with isoflurane. After a midline laparotomy, intestinal ischemia was produced for 20 min, followed by 3 h of reperfusion as previously described (7, 8). Sham-operated controls underwent the same surgical procedures without GI/R. C1q KO and C2/fB KO mice were backcrossed 10 generations onto the C57BL/6 background, whereas the MBL-null mice were still on a mixed background with ~70–80% C57BL/6. All animal experiments were conducted under a protocol approved by the HMA standing committee on animals at Brigham and Women’s Hospital.

C2, MBL, and anti-MBL treatment

Human C2 (Advanced Research Technologies) or an equivalent volume of saline was administered by penile vein injection at a dose of 25 μg. 10 min before ischemia. The ability of human C2 to restore functional C2 levels was assessed by measuring serum hemolytic activity as described previously (30). Briefly, serum from C2 or saline-treated C2/fB KO or WT mice was serially diluted 1/2 (v/v) in GVB buffer (gelatin veronal-buffered saline: 0.1% gelatin, 141 mmol/L NaCl, 0.5 mmol/L MgCl2, 0.15 mmol/L CaCl2, and 1.8 mmol/L sodium barbital) and added in triplicate (50 μl/well) to a 96-well plate. Classical complement pathway hemolytic assays (CH50) using sensitized chicken RBC were performed as previously described (30). All experiments were performed in triplicate.

Recombinant human MBL (rhuMBL; gift from NatImmune) was administered i.p 18 h and 10 min before ischemia at 75 μg to restore the MBL pathway in MBL-null mice (29). Alternatively, rhuMBL was combined with a humanized, functionally inhibitory, anti-human MBL mAb 3F8 (31) at ~1:1 (molar ratio) and administered i.p. 18 h and 10 min before ischemia.

Histological sections

Tissue samples for histological samples were fixed in 10% formalin PBS at 4°C overnight. The samples were dehydrated and embedded in paraffin. Sections (7 μm) were cut and stained with H&E. Intestinal tissue sections were scored using a semiquantitative grading system adapted from Chiu et al. (32) to quantify the extent of mucosal damage. Injury was classified using the following five-point scale where a numerical score was assigned based on the degree of mucosal and submucosal damage. The five-point scale progresses from normal (i.e., 0) to development of apical subepithelial space (i.e., 1), epithelial lifting and denuded villi (i.e., 2–3), and cellular infiltration (i.e., 4), to disintegration of lamina propria, hemorrhage and ulceration (i.e., 5). In addition, a semiquantitative assessment of lung tissue sections were scored using a three-point scale adapted from Johnson et al. (33) for each of the following alveolar wall features: thickness, capillary congestion, and cellularity. The alveolar wall’s thickness was classified based on the majority of the section exhibiting: 0) normally thin alveolar walls, 1) mild thickening, 2) clearly thickened walls, or 3) thickening of the wall, with 50–100% being extremely thick. The capillary congestion of the alveolar wall was quantified as: 0) exhibiting a normal red cell density, 1) mildly congested, 2) moderately congested, or 3) being severely congested. Cellularity of the alveolar wall was classified based on the section exhibiting: 0) normal variable but small number of cells, 1) mild increase in cellularity, 2) mild to moderately increased cell number in 50–75% the section or 3) an increase in cellularity of all alveolar walls. For each section, an average score was calculated based on individual scores for thickness, capillary congestion, and cellularity. All sections were scored in a blinded manner.

Mouse C3 deposition was evaluated with a FITC-conjugated goat anti-rat C3 Ab (ICN). After incubation with the Ab, slides were washed (three times for 10 min each), coated with anti-fade mounting medium (Molecular Probes), covered, and analyzed with a Zeiss confocal microscope as previously described using the same pinhole, voltage, and laser settings for each tissue examined (8, 34). C3 deposition was performed on formalin-fixed tissue and assessed in a blinded manner.

Alanine aminotransferase (ALT) serum activity

Serum ALT levels were measured using a microtiter plate adaptation of a commercially available kit (Teco Diagnostics) (35). Intestinal permeability

Intestinal permeability was assessed by luminal enteral administration of FITC-conjugated dextran 4000 (FITC-dextran; Sigma-Aldrich) (36). All animals were gavaged 5 min before ischemia with FITC-dextran (2 mg/10 g body weight). Whole blood was obtained by cardiac puncture at the time...
of euthanasia. Blood samples were allowed to clot overnight at 4°C and centrifuged (3000 rpm) for 10 min. Serum FITC-dextran concentrations were determined by fluorescence spectrophotometry using a standard curve performed in duplicate.

