

The Differing Roles of the Classical and Mannose-Binding Lectin Complement Pathways in the Events following Skeletal Muscle Ischemia-Reperfusion¹

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Complement is an important mediator of the injuries observed after skeletal muscle ischemia and subsequent reperfusion. Although the classical pathway had been assumed to be the major pathway of activation leading to injury, the mannose-binding lectin (MBL) pathway might also play a contributing role. In this study, we found that MBL-deficient mice had significant protection after skeletal muscle reperfusion injury compared with wild-type, classical pathway-specific C1q-deficient mice, or MBL-deficient mice reconstituted with recombinant human MBL. MBL-deficient mice, however, were not protected from permeability edema or secondary lung injury after ischemia-reperfusion. These data indicate that blockade of the classical pathway alone (C1q) is protective against permeability edema and remote pulmonary injury but not protective against histologic muscle injury. In contrast, blocking the MBL pathway alone protects against histological injury but is not protective against permeability edema or lung injury. Thus, the activation of both pathways is likely responsible for the full spectrum of injuries observed after skeletal muscle reperfusion injury. *The Journal of Immunology*, 2006, 177: 8080–8085.

The local and systemic injury that ensues after reperfusion of ischemic skeletal muscle is not only an important clinical problem in its own right, but also serves as a model-system of the events that might occur after reperfusion of other ischemic organs or after severe hemorrhagic shock. Initially assumed to be an injury exclusively caused by exhaustion of metabolic substrates, it now appears that amplification of nascent metabolic injury by the host inflammatory system causes the bulk of the damage. The defined elements of injury after reperfusion of ischemic skeletal muscle are disruption of muscle fibers (1), increased vascular permeability (2), and systemic neutrophil activation (3) as reflected by pulmonary injury. The inflammatory basis of this reperfusion injury has been established by immunologic studies that have identified the formation of immune complexes and activation of complement as pivotal events (1).

Both wild-type mice (C57BL/6) treated with sCR1, an inhibitor of the major complement protein C3, and C3 knockout mice, after lower extremity reperfusion injury, demonstrate attenuated vascular permeability, histological muscle injury, and mast cell degranulation (2, 3). The activation of complement that produces C3 cleavage proceeds via Factor B of the alternative pathway or via

C4 of the classical and mannose binding lectin (MBL)³ pathways. C4 knockout mice, deficient in both the classical and MBL pathways, demonstrate as much attenuation of injury parameters after hind limb ischemia-reperfusion (HLIR) as do C3 knockout mice. Thus, permeability, cytotoxicity, and mast cell degranulation are also dependent on the classical and/or lectin pathways.

The classical pathway of complement is activated by Ab binding to target Ags, followed by C1q binding to the Ab leading to C4 cleavage. MBL, conversely, is believed to be initiated on cell surfaces rich in *N*-acetylglucosamine and mannose residues in microbial rather than mammalian cells. MBL shares significant homology with the C1q subunit of C1, whereas MBL-associated serine protease (MASP)-2 and MASP-3 are homologous to C1r and C1s, respectively. The three MASPs (MASP-1, MASP-2, and MASP-3) form complexes with MBL in circulation and can activate complement (4). Recently, M-ficolin, L-ficolin, and H-ficolin have also been found to form complexes with MASPs to activate complement, similar to MBL (5). For instance, ficolin-A binding to MASP-2 initiates the complement cascade in mice independent of MBL (6). In humans, MBL is encoded by a single gene, whereas in rodents, MBL is encoded by genes *mb11* and *mb12* (7).

The demonstration that RAG knockout mice deficient in Ab produces attenuated injury subsequent to muscle reperfusion has led to the hypothesis that C1q and the classical pathways are the relevant pathways involved in complement activation. This hypothesis was strengthened by the finding that endogenous IgM was a critical element in the initiation of reperfusion injury (2). However, recent work by others has indicated that reperfusion injury could partially result from activation of the MBL pathway (8, 9). In this study, we have re-examined the Ab dependency hypothesis using MBL-deficient and C1q-deficient mice. Our results indicate that there are significant differences in the contribution of MBL and classical pathways to the pathogenesis of reperfusion injury.

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³ Abbreviations used in this paper: MBL, mannose-binding lectin; rhMBL, recombinant human MBL; HLIR, hind limb ischemia-reperfusion; MASP, MBL-associated serine protease.

