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Activation of the Lectin Pathway by Natural IgM in a Model of Ischemia/Reperfusion Injury¹

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Reperfusion of ischemic tissues elicits an acute inflammatory response involving serum complement, which is activated by circulating natural IgM specific to self-Ags exposed by ischemia. Recent reports demonstrating a role for the lectin pathway raise a question regarding the initial events in complement activation. To dissect the individual roles of natural IgM and lectin in activation of complement, mice bearing genetic deficiency in early complement, IgM, or mannan-binding lectin were characterized in a mesenteric model of ischemia reperfusion injury. The results reveal that IgM binds initially to ischemic Ag providing a binding site for mannan-binding lectin which subsequently leads to activation of complement and injury. *The Journal of Immunology*, 2006, 177: 4727–4734.

Acute mesenteric ischemia represents a severe medical problem with a mortality rate of 70–90% (1). A current view (2) is that injury results from both intrinsic and extrinsic pathways following initial ischemia and reperfusion. In the intrinsic pathway, ischemia and subsequent reperfusion lead to a number of intracellular changes including mitochondrial dysfunction (3, 4) and production of reactive oxygen species (5–7) with downstream cellular injury that left unchecked leads to cell death (8–11). However, ischemic cells are also susceptible to a second phase of injury, i.e., the extrinsic pathway, which is mediated by natural IgM and complement. Accordingly, alterations in cell morphology are recognized by the innate immune system resulting in an acute inflammatory response (12).

A large body of evidence has accumulated over the past two decades implicating a major role for the complement system in ischemia/reperfusion (I/R)⁴ injury in humans (13–16) and animals (12, 17–23). Studies (24–26) using mice deficient in complement C4, C3, total Ig (Rag7^{-/-}), or a subset of B-1 lymphocytes (Cr2^{-/-}) found they were protected in the hind limb and intestinal I/R models. Moreover, reconstitution of mice totally deficient in Ab (Rag1^{-/-}) with IgM prepared from wild-type (WT) sera restored injury (26). A single I/R-specific natural IgM clone, CM22,

was identified in the intestinal and hind limb models (27, 28). Recently, highly conserved self-Ags, namely, non-muscle myosin H chain type II (NMHC-II) A and C, were identified (2) as the self-targets, confirming a crucial link in the mechanism of complement-mediated induction of I/R injury. NMHC-II is expressed in all eukaryotic cells and three forms (A, B, and C) of NMHC-II have been identified (29, 30). They are highly conserved among mice and humans but the distribution of the three isoforms varies among tissues. Functionally, NMHC-II isoforms bear ATPase activity, form molecular motors within the cell, and are thought to be important in regulating cytokinesis, cell motility, and cell polarity (31).

Although the above evidence suggests a pivotal role of the classical pathway of complement for reperfusion injury, studies of the lectin pathways have suggested it is also involved (32, 33). In vitro studies of endothelium found that both classical and lectin pathways may be activated under hypoxia/reperfusion condition. In addition, MBL was found as a deposit throughout the ischemic area at risk in myocardial (33) and renal (34) I/R models. A study (35) in a stroke model also found that the powerful neuroprotective action of C1 inhibitor (C1-INH) on brain I/R injury does not require C1q, an early component in the classical pathway of complement. Recently, Stahl and colleagues (36, 37) reported that MBL-deficient mice displayed limited injury in the intestinal or myocardial I/R models. They showed that C2-knockout, but not C1q-deficient, mice were protected in the intestinal I/R model (36). Similarly, C1q-deficient mice were not protected in a myocardial model (37). Their studies suggest that C2 and MBL, but not C1q, are necessary for I/R injury. In addition, Moller-Kristensen et al. (38) reported that the MBL-deficient mice were protected in the renal I/R model. Thus, these reports raised a question regarding the initial events following I/R.

The classical pathway can be initiated by Ab-Ag interaction followed by the activation of C1 and downstream components (C4, C2, and C3). Characterization of the lectin pathway is relatively recent. It is triggered by MBL recognizing certain patterns of carbohydrate structures (39–43). MBL naturally exists in a complex with the MBL-associated serine proteases (MASPs) (44–48). The MASPs are activated when MBL binds to a fitting carbohydrate pattern, resulting in cleavage of the polypeptide chains of the

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⁴ Abbreviations used in this paper: I/R, ischemia/reperfusion; WT, wild type; NMHC-II, nonmuscle myosin H chain type II; MASP, MBL-associated serine protease; Tw, Tween 20.