**Myeloperoxidase (MPO) activity**

MPO activity, an index of neutrophil infiltration, was measured in tissue homogenates as described previously (7, 8, 30).

**Statistical analysis**

All values are presented as the mean ± SEM of three to five mice per group. IR, Ischemia and reperfusion; N/A, not applicable.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>IR</th>
<th>IR + MBL</th>
<th>IR + MBL + 3F8</th>
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<td><strong>Intestinal score</strong></td>
<td></td>
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<tr>
<td>WT</td>
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<td>2.5 ± 0.0₂</td>
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<td>N/A</td>
</tr>
<tr>
<td>C1q KO</td>
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<td>3.0 ± 0.3³</td>
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<td>N/A</td>
</tr>
<tr>
<td>C2/B KO</td>
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<td>1.5 ± 0.5⁴</td>
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<td>N/A</td>
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<tr>
<td>MBL KO</td>
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<td>0.8 ± 0.4</td>
<td>2.9 ± 0.4⁵</td>
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<td><strong>Lung score</strong></td>
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<tr>
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<tr>
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<tr>
<td>C2/B KO</td>
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<tr>
<td>MBL KO</td>
<td>1.0 ± 0.2</td>
<td>2.5 ± 0.3⁸</td>
<td>2.5 ± 0.4⁹</td>
<td>0.9 ± 0.3</td>
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</table>

*Results are the mean ± SEM of three to five mice per group. IR, Ischemia and reperfusion; N/A, not applicable.

₁p < 0.05 compared to the respective sham group.

₂p < 0.05 compared to the WT and C1q KO IR groups.

₃p < 0.05 compared to the respective IR group.

₄p < 0.05 compared to the respective sham group.

₅p < 0.05 compared to the respective WT and IR + MBL groups.

We previously demonstrated in factor D KO mice that C3 is deposited in the intestine and lung after GI/R (8), suggesting that either the lectin and/or classical pathway initiates complement activation. To verify these results, we reconstituted the lectin and classical pathways in C2/B KO mice with human C2. To confirm restoration of functional C2 in C2/B KO mice, the serum hemolytic activity of C2/B KO mice administered C2 or saline, compared to WT mice, was measured. Fig. 2A demonstrates that human C2 restored complement hemolytic activity comparable to WT mice. C2 administration significantly increased serum ALT activity (Fig. 2B), intestinal permeability (Fig. 2C), and pulmonary inflammation (Fig. 2D) after GI/R compared with sham-operated controls or mice given saline. Furthermore, restoration of the classical/lectin pathways in C2/B KO mice undergoing GI/R resulted in injury comparable to that in WT I/R mice (Fig. 2, B–D). These data demonstrate that either lectin and/or classical pathways initiate complement activation during GI/R.

The specific role of the classical complement pathway in GI/R injury was evaluated using C1q KO mice. GI/R resulted in loss and shortening of villi and marked epithelial cell denudation in C1q KO mice (Table I and Fig. 1F) compared with sham controls (Table I and Fig. 1E). Marked pulmonary congestion after GI/R was also observed in C1q KO mice (Table I and Fig. 1L) compared with sham-operated controls (Table I and Fig. 1K). C1q KO mice demonstrated similar levels of intestinal tissue injury (Table I and Fig. 1F) and pulmonary congestion (Table I and Fig. 1L) as WT mice (Table I and Fig. 1, B and H, respectively) after GI/R. These data suggest that C1q KO mice are not protected from GI/R-mediated injury.

Confocal micrographs of C3 deposition in the intestine (A–F) and lung (G–L) are presented in Fig. 3. Complement C3 deposition increased in the intestine (Fig. 3B) and lung (Fig. 3H) in WT mice after GI/R compared with sham-operated controls (Fig. 3, A and G, respectively). C2/B KO mice showed no C3 staining after GI/R compared with sham-operated controls (Fig. 3, D and J vs C and I, respectively). C1q KO mice also demonstrated increased C3 deposition in the intestine (Fig. 3F) and lung (Fig. 3L) after GI/R compared with controls (Fig. 3, E and K, respectively). These data demonstrate that complement is activated and deposited in the lung and intestine in WT and C1q KO mice after GI/R, suggesting that C1q is not involved in the complement activation of this model.