Materials and Methods

Animals

Male C57BL/6 mice (Taconic Farms) were used as parental strain controls (wild-type) in all experiments. C1q^{-/-} mice were bred in the laboratory of Dr. M. Carroll (Center for Blood Research, Boston, MA) as previously described by Botto et al. (10). MBL A/C^{-/-} mice were produced in our laboratory as previously described (7), on a mixed C57BL/6 × 129 background and backcrossed into C57BL/6 for seven generations. The backgrounds of these mice were verified using microsatellite analysis.

Mice were maintained in accordance with the guidelines of the Harvard Medical School Committee on Animals and of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council as found in Department of Health, Education and Human Services, Publication no. 85-23 (National Institutes of Health).

Animal experiments

Mice (25–30 g), ages 6–8 wk, were anesthetized by i.p. injection of pentobarbital (60 mg/kg) and subjected to 2 h of hind limb ischemia followed by 3 h of reperfusion. Longer periods of ischemia resulted in increased mortality. Hind limbs were elevated for 2 min before the initiation of ischemia. After which, a tourniquet was applied above the greater trochanter using bilateral double rubber bands (Latex O-rings) from the McGivney Hemorrhoid Ligator kit (MilteX). Control mice did not undergo banding. Mice were maintained in a supine position and kept anesthetized throughout the course of the experiment. At the end of the experiment, mice were sacrificed by pentobarbital (120 mg/kg) injection and their hind limbs and lungs were harvested.

Reconstitution of the MBL-deficient mice was performed 1 h before the initiation of ischemia by injecting 75 μg of recombinant human (rh)MBL into the tail vein (11).

Permeability studies

Five minutes before tourniquet release, animals were reperfused with 1 μCi of ¹²⁵I-labeled human albumin (ICN Pharmaceuticals) in 0.3 ml of normal saline via tail vein injection. Three hours after reperfusion, blood was aspirated from the right ventricle of the mice, and gamma radioactivity in the blood was quantitated by liquid scintillation counting (Packard Instrument). Muscle was harvested from both hind limbs, its radioactivity measured by liquid scintillation counting, and then dried to a constant weight in a gravity convection oven (Precision Scientific Group) at 100°C for two nights. The vascular permeability index, which represents extravasation of ¹²⁵I-albumin, was determined as the ratio of radioactivity per gram of dry muscle to radioactivity per gram of blood.

Pathological processing

Muscle and lung sections were fixed in 4% paraformaldehyde in PBS for 18 h followed by incubation in JB-4 glycolmethacrylate overnight. Sections were then stained with Masson's Trichrome for histological assessment of muscle fiber integrity. Fifty individual muscle fibers were counted in each histological section and the extent of injury was expressed as the number of damaged fibers divided by 50 and averaged over 5 fields per animal in blinded counts by a pathologist. The final injury score is presented as the average of the number of animals used in the experiment, with its corresponding SE. Lungs were stained with chloroacetate esterase for the assessment of pulmonary neutrophils. Quantification of lung injury was performed by counting the number of pulmonary neutrophils per high-power field (magnification, ×40) for 5 fields per animal in a blinded test by a pathologist.

To identify mast cells, tissue sections were incubated with chloroacetate esterase, which stains protease/proteoglycan macromolecular complexes bright red. Both macromolecular complexes found within granules in mast cells as well as those found extracellularly from degranulated mast cells are stained in this assay. A section through an intact mast cell normally contains over 75 secretory granules. When three or more secretory granules were found outside the cell, the cell was considered degranulated. We had previously noted that degranulation was usually limited to mast cells within injured skeletal muscle bundles (3).

Immunohistochemistry

Sections for immunohistochemical studies were prepared from the gastrocnemius muscle. The muscle was embedded in paraffin before sectioning. Sections were heated at 60°C for 1 h before deparaffinization and hydration. Sodium citrate 10 mM (pH 6.0) was then added to the sections and heated to 100°C for 10 min for Ag retrieval.