MASPs (49). The activated MASPs further cleave relevant substrates, i.e., C4 and C2 for MASP-2; C3 and C2 for MASP-1 (50). The activities of MASP-1 and MASP-2 can be regulated by C1-INH (51, 52). It was thought that immobilized IgM may activate the classical but not the lectin pathway as reported (39) in an *in vitro*-binding system using human myeloma IgM and patient serum with or without MBL. However, both murine and human IgM can directly associate with rabbit MBL under certain *in vitro* conditions that are widely used to purify IgM (53). Furthermore, a recent study (54) found that 20% of human serum IgM could bind to immobilized human MBL *in vitro*. Nevertheless, it is not clear whether MBL binds IgM *in vivo* and whether this interaction results in complement activation.

To address this question, mice genetically deficient in early components of either the classical or lectin pathways were characterized in the mesenteric model of I/R injury. Our results reveal colocalization of MBL and IgM in the injured tissues in WT, as well as Ab-deficient, mice reconstituted with I/R-specific IgM. Moreover, MBL^{-/-} but not C1q^{-/-} mice were protected from I/R injury. The finding of IgM bound to ischemic Ag within 15 min of reperfusion in MBL^{-/-} animals supports a model in which IgM binds initially followed by MBL. In addition, *in vitro* experiments determined that murine MBL could bind to natural IgM. Thus, this study suggests a novel sequence of events in which the lectin pathway is activated following Ag recognition by natural IgM.

Materials and Methods

Animals and intestinal model of ischemia reperfusion injury

WT C57BL/6 and Rag1^{-/-} mice (C57BL/6 genetic background) were purchased from The Jackson Laboratory and bred under specific pathogen-free conditions. MBL-A^{-/-} knockout mice were generated and maintained as described previously (55). C1q^{-/-} mice are bred onto the 10th generation of the C57BL/6 background (56).

Surgical protocol for I/R was performed as previously described (2, 26, 27). Briefly, after anesthesia, a laparotomy is performed, and the microclip (125 g of pressure) was applied to the superior mesenteric artery. After 40 min of ischemia, the microclip was removed, and all animals were kept warm during reperfusion. At the end of experiment, the ischemic segment of the jejunum was harvested and the central 4 cm was excised for pathological analysis.

Histopathology and immunohistochemistry analysis

Cryostat sections of intestinal tissues were stained with H&E, blind-coded by a different person, and examined by light microscopy for mucosal damage. The pathology score was assessed based on a procedure modified from

Chiu (57, 58), which included a direct inspection of all microvilli over a 4-cm stretch of jejunum as described (27).

For immunofluorescence staining, biotin-labeled goat anti-mouse IgM (Southern Biotechnology Associates) was incubated overnight on cryosections (fixed with 4% paraformaldehyde; Sigma-Aldrich) followed by staining 1 h with streptavidin-Alexa-568 (Molecular Probes). Colocalization of MBL was achieved by staining with monoclonal rat anti-mouse MBL-C (59), followed by goat anti-rat IgG-FITC (Caltag Laboratories). The colocalization of C3 was performed by using FITC-labeled anti-C3 (DakoCytomation). Sections were mounted in Antifade Mounting Medium with 4',6'-diamidino-2-phenylindole (DAPI; Molecular Probes). Fluorescent images were made with a Leica digital imaging system (Leica).

Immunoprecipitation and protein identification by tandem mass spectrometry

Immunoprecipitation was performed using a standard protocol (2, 60). Briefly, intestinal tissues were flushed with sterile saline and immediately cryofrozen. Intestines were homogenized and lysates were precleared with cyanogen bromide-activated Sepharose 4B (Amersham Biosciences) and Sepharose coupled with goat anti-rat IgG (Caltag Laboratories). Post-cleared supernatants were incubated with Sepharose conjugated with goat anti-mouse IgM (Caltag Laboratories) to capture IgM-bound immunocomplexes. The bound samples were eluted and subsequently analyzed on SDS-PAGE (6%) under reducing conditions. Protein bands were visualized by staining with GelCode Blue (Pierce). Individual Coomassie blue-stained bands were excised from SDS gels, destained, and subjected to enzyme digestion as described previously (61). The peptides were separated using a Nanoflow Liquid Coupled Chromatography System (Waters Cap LC), and amino acid sequences were determined by tandem mass spectrometer (Q-TOF micro). Mass spectrometry data were processed and subjected to database searches using Mascot (Matrixscience) against Swissprot, TREMBL/New, or the National Center for Biotechnology Information nonredundant database.