**FIGURE 2.** Reconstitution of C2/B KO mice with human C2. Mice were injected i.v. with saline (IR) or 25 µg of human C2 (IR+C2) before GI/R. A, CH50 using serum from C2- or saline-treated C2/B KO or WT mice. Results are the mean ± SEM of two mice per group performed in triplicate. B, Serum ALT concentration. C, Intestinal permeability as measured by enteral administration of FITC-dextran. D, Pulmonary MPO activity after GI/R. Results (B–D) are the mean ± SEM of four to eight mice per group. *p < 0.05 compared with sham and C2/B KO IR groups.
Biochemical analysis demonstrated that GI/R significantly increased serum ALT concentrations in C1q KO and WT I/R mice compared with controls (Fig. 4). Although ALT levels in C2/fB KO mice undergoing GI/R were significantly increased compared with those in sham-operated controls, C2/fB KO mice demonstrated significantly less serum ALT compared with C1q KO or WT mice during the same procedure. These data suggest that local and remote injuries after GI/R are largely complement dependent, but do not require C1q.

Intestinal permeability analysis also revealed significant elevations of serum FITC-dextran concentrations in WT and C1q KO mice after GI/R compared with controls (Fig. 5). However, FITC-dextran concentrations in C2/fB KO mice after GI/R were not significantly different from control values. Furthermore, C2/fB KO mice demonstrated significantly reduced serum FITC-dextran compared with C1q KO or WT mice after GI/R. Thus, C1q KO mice are not protected from intestinal barrier dysfunction after GI/R.

Intestinal (Fig. 6A) and pulmonary (Fig. 6B) MPO activities were also significantly increased in C1q KO and WT I/R mice compared with sham controls. C2/fB KO mice after GI/R demonstrated significantly less MPO activity in the lung compared with WT or C1q KO mice. There was no significant increase in tissue MPO activity in C2/fB KO mice after GI/R compared with sham-operated control mice. These data demonstrate that neutrophil infiltration into intestinal or pulmonary tissue after GI/R is not dependent on C1q, but is complement dependent, as we have shown previously (7, 8).

Our data demonstrate that GI/R injury is complement dependent, but does not involve C1q, suggesting that initial complement activation occurs via the lectin complement pathway, correlating with our previous findings in myocardium (24). To investigate the
FIGURE 6. Intestinal and pulmonary MPO activities after GI/R. Neutrophil accumulation as measured by intestinal (A) and pulmonary (B) MPO activities from WT, C1q KO, or C2/IB KO mice after GI/R (IR) or sham-operated control mice (sham). Results are the mean ± SEM of eight mice per group. *, p < 0.05 compared with respective sham group; **, p < 0.05 compared with all IR groups.

FIGURE 7. Intestinal and pulmonary injuries in MBL-null mice after GI/R. H&E of jejunum (A) and lung (C). Neutrophil accumulation as measured by intestinal (B) and pulmonary (D) MPO activities in MBL-null mice after GI/R with or without MBL and with or without anti-MBL (3F8). Results are the mean ± SEM of eight mice per group. *, p < 0.05 compared with sham and IR groups. Each micrograph is representative of three to five mice. Original magnification, ×200.

FIGURE 8. Tissue injury assessment of MBL-null mice after GI/R (IR). Serum ALT (A) and intestinal permeability (B), as measured by enteral administration of FITC-dextran from MBL-null mice that underwent IR with or without MBL and with or without anti-MBL (3F8). Results are the mean ± SEM of eight mice per group. *, p < 0.05 compared with sham, IR, and IR + MBL + 3F8 groups.
role of the lectin complement pathway in GI/R, we used MBL-null mice. As presented in Table I and Fig. 7, MBL-null mice after GI/R were protected from intestinal injury (Table I and Fig. 7A) and neutrophil infiltration (Fig. 7B) compared with sham controls. Intestinal I/R injury and neutrophil infiltration were enhanced by the addition of recombinant human MBL (IR+MBL) to MBL-null mice before ischemia (Table I and Fig. 7, A and B, respectively). Furthermore, treatment with a humanized anti-human MBL mAb (IR+MBL+3F8) prevented intestinal injury (Table I and Fig. 7A) and inflammation (Fig. 7B). However, as demonstrated in Table I and Fig. 7, C and D, MBL-null mice were not protected from GI/R-induced lung injury, because similar pulmonary congestion and neutrophil infiltration were observed in MBL-null mice reconstituted with rhuMBL.