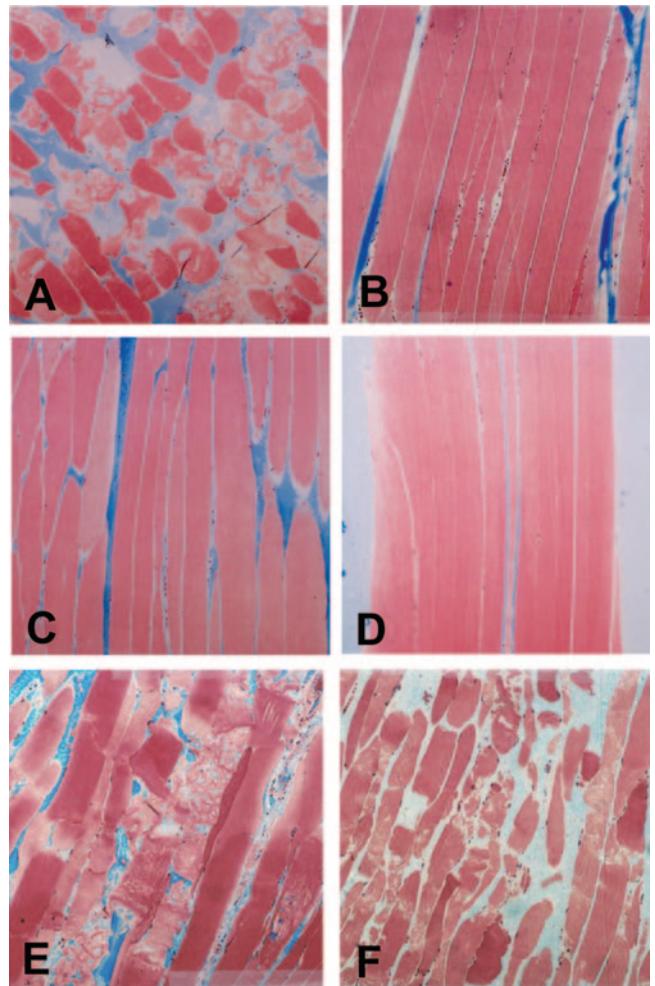


FIGURE 1. MBL deficiency prevents muscle fiber disruption after HLIR. Photomicrographs (original magnification, ×40) of murine gastrocnemius muscle in longitudinal orientation stained with Masson's Trichrome. *A*, C57BL/6 mice subjected to HLIR. *B*, C57BL/6 mice subjected to anesthesia without injury. *C*, MBL-deficient mice subjected to HLIR. *D*, MBL mice subjected to anesthesia without injury. *E*, MBL mice reconstituted with rhMBL and then subjected to HLIR. *F*, C1q-deficient mice subjected to HLIR. In response to reperfusion injury, gross disruption of skeletal muscle fibers is seen in C57BL/6 mice, reconstituted MBL mice, and C1q-deficient mice, but not in MBL-deficient mice.

For IgM staining, sections were blocked in 1.5% horse serum for 1 h and incubated in biotinylated goat anti-mouse IgM (1/25 dilution; The Jackson Laboratory) for 1 h, and developed with peroxidase-conjugated avidin-biotin complex using diaminobenzidine as a substrate (Vectorstain ABC; Vector Laboratories).

For C3 staining, sections were blocked with 5% rabbit serum and incubated overnight at 4°C with 1 μg/ml goat anti-mouse C3 (a gift from L. Kobzik, Harvard School of Public Health, Boston, MA). Peroxidase-conjugated rabbit anti-goat IgG (1/50 dilution) was then added and incubated further for an hour. Immunoreactivity was visualized directly by peroxidase assay using diaminobenzidine as a substrate or after enhancement of signal with goat peroxidase-antiperoxidase (Sternberger Monoclonals) and using diaminobenzidine as a substrate. To control for nonspecific secondary Ab binding, primary Ab was omitted from control sections.

Statistical analyses

Results are presented as the mean ± SE. Comparisons between groups were performed by one-way ANOVA, and when significant difference was found, Student's *t* test with the Bonferroni correction for multiple comparisons was applied.