In vitro MBL-binding assay

Purified CM31 and CM22 monoclonal IgMs were coated in polystyrene microtiter wells (Maxisorp; Nunc) in concentrations at 0.1, 1, and 10 µg/ml diluted in 150 mM NaCl/ 20 mM Tris-HCl (pH 9.4) by incubation overnight at 4°C. Similarly, mannan was coated into wells at a concentration of 10 µg/ml. The wells were washed three times in 150 mM NaCl, 15 mM Na₃, 10 mM Tris-HCl (pH 7.4; TBS) with 0.05% (v/v) Tween 20 (TBS/Tw) and blocked for 1 h at room temperature with 0.1% (w/v) human serum albumin (State Serum Institute) diluted in TBS. The wells were washed three times either in TBS/Tw with 1 mM CaCl₂ (TBS/Tw/Ca²⁺) or in TBS/Tw with 1 mM EDTA (TBS/Tw/EDTA). Mouse serum (M-5905; Sigma-Aldrich) was diluted 10-, 100-, 1,000-, 10,000-, and 100,000-fold in TBS/Tw/Ca²⁺ or in TBS/Tw/EDTA. Following incubation overnight at 4°C, the wells were washed three times in either TBS/Tw/Ca²⁺ or TBS/Tw/EDTA. Bound murine MBL-A and MBL-C was detected by time-resolved immunofluorometry as described elsewhere (62). In brief, biotinylated mAbs, recognizing either murine MBL-A (I3H6) or MBL-C

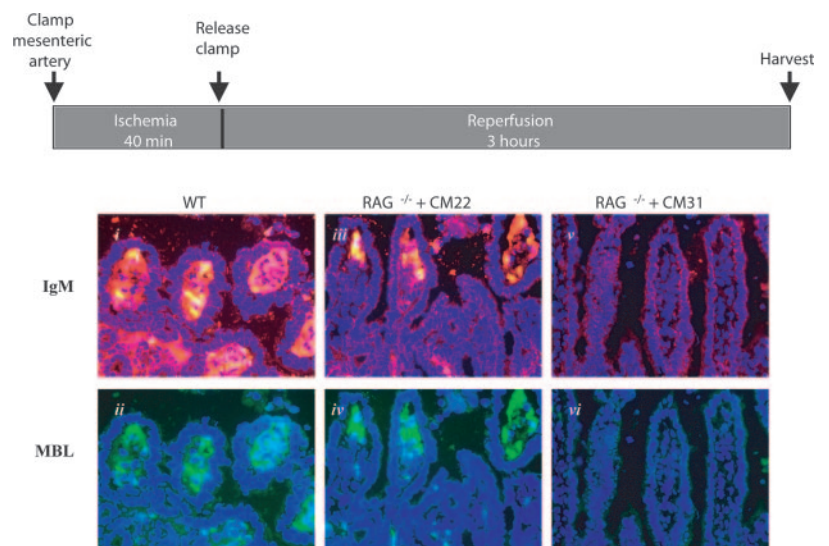
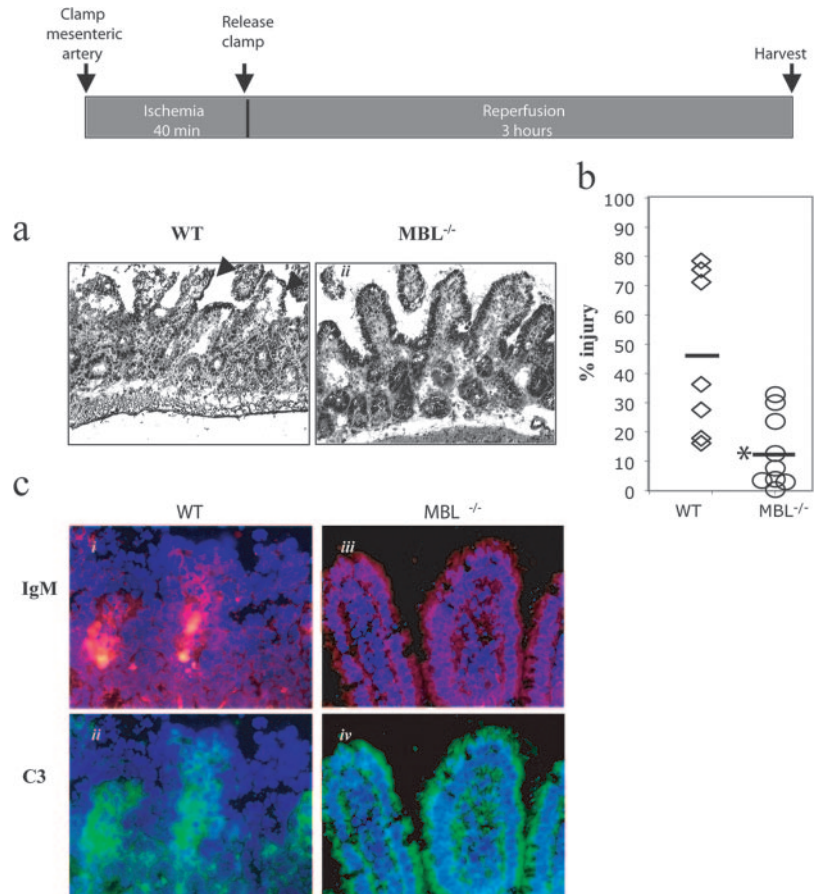