Examination of serum ALT and FITC-dextran concentrations from MBL-null mice demonstrated that MBL-null mice were also protected from tissue injury and intestinal barrier dysfunction after GI/R (Fig. 8, A and B, respectively). Addition of MBL to MBL-null mice undergoing GI/R increased ALT levels (Fig. 8A) and intestinal barrier dysfunction (Fig. 8B). Anti-human MBL mAb treatment significantly inhibited these alterations, demonstrating the specificity of MBL-induced injury in this model.

These results suggest that GI/R injury occurs via a C2-dependent, C1q-independent mechanism. Additionally, these results demonstrate that MBL-null mice are protected from intestinal, but not secondary, lung injury. To further investigate the contribution of MBL in GI/R, we examined at C3 deposition in MBL-null mice. MBL-null mice showed minimal C3 staining in the intestine after GI/R compared with sham controls (Fig. 9, B and A, respectively). However, MBL-null mice reconstituted with rhuMBL demonstrated increased C3 deposition in the intestine (Fig. 9C), and this deposition was inhibited by anti-MBL treatment (Fig. 9D). Surprisingly, MBL-null mice, after GI/R, demonstrated increased C3 deposition in the lung, similar to MBL-null mice reconstituted with rhuMBL (Fig. 9, F and G, respectively) compared with sham controls (Fig. 9E). Anti-MBL treatment concomitant with addition of rhuMBL appeared to slightly attenuate GI/R-mediated C3 deposition in the lung (Fig. 9H). These data indicate that MBL is responsible for initiating complement activation in the intestine, but not in the lung, after GI/R.

**Discussion**

Collectively, our findings suggest that the lectin complement pathway, not the classical complement pathway, is a key initiator of GI/R injury. The present study shows that selective inhibition of the classical pathway does not attenuate C3 deposition, tissue injury, or neutrophil infiltration in the intestine or lung, nor does it prevent intestinal permeability changes. Furthermore, this study shows that mice devoid of MBL, yet maintaining intact classical and alternative pathways, are protected from intestinal injury, neutrophil infiltration in the intestine, intestinal permeability dysfunction, and secondary liver injury, as measured by ALT, whereas addition of MBL reconstitutes injury. Interestingly, this study also demonstrates that MBL-null mice are not protected from secondary lung injury, and complement is deposited in the lung.

Although previous studies using mice deficient in C4, C3, IgM (RAG1−/−), or Cr2 or wild-type mice treated with soluble complement receptor 1 suggested that the classical complement pathway mediates complement activation after GI/R (13, 19, 20), the possible involvement of the lectin complement pathway in these studies could not be ruled out. Williams et al. (13) suggested a predominant role of the classical pathway for initiation of I/R injury in the intestines of mice by showing reduced organ staining for C3 and protection from injury in C4 KO and IgM-deficient (RAG1−/−) mice. However, loss of C4 inhibits both lectin and classical pathways. Soluble Cr1 binds and inhibits both C3 and C5 convertases, thus inhibiting all complement pathways. Although RAG1−/− or Cr2 mice reconstituted with IgM show increased staining and colocalization of C3 with IgM, Ig deposition alone may not demonstrate classical pathway-dependent cause and effect. If the classical pathway were involved in GI/R by natural Ab, then deletion of C1q should demonstrate tissue protection. A recent report shows that IgM from a single self-reactive hybridoma clone was sufficient to restore GI/R injury in RAG1−/− mice (22).