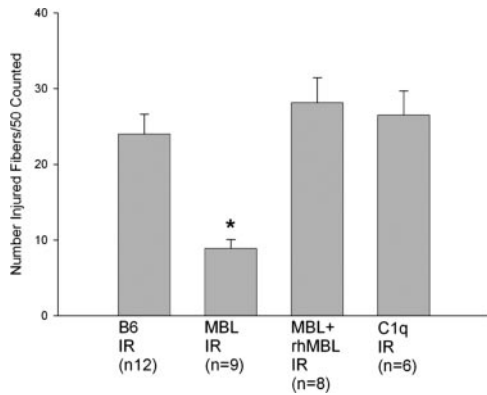


FIGURE 2. MBL deficiency prevents muscle fiber disruption after HLIR. Representative cross-sections of the gastrocnemius muscle from mice subjected to HLIR were used to quantitate muscle fiber disruption. Mice shown are C57BL/6 mice subjected to HLIR (B6 IR), MBL-deficient mice subjected to HLIR (MBL IR), MBL mice reconstituted with rhMBL and subjected to injury (MBL + rhMBL IR), and C1q-deficient mice subjected to HLIR (C1q IR). Uninjured, anesthetized mice had 0 fibers disrupted/50 counted (data not shown). The MBL IR group was significantly less injured than C57BL/6, reconstituted MBL, and C1q-deficient groups. Error bars depict SEM. Statistical significance is indicated by *, $p < 0.05$.

Results

MBL, but not C1q, deficiency protects against histological muscle injury after HLIR

Mice deficient in MBL exhibited significant protection from muscle necrosis after HLIR. In these mice, the majority of mus-

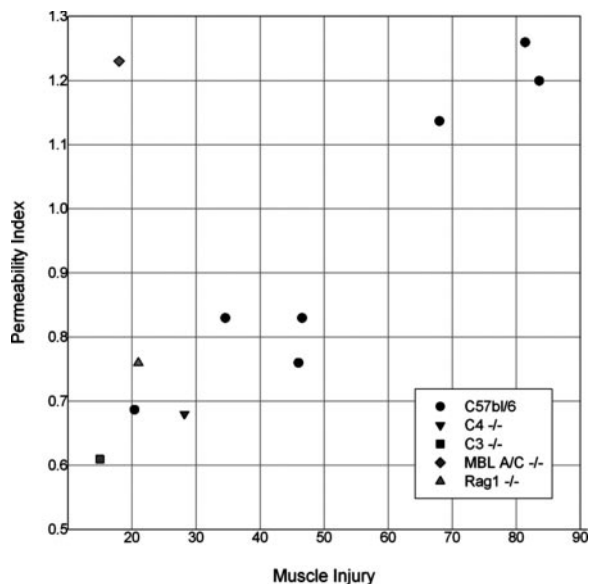


FIGURE 3. No correlation determined between muscle fiber disruption and muscle permeability in MBL-deficient mice after HLIR. Plot showing the linear relationship between increasing hind limb permeability index and increasing quantitative muscle injury in multiple strains of mice analyzed after HLIR. Hind limbs from anesthetized, uninjured mice have a permeability index of 0.1–0.2. Muscle injury is plotted as the percentage of the maximum seen at 3 h of reperfusion. Mice tested include: C57BL/6 mice after increasing periods of reperfusion up to 24 h (●); C4^{-/-} mice (▼); C3^{-/-} mice (■); MBL^{-/-} mice (◆); and Ab-deficient RAG1^{-/-} (knock-out) mice (▲). Data show that MBL-deficient mice exhibit increased permeability edema after HLIR despite a lack of histological muscle injury. Data shown for C3^{-/-}, C4^{-/-}, and Ab-deficient mice were extracted from our previously published work (3).