FIGURE 1. Correlation of IgM and MBL deposition in the intestine tissues of I/R-treated animals. Representative cryosections of intestinal tissues were harvested following intestinal I/R. WT mice (*i* and *ii*), IgM^{CM22}-reconstituted RAG-1^{-/-} mice (*iii* and *iv*), and IgM^{CM31}-reconstituted RAG-1^{-/-} mice (*v* and *vi*). *i*, *iii*, and *v*, Stained with anti-IgM-biotin followed by streptavidin-Alexa-568 (red). *ii*, *iv*, and *vi*, Stained with rat anti-mouse MBL followed by goat anti-rat IgG-FITC (green). All sections were counterstained with DAPI (violet). Original magnification, ×400.

FIGURE 2. MBL^{-/-} mice are protected from intestinal reperfusion injury. *a*, Representative sections from I/R-treated WT and MBL^{-/-} mice (both on the 129/B6 genetic background) were stained with H&E. Arrows indicate typical pathology features of injury. Original magnification, ×200. *b*, Pathology scores were assigned based on the degree of injury. Each symbol represents an individual mouse. The horizontal bars represent the arithmetic means of the injury scores. Asterisks indicate statistical significance (Student's *t* test; *p* < 0.05). *c*, IgM and complement C3 were absent within the microvilli of MBL^{-/-} mice. Representative cryosections were stained with Ab specific for mouse IgM or C3. Original magnification, ×400. *i* and *ii*, WT mice and MBL^{-/-} mice (*iii* and *iv*). *i* and *iii*, Stained with anti-IgM-biotin followed by streptavidin-Alexa-568 (red). *ii* and *iv*, Stained with anti-C3-FITC (green). All sections were counterstained with DAPI (violet).



(I4D12), were applied to wells and diluted in TBS/Tw/Ca²⁺ to concentrations of 0.4 and 0.25 μg/ml, respectively. After three washes, the wells received Eu³⁺-labeled streptavidin diluted in TBS with 25 μM EDTA and, after three washes, signals were read in a Delphia fluorometer (Delphia).

Results

To study the role of the lectin pathway in the mesenteric model of I/R injury, we examined the deposition of MBL in injured tissues. Jejunum tissues isolated from WT animals treated for 40 min of ischemia and 3 h of reperfusion were analyzed by immunohistochemistry for IgM and MBL deposition. As reported previously (27), IgM deposited within the injured microvilli. Moreover, staining with MBL-specific Ab identified colocalization of MBL and IgM (Fig. 1, *i* and *ii*). To test whether MBL deposition is directly related to IgM binding, mice lacking Ig (Rag1^{-/-}) were reconstituted with the I/R-specific monoclonal IgM (CM22) or control IgM (CM31) (27). Previous studies (26, 27) demonstrated that Rag1^{-/-} mice were protected from intestinal I/R injury and protection was abrogated by reconstitution with WT IgM or CM22 IgM before I/R. After 40 min of ischemia and 3 h of reperfusion, the jejunum sections of CM22 reconstituted Rag1^{-/-} mice revealed specific IgM deposition in the injured microvilli, which colocalized with MBL (Fig. 1, *iii* and *iv*). In contrast, no deposits of IgM or MBL were present in Rag1^{-/-} mice receiving the irrelevant IgM, i.e., CM31 (Fig. 1, *v* and *vi*).

To explore further the role of MBL in this model, mice lacking both MBL-A and MBL-C were characterized (55, 59). Although the mice are totally deficient in MBL, they retain an intact classical pathway of complement (55). Analysis of intestine pathology after 3 h of reperfusion revealed that the deficient mice failed to develop injury compared with MBL-sufficient littermates (injury scores: 13 ± 13% vs 46 ± 28%, respectively; *p* < 0.05; Fig. 2, *a* and *b*),

consistent with an earlier report (36). Furthermore, the protection in MBL^{-/-} mice correlated with the absence of IgM and C3 deposition (Fig. 2c). It is also interesting to note that the I/R injury