Thus, a role of natural Abs may be correct, but it may be their interaction with MBL and not C1q that mediates complement activation. It is well known that MBL and Igs interact. For example, IgM, IgG, or IgA can enhance lectin pathway-mediated hemolysis (37, 38). Furthermore, polymeric IgA binds MBL and activates the lectin pathway (39). MBL can also enhance phagocytosis of IgG-or IgG- and C4b-opsonized erythrocytes (40). Additionally, IgM purification using MBL-agarose beads (Pierce) used EDTA to elute IgM, suggesting that MBL/IgM interactions occur via MBL’s carbohydrate recognition domains and not collagen tails (41). Karpel-Massler et al. (14) demonstrated that C1 inhibitor protects against intestinal I/R injury. However, as also described by these investigators

**FIGURE 9.** Confocal micrographs of C3 deposition in MBL-null mice. C3 deposition in jejunum (left) and lung (right) sections is shown. Each micrograph is representative of three to five mice. Original magnification, ×200.
liver; however, L-ficolin is also produced in the lung by alveolar
Ag) and L-ficolin (p35) (52). Ficolins are mainly synthesized in the
MBL, the lectin pathway can be activated by H-ficolin (Hakata
given saline, after GI/R. Thus, lung injury in mice after GI/R is
mice as well as C2/fB KO mice given C2, but not C2/fB KO mice
ment (C3) was deposited in the lungs of C1q KO and MBL-null
lung injury and pulmonary neutrophil infiltration as MBL-null
shows that inhibition of complement provides better tissue
dependent, as we have shown previously (7, 8). These
levels was observed after GI/R compared with those in sham con-
with regard to tissue injury in the gastrointestinal tract remains to
report that mucosal barrier failure after gastrointestinal injury is not
dependent on C1q, but is dependent on MBL. Furthermore, addition of
MBL to MBL-null mice and its inhibition by anti-MBL treat-
ment demonstrated the specificity of MBL and its actions on gut
permeability changes after GI/R.

Intestinal I/R-induced mucosal barrier failure is characterized by
translocation of enteric bacteria through the intestinal barrier to
extraintestinal sites and the systemic circulation (49, 50). A severe
complication of GI/R, most likely due to changes in intestinal perme-
ability, is multiorgan failure, also referred to as remote or sec-
ondary organ injury (2, 4, 5). Significant elevations of serum
FITC-dextran concentrations were observed in WT and C1q KO,
but not C2/IB KO mice after GI/R. Furthermore, C1q KO mice
after intestinal I/R demonstrate neutrophil sequestration within the
lung similar to that observed in WT I/R mice. Restoration of C2
in C2/IB KO mice with human C2 increased GI/R-induced intestinal
permeability and neutrophil sequestration within the lung. Previous
studies showed that C4 and terminal complement components are
important for increased gastrointestinal permeability (13, 17).
The present study extends these observations by demonstrating that
mucosal barrier failure after gastrointestinal injury is not de-
pendent on C1q, but is dependent on MBL. Furthermore, addition of
MBL to MBL-null mice and its inhibition by anti-MBL treat-
ment demonstrated the specificity of MBL and its actions on gut
permeability changes after GI/R.

Intestinal MPO levels are extremely low at baseline (sham) and
after I/R. A significant increase in WT and C1q KO intestinal MPO
levels was observed after GI/R compared with those in sham con-
trols. Whether this significant increase has biological importance
with regard to tissue injury in the gastrointestinal tract remains to
be identified. Additionally, C1 inhibitor has been previously
shown to provide more protection from I/R-mediated tissue dam-
age than that conferred by anti-CD18 interventions (51). These
data suggest that inhibition of complement provides better tissue
protection than inhibition of neutrophil function. Regardless, there
was not a significant increase in intestinal tissue MPO activity in
C2/IB KO mice after GI/R compared with sham-operated control
mice, collectively suggesting that neutrophil infiltration into in-
testinal tissue after GI/R is not dependent on C1q, but is complement
dependent, as we have shown previously (7, 8).

C1q KO and MBL-null mice demonstrated similar secondary
lung injury and pulmonary neutrophil infiltration as MBL-null
mice reconstituted with MBL after GI/R. Interestingly, comple-
ment (C3) was deposited in the lungs of C1q KO and MBL-null
mice as well as C2/IB KO mice given C2, but not C2/IB KO mice
given saline, after GI/R. Thus, lung injury in mice after GI/R is
C1q and MBL independent, but C2 dependent. In addition to
MBL, the lectin pathway can be activated by H-ficolin (Hakata
Ag) and L-ficolin (p35) (52). Ficolins are mainly synthesized in the
liver; however, L-ficolin is also produced in the lung by alveolar

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