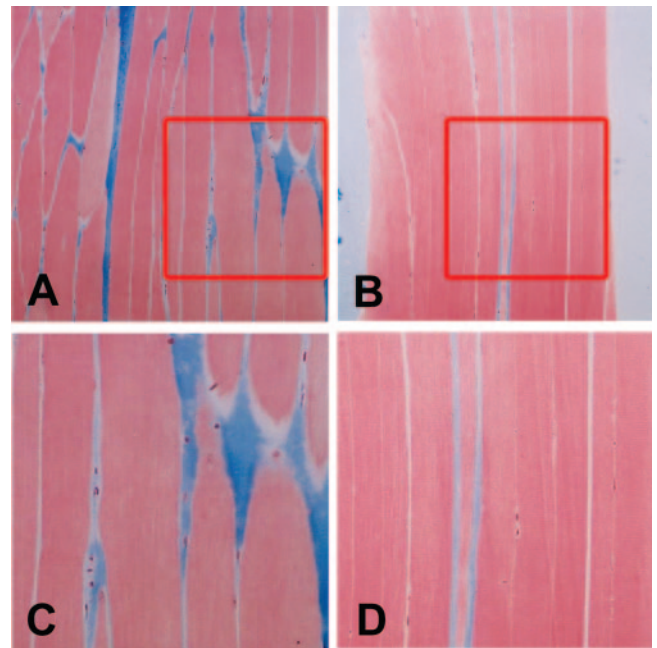


FIGURE 4. MBL deficiency increases intracellular and extracellular edema after HLIR. Photomicrographs of Masson's Trichrome-stained sections of gastrocnemius muscle from MBL-deficient mice subjected to HLIR (A and C) or to anesthesia without injury (B and D) at original magnification $\times 40$ and $\times 60$, respectively. Photomicrographs show nonfiber disruptive histologic changes (muscle fiber swelling and increased intercellular gaps) in MBL-deficient mice (A and C).

cle fibers were intact, as opposed to widespread muscle fiber disruption seen in C57BL/6 mice subjected to the same injury (Fig. 1). Quantitative assessment of muscle injury in cross-sections (Fig. 2) revealed that MBL-deficient mice ($n = 9$) had a significantly lower number of injured fibers (8.9 ± 1.2 injured fibers/50 counted) compared with C57BL/6 mice ($n = 12$) (24 ± 2.6 injured fibers/50 counted) ($p < 0.05$). This protection is reverted to normal injury levels in MBL-deficient mice reconstituted with rhMBL ($n = 8$) before the beginning of the experiment (28 ± 3.3 injured fibers/50 counted). Human MBL has been previously shown to reproduce the MBL phenotype in MBL-deficient mice (12). In contrast to MBL-deficient mice, C1q-deficient mice ($n = 6$) had similar injury (27 ± 3.2 injured fibers/50 counted) to that seen in C57BL/6 mice.

C1q, but not MBL, deficiency protects against muscle permeability edema after HLIR

When permeability edema was used as the measure of injury, C57BL/6 mice had a significant increase in permeability (1.2 ± 0.1) over uninjured controls (0.11). Interestingly, MBL-deficient mice also displayed a similar increase in permeability (1.2 ± 0.2) over uninjured controls (0.27). Although increased tissue permeability has been previously shown to have a direct correlation with muscle cytotoxicity, MBL-deficient mice did not exhibit this relationship (Fig. 3). MBL-deficient mice instead had increased tissue permeability without histological muscle injury. This increases permeability was also evident by the presence of enlarged muscle fibers and widened intercellular spaces in MBL-deficient mice after ischemia-reperfusion compared with uninjured mice (Fig. 4).

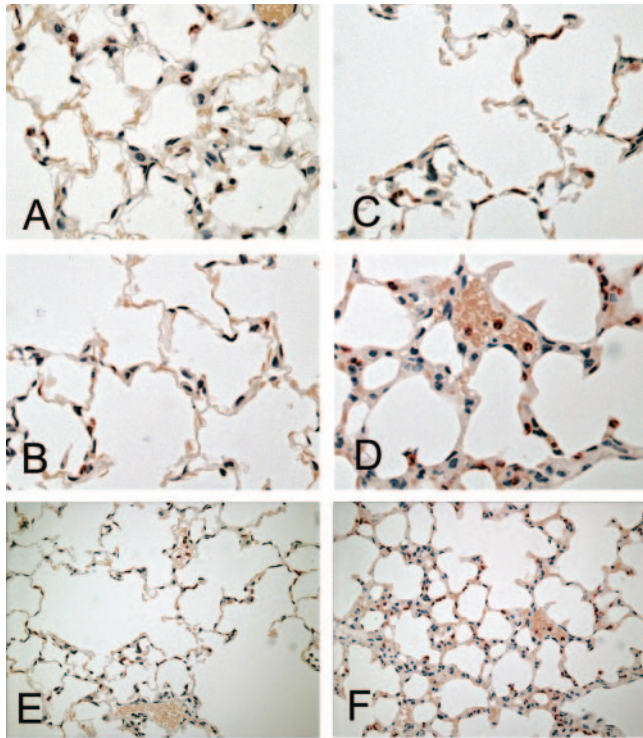


FIGURE 5. MBL deficiency increases pulmonary neutrophils after HLIR. Photomicrographs (original magnification, $\times 60$) of lung parenchymal sections are stained with chloroacetate esterase to detect pulmonary neutrophils. *A*, C57BL/6 mice subjected to HLIR. *B*, C57BL/6 mice subjected to anesthesia without injury. *C*, C1q-deficient mice subjected to HLIR. *D*, MBL-deficient mice subjected to HLIR. *E* and *F* are magnification $\times 40$ of *C* and *D*, respectively. Parenchymal edema and neutrophil accumulation are observed in injured C57BL/6 and MBL-deficient mice, but not in C1q-deficient mice.