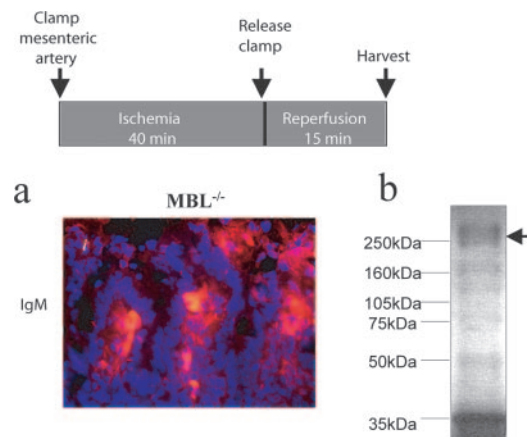


FIGURE 3. *a*, Deposition of IgM in microvilli during the early phase of reperfusion in MBL^{-/-} mice. Animals underwent 40 min of ischemia followed by 15 min of reperfusion. Represented intestinal sections were stained with anti-IgM-biotin/streptavidin-Alexa-568 (red) and rat anti-mouse MBL/goat anti-rat IgG-FITC (green). *b*, Immune precipitation of IgM self-Ags complex at early reperfusion. Intestinal lysates were prepared from MBL^{-/-} mice subjected to 40 min of ischemia/15 min of reperfusion. IgM-Ag immunocomplexes were isolated as described in *Materials and Methods*, and separated on 6% polyacrylamide SDS-PAGE (reducing gel) followed by Coomassie brilliant blue staining. Apparent molecular weight (kDa) markers are indicated on the left. The arrow indicates the dominant band at 250 kDa that was cut and used for subsequent protein sequencing by tandem mass spectrometry.

Table I. Mass spectrometry results^a

Matched Proteins	Mass Spectrometry, Sequenced Peptides
NMHC-II-A (gi/20137006)	VVFQEFR
Total score = 486; peptides matched = 14	FDQLLAEEK
	KFDQLLAEEK
	KEEELQAALAR
	EQADFAIEALAK
	LDPHLVLDQLR
	VISGVLQLGNIAFK
	NTDQASMPDNTAAQK + Oxidation (M)
	QLLQANPILEAFGNAK
	QLLQANPILEAFGNAK
	LQQEELDLLVLDLHQR
	TQLEELEDELQATEDAK
	IIGLDQVAGMSETALPGAFK + Oxidation (M)
	LQQLFNHTMFILEQEEYQR + Oxidation (M)
	DFSALESQQLDTQELLQEENR

^a Tandem mass spectrometry result of isolated ischemic tissue. Immunoprecipitations were performed as described in *Materials and Methods*. Bands obtained from SDS-PAGE were digested and extracted for protein identification by tandem mass spectrometry. Peptides identified from the I/R-specific 250-kDa band were matched to isoform A of NMHC-II. The Mascot score is defined as $-10 \times \log(p)$, where p is the probability that the observed match is a random event. Individual ion score > 53 indicates identity or extensive homology ($p < 0.05$).

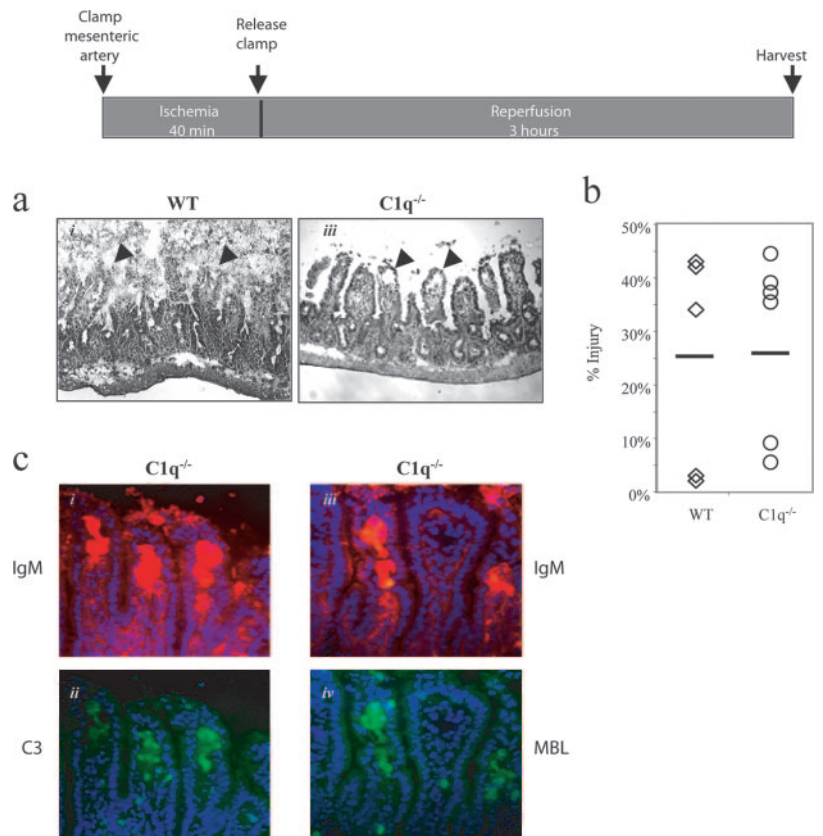
scores of WT mice have a spectrum of variation as observed previously (2). This is likely due to the different pathogenic natural IgM levels in individual WT animals. In other words, those mice with a high level of pathogenic natural IgM may have a more severe reperfusion injury, whereas those with a low level of pathogenic natural IgM may be less injured.