C1q, but not MBL, deficiency attenuates pulmonary neutrophil accumulation after HLIR

After HLIR injury, MBL-deficient mice demonstrated sequestration of neutrophils in the lungs similar to C57BL/6 mice, but different from C1q-deficient mice (Fig. 5). When the number of pulmonary neutrophils was determined as a measure of remote lung

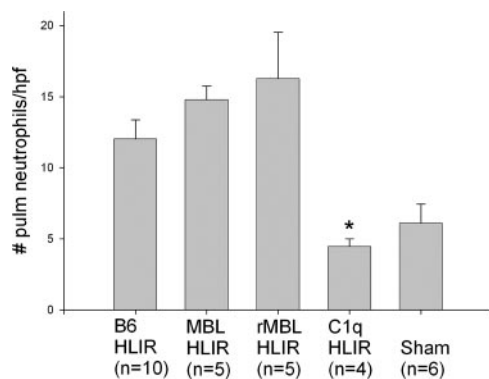


FIGURE 6. MBL deficiency increases pulmonary neutrophils after HLIR. Quantitative assessment of lung injury is expressed as the number of pulmonary neutrophils per high-power field. HLIR increased pulmonary neutrophils in C57BL/6 (B6 HLIR) and MBL-deficient mice (MBL HLIR) but not in C1q-deficient mice (C1q HLIR). Reconstitution of MBL-deficient mice with rhMBL produces little additional neutrophil accumulation. Control, anesthetized, uninjured C57BL/6 mice (sham) are indicated. Error bars depict SEM. Statistical significance is shown *, $p < 0.05$.

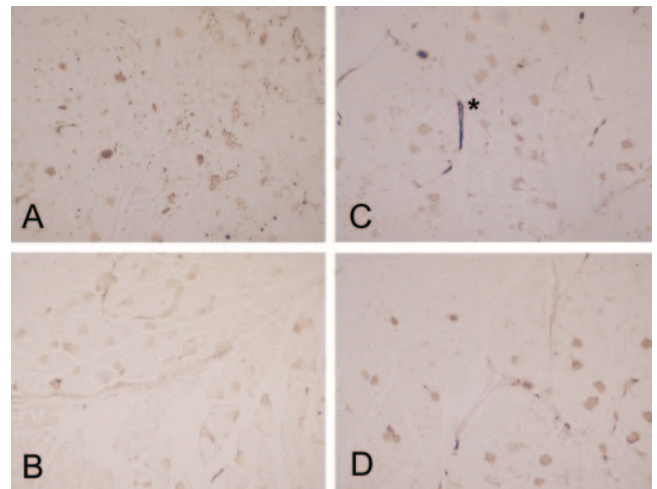


FIGURE 7. Deposition of innate immunity components after HLIR in MBL-deficient mice. Photomicrographs (original magnification, $\times 40$) of murine gastrocnemius muscle after HLIR, probed with Abs for the presence of C3 and IgM. *A* and *B*, C57BL/6 mice are subjected to ischemia and reperfusion injury and stained for IgM and C3 deposition, respectively. *C* and *D*, MBL-deficient mice are subjected to HLIR and stained for IgM and C3 deposition, respectively. Brown color in the shape of muscle fiber represents specific binding of either anti-C3 or anti-IgM. A central blood vessel staining positive for IgM (*) is marked.

injury, C57BL/6 mice had a significant increase in pulmonary neutrophils (12 ± 1.3) over uninjured controls (6.1 ± 1.4) ($p < 0.05$). MBL-deficient mice as well as MBL-deficient mice reconstituted with rhMBL showed no attenuation of pulmonary neutrophil accumulation, with 15 ± 0.9 and 16 ± 3.2 neutrophils, respectively, compared with C57BL/6 mice. The lack of protection against remote pulmonary injury is particularly notable in the setting of protection against local muscle disruption. In contrast, classical pathway-deficient mice (C1q knockouts) showed significant attenuation of pulmonary neutrophil accumulation (4.5 ± 0.53) compared with C57BL/6 mice ($p < 0.05$) (Fig. 6).

MBL-deficient mice demonstrate IgM and C3 deposition on muscle fibers after HLIR

MBL-deficient mice after HLIR demonstrated IgM and C3 levels on muscle fibers that were similar to those found in MBL-sufficient C57BL/6 mice, suggesting an activated classical complement pathway despite a lack of histological injury (Fig. 7).

MBL-deficient mice demonstrate mast cell activation after HLIR

We have previously shown that tissue mast cells degranulate following HLIR (3). In the present study, C57BL/6 mice had a greater number of intact mast cells before HLIR (18 ± 0.7) vs after HLIR (12 ± 2.4) ($p = 0.12$). This translates to a 33% rate of degranulation after HLIR. Similarly, there were more intact mast cells among MBL-deficient mice before reperfusion (22 ± 1.8) vs after reperfusion (16 ± 2.2) ($p = 0.09$), representing a 27% rate of degranulation.

Discussion

Previous studies using complement C3- and C4-deficient mice, as well as complement-inhibited rats, have emphasized the role of complement in the production of cytotoxic muscle injury, local vascular permeability, and remote lung injury following ischemia-reperfusion (2, 13, 14). These studies do not, however, completely distinguish the relative contributions of the two complement pathways leading to C4 cleavage, namely classical or C1q-dependent

and MBL-dependent pathways. In the present study, we show that both the classical and the MBL pathways are activated, but they are responsible for different features of reperfusion injury. To dissect the roles of the two C4-activating complement pathways, knockout mice specific to each pathway were used. C1q-deficient mice lack the classical pathway but retain robust MBL and alternative pathways (10), whereas MBL-deficient mice have a robust classical and alternative pathway while lacking MBL (7, 12).

We observed a lack of protection against cytotoxic muscle injury in C1q-deficient mice compared with protection against cytotoxicity seen in MBL-deficient mice (Figs. 1 and 2). Restoration of cytotoxic injury in MBL-deficient mice after reconstitution with rhMBL suggests that activation of the MBL pathway is both necessary and sufficient in the development of local cytotoxic injury. Our results are concordant with those published recently by the Stahl group (8, 9) using murine models of gastrointestinal and cardiac ischemia-reperfusion. Taken together, these findings suggest that the activation of the MBL pathway plays a key role in mediating the cytotoxic effects of reperfusion injury.

Another characteristic feature of reperfusion injury is the development of local vascular injury, demonstrated most commonly by increased vascular permeability in the zone of injury. In our experiments, vascular injury appears to be mediated by the classical pathway and not the MBL pathway. MBL-deficient mice, with an intact classical pathway, demonstrated an increased vascular permeability that was also seen in C57BL/6 mice after reperfusion injury. Vascular leakage, however, was not observed in C1q-deficient mice subjected to the same injury (Fig. 4). In fact, the general relationship between increasing muscle fiber necrosis and increasing muscle edema was not seen in MBL-deficient mice (Fig. 3). This permeability is unlike the combined cytotoxic and vascular protection afforded by C3-, C4-, and (RAG^{-/-}) Ab-deficient mice (2). This result may indicate that the MBL-dependent pathway directs C4 and C4 deposition to the muscle bundles and that C1q-dependent pathway directs deposition to the vascular endothelium. In addition, the protection against both cytotoxic and vascular injury afforded by Ab deficiency may indicate that the MBL pathway is activated by Ab deposition on ischemic muscle bundles, whereas the C1q pathway is activated by Ab deposition on injured vascular endothelium.

A final characteristic of reperfusion injury is remote pulmonary injury (15). This systemic aspect of a major local injury is most commonly measured as increased extravasation of intravascular markers into bronchoalveolar lavage fluid (14), a technique that is difficult to perform reproducibly in mice. The generally accepted sequence of remote pulmonary injury is the activation of both neutrophils and pulmonary endothelium leading to leukocyte adherence and extravasation in the lungs (16). We have used the early accumulation of neutrophils in the lung, determined histologically, as an indicator of remote injury in this study. After HLIR, MBL-deficient mice and C57BL/6 mice demonstrated a similar degree of increased pulmonary neutrophil accumulation. C1q-deficient mice did not show this remote response to local reperfusion injury (Figs. 5 and 6). To the extent that remote injury must reflect neutrophil activators being released into the circulation or neutrophil-activating surfaces being exposed at the site of injury, it is not surprising that C1q-deficient mice, which appeared protected from the vascular component of reperfusion injury, also did not exhibit intravascular remote pulmonary injury.