The finding that MBL^{-/-} mice were protected from I/R injury with the absence of IgM deposition 3 h postreperfusion raised the question whether IgM bound initially but was released in the absence of MBL or whether the deficient mice have an altered repertoire of natural Ab, i.e., missing pathogenic IgM. To determine

whether IgM-ischemic Ag complexes form in the early phase of reperfusion, MBL^{-/-} mice were subjected to 40 min of ischemia and the tissues were harvested at an early time point of reperfusion (15 min). Immunostaining identified deposits of IgM within the intestinal villi (Fig. 3a). The presence of IgM in the early but not late time points of reperfusion suggests that pathogenic IgM is present and binds transiently but does not lead to injury in the absence of MBL, at least under the conditions of this model.

In a recent report (2), one major target of IgM in the mesenteric model was identified as nonmuscle myosin H chain (NMHC-II-A and -C). To determine whether a similar target was involved in the

FIGURE 4. C1q^{-/-} mice are not protected from intestinal reperfusion injury. *a, i* and *ii*, Representative sections (stained with H&E) from I/R-treated WT and C1q^{-/-} mice (both on the B6 genetic background). Arrows indicate pathologic features of injury. Original magnification, $\times 200$. *b*, Scatter plot shows the pathology scores of each treated group. Statistical analysis by Student's *t* test found no significance between the C1q^{-/-} vs WT groups. *c*, IgM colocalize with complement C3 and MBL within the microvilli of C1q^{-/-} mice. *i* and *iii*, Stained with anti-IgM-biotin followed by streptavidin-Alexa-568 (red). *ii*, Stained with anti-C3-FITC (green); was stained with anti-MBL followed by goat anti-rat-FITC (green; *iv*). All sections were counterstained with DAPI (violet). Original magnification, $\times 400$.



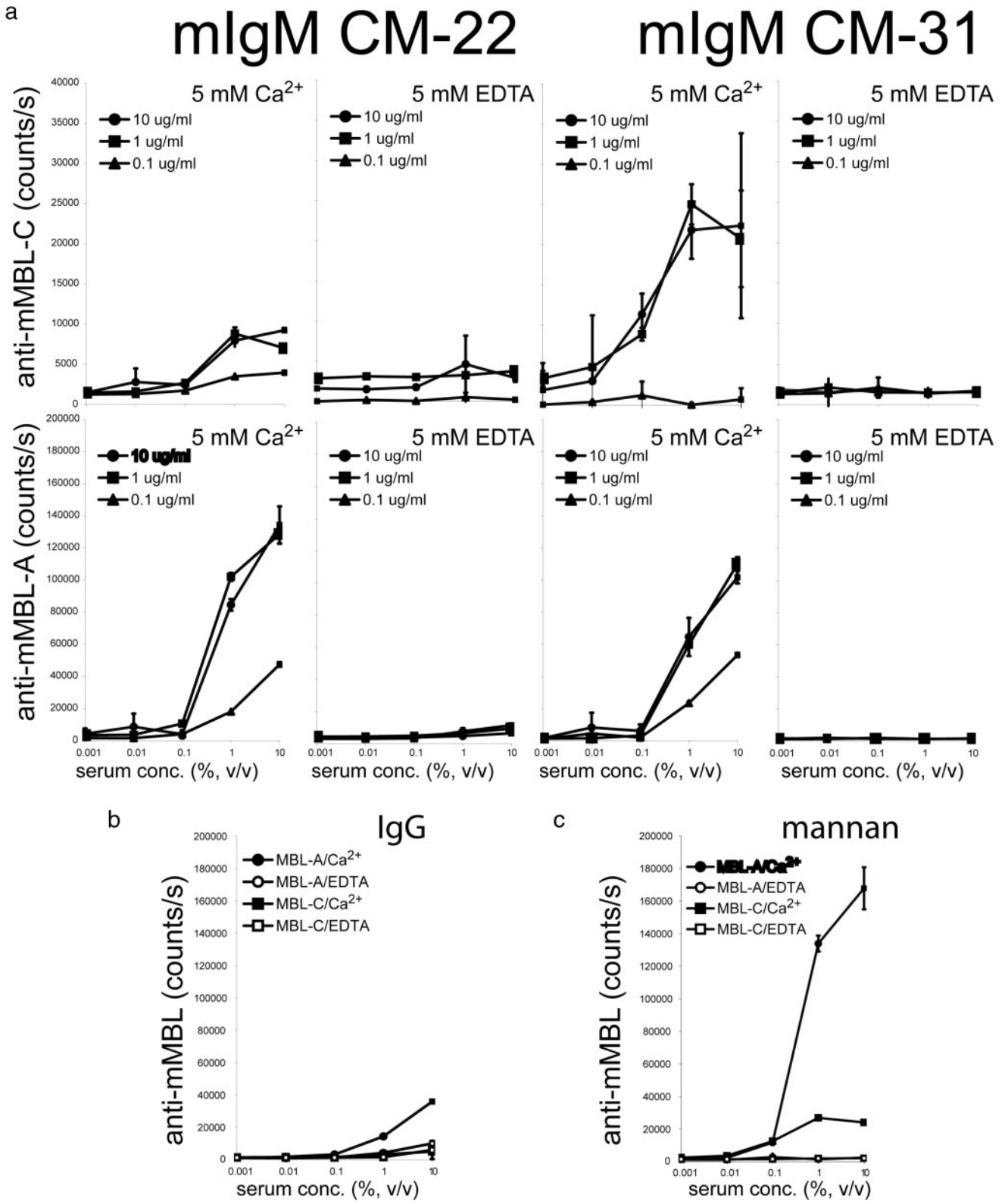
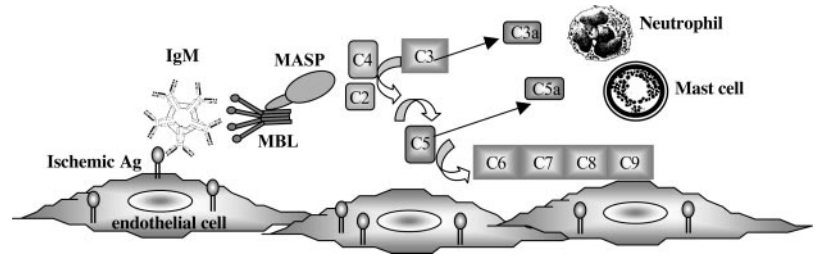