A more recently described feature of reperfusion injury is local mast cell degranulation (3). Mast cell degranulation, initiated after reperfusion, is not observed in C3- or C4-deficient mice. This suggests that mediators released from complement activation are likely initiators of mast cell degranulation. Furthermore, disruption

of muscle fibers after HLIR is not seen in complement-sufficient, mast cell-deficient mice and in mice lacking a mast cell granule enzyme that is similar to human elastase (3). Thus, tissue necrosis after HLIR is dependent on mast cell constituents as well as on the MBL pathway of complement activation. However, as both C57BL/6 mice and MBL-deficient mice demonstrated mast cell degranulation after HLIR, the C1q-dependent pathway is also likely to contribute to mast cell degranulation.

On longitudinal sections, muscle fiber injury after HLIR was observed as multiple points of disruption on single fibers. On cross-sections, injury was observed in patches within a given muscle. Additionally, only a minority of the fibers was injured and intact fibers were found adjacent to disrupted fibers in a random distribution. Our group has previously reported that this pattern of injury is associated with the type of myosin within a particular muscle fiber (17) and that disruptive injury in this model is limited exclusively to fibers containing Fast Twitch myosin (Type IIB fibers). In addition, deposition of C3 and IgM is also limited to these fibers and occurs with reperfusion (not ischemia) and before disruption begins (18). Fig. 7 shows IgM and C3 deposition on injured gastrocnemius muscle (that has a relatively high content of Type IIB fibers) by immunoperoxidase staining. Both C57BL/6 and MBL-deficient mice had equivalent numbers of fibers with IgM deposition, but MBL-deficient mice had fewer fibers with C3 deposition. This again indicates the possible dependency of muscle injury on the MBL pathway. The residual C3 complement activation in MBL-deficient mice might be accounted for by associations of ficolins with MASPs in an MBL-independent manner.

IgM is believed to play a pivotal role as an early effector of the ischemia-reperfusion cascade. In Ab-deficient RAG1^{-/-} mice and Ab-defective cr2^{-/-} mice, ischemia-reperfusion injury is attenuated (19–21), but is restored to wild-type level after i.v. administration of IgM (22, 23). Moreover, blockade of IgM with peptide antagonists also attenuates this injury (23). Based on these data and on our current findings, we hypothesize the following sequence of events to occur after ischemia-reperfusion injury. Ischemia induces expression of antigenic epitopes on the endothelium and on specific muscle fibers. IgM binding to epitopes on the endothelium activates complement via C1q resulting in vascular leakage and intravascular neutrophil activation. IgM binding to epitopes on reperfused muscle bundles activates complement via the MBL pathway leading to mast cell degranulation and necrosis of susceptible muscle fiber types. The activation of complement by C1q binding to bound IgM has been previously demonstrated (24). In contrast, binding of MBL to bound IgM to activate complement has not yet been demonstrated. However, there is indirect evidence for this association. Firstly, MBL shares significant structural similarities to C1q. Secondly, MBL-agarose columns are well known for their use in IgM purification (25). It is therefore plausible that IgM is a common initiator of both the classical and MBL pathways.

Our results demonstrate for the first time that an increase in permeability after reperfusion injury can occur in the absence of histological muscle injury. Histological injury implies muscle necrosis, breakdown of muscle fibers, degradation of intracellular protein, and breakdown of the muscle plasma membrane. Permeability, in contrast, implies an endothelial injury, with loss of integrity of the endothelial basement membrane and intracellular tight junctions. Therefore, specific injury to the muscle or the endothelial compartment will lead to divergent pathophysiological events. The reason for the preferential activation of the classical pathway in the endothelial compartment and the MBL pathway in

the muscle compartment is unclear. The implications of this finding, though, are clear. Effective therapy to prevent reperfusion injury must address blockade of the classical as well as the MBL pathways.

Disclosures

The authors have no financial conflict of interest.

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