FIGURE 5. Murine MBL binding to monoclonal IgMs. *a*, The binding of mMBL-A or mMBL-C was tested by incubating wells coated at 0.1, 1, or 10 $\mu\text{g/ml}$ with either CM22 or CM31 IgM. Murine serum, diluted in buffer with either 1 mM Ca^{2+} or 5 mM EDTA, was added to the wells in concentrations from 0.001 to 10% (v/v). The bound MBL was detected by incubation with rat mAbs to mMBL-A or mMBL-C. *b*, Wells were coated at 10 $\mu\text{g/ml}$ with protein A-purified murine IgG and the binding of murine MBL were tested as in *a*. *c*, For comparison the binding of murine MBLs to mannan coated at 10 $\mu\text{g/ml}$ was tested as for the IgGs in *a* and *b*.

MBL^{-/-} mice, a biochemical approach was used to analyze IgM-immune complexes. Earlier studies (2) determined that the optimal time point for IgM-NMHC-II complexes was 15 min post-

reperfusion. Therefore, intestinal tissues were harvested from MBL^{-/-} mice at 15 min postreperfusion, and preformed IgM-Ag complexes were isolated by incubating the lysates with beads

FIGURE 6. A model illustrates the activation of the lectin pathway by natural IgM in I/R injury. IgM binds to the neoepitope in self-Ag and activates the lectin pathway of complement. The downstream events include the releasing of proinflammatory factors C3a and C5a, deposition of the membrane attack complex C5–C9, recruitment of inflammatory cells, and leading to direct cell damage.



coated with Ab specific for murine IgM. Analysis of the isolated immune-complexes identified a dominant band at ~250 kDa (Fig. 3*b*). Protein sequence analysis by mass spectrometry identified NMHC-II A as the major protein (Table I). Thus, as reported earlier, nonmuscle myosin H chain is a major target for natural IgM in the early stage following the reperfusion of ischemic jejunum (2).

The finding that MBL is required for complement activation raises the question whether the C1q-dependent classic pathway functions in this model of reperfusion injury. To address this question, mice genetically deficient in C1q were characterized in the mesenteric I/R model. Because C1q^{-/-} mice were in the C57BL/6 genetic background and MBL^{-/-} mice were in the 129/B6 genetic background, WT mice with a C57BL/6 genetic background were used as controls for C1q^{-/-} mice to prevent a biased conclusion influenced by genetic background (63–66). In contrast to MBL^{-/-} mice, the injury levels in C1q^{-/-} mice were similar to those of WT animals (pathology scores: 25 ± 21% vs 22 ± 17%, respectively; Fig. 4, *a* and *b*). In addition, the variation pattern of injury scores among C1q^{-/-} group is similar to that of the WT group, which indicates both groups have a similar pathogenic natural IgM repertoire. Moreover, immunohistochemistry analysis showed that MBL and C3 codeposited with IgM in the microvilli of I/R-treated C1q^{-/-} mice (Fig. 4*c*). Thus, mice deficient in C1q are not protected in the mesenteric model because the presence of MBL is sufficient to initiate injury. These results are consistent with the previous report by Hart et al. (36) that injury in the mesenteric model was independent of C1q. Although C1q does not appear to be required for local injury, immunohistochemistry analysis of sections prepared from I/R-treated WT mice identified C1q colocalization with IgM within the microvilli (data not shown).

These *in vivo* results suggest that MBL functions downstream of IgM-ischemic Ag interaction and possibly through direct binding of MBL to IgM. To test this possibility, we used a time-resolved immunofluorometric assay-based binding assay (62). Monoclonal murine IgMs were immobilized in polystyrene plates followed by the addition of purified murine MBL-C or MBL-A protein. The bound MBL on immobilized IgM were detected by specific Ab against MBL (62). To verify whether the binding depended on Ca²⁺, 1 mM CaCl₂, or 5 mM EDTA, was added to the buffer. Murine IgG was used as a negative control in the binding assays, and directly immobilized mannan was included as a positive control (Fig. 5, *b* and *c*). Our results indicate that both MBL-C and MBL-A bind to immobilized natural IgM clones in a dose- and calcium-dependent manner (Fig. 5*a*).

Discussion

Until recently, it was generally held that complement-dependent injury in the mesenteric (and skeletal muscle) models of I/R injury were mediated via IgM activation of the classical pathway. The recent observation that the lectin pathway was also essential raised the question of the order of initiating events. Using mice deficient

in early components of complement, IgM, or MBL, we found that both IgM and MBL are required in a mesenteric model of I/R. Moreover, results from biochemical analyses indicate that IgM binding of target host ligands within 15 min postreperfusion is independent of MBL. Thus, we conclude that recognition of host ligands by natural IgM enhances binding of MBL and subsequent activation of the lectin pathway (Fig. 6).

In vitro-binding studies by others (53, 54) and as indicated in Fig. 5 of this study indicate that murine MBL can directly bind to murine IgM. The responsible binding site(s) on murine IgM is unclear, but it is likely that certain carbohydrate patterns exist on IgM, and such glycosylation could take place when natural Abs are produced. Indeed, the observation that the binding is calcium dependent supports that the interaction between IgM and MBL is linked through carbohydrate binding. The central role of the calcium ion in mediating contact to carbohydrate ligands in the MBL C-type carbohydrate recognition domain is structurally well characterized, and all carbohydrate interactions are thus calcium dependent. In humans, 20% of the total serum IgM can bind to immobilized MBL and these MBL-binding glycoforms contain 97% more GlcNAc-terminated glycans (54), which are strong ligands for MBL.

Our results do not exclude the possibility that IgM and MBL may work in a synergistic way, in which the interaction between IgM and ischemic Ag leads to the exposure of binding sites for MBL. Such targets could be either ischemic Ags or other candidates on ischemia-assaulted cells. It is known (8, 10) that ischemia causes rapid apoptosis in intestinal mucosa, and MBL can interact with structures exposed on apoptotic cells to initiate activation of complement C4 on the targets (67, 68). Nevertheless, in view of the *in vitro* data on the binding of MBL to IgM, the inability of CM22 IgM to induce injury in the absence of MBL, and the inability of MBL to induce damage in the absence of IgM, it appears that the most parsimonious account of the complement activation described in our study is that the IgM deposition triggers complement activation through MBL.

The finding that C1q was not essential for local injury but colocalized with IgM deposition suggests that it could have another role such as amplification of remote injury. Indeed, in a skeletal muscle model where remote lung injury is a prominent outcome of I/R, C1q appears to be important in severity of lung inflammation (R. Chan, F. D. Moore, M. Carroll and W. Austen, unpublished observations).

In summary, these results identify a new synergistic interaction between natural IgM and MBL leading to activation of the lectin pathway and acute inflammation in the mesenteric model of I/R injury. It will be important to learn whether these two important recognition proteins of innate immunity participate in other examples of synergy either in host protection or sterile response to self-targets (innate immune reaction in the absence of foreign Ag, *i.e.*, bacteria).

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Disclosures

M. C. Carroll, F. D. Moore, and E. M. Alicot have equity in DecImmune Therapeutics.

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