

Absence of Mannose-Binding Lectin Prevents Hyperglycemic Cardiovascular Complications

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Diabetes, stress, pharmaceuticals, surgery, and physical trauma can lead to hyperglycemic conditions. A consistent relationship has been found between chronic inflammation and the cardiovascular complications of hyperglycemia. We hypothesized that cardiomyopathy and vasculopathy resulting from acute hyperglycemia are dependent on mannose-binding lectin (MBL) and lectin complement pathway activation. Hyperglycemia was induced in wild-type (WT) C57BL/6 and MBL-null mice after streptozotocin administration. Echocardiographic data and tissue samples were collected after 4, 7, or 14 days of acute hyperglycemia. Hyperglycemic WT mice demonstrated dilated cardiomyopathy with significantly increased short and long axis area measurements during systole and diastole compared to hyperglycemic MBL-null mice. The EC₅₀ for acetylcholine-induced relaxation of mesenteric arterioles in WT mice after 4 days of hyperglycemia demonstrated a significant loss of nitric oxide-mediated relaxation compared to normoglycemic WT or hyperglycemic MBL-null mice. Myocardial histochemistry and Western blot analysis revealed a significant influx of macrophages, altered morphology, and increased elastin and collagen deposition in hyperglycemic WT hearts compared to MBL-null hearts. Serum transforming growth factor- β 1 levels were significantly lower in hyperglycemic MBL-null compared to WT mice, suggesting decreased profibrotic signaling. Together, these data

suggest that MBL and the lectin complement pathway play a significant role in vascular dysfunction and cardiomyopathy after acute hyperglycemia. (*Am J Pathol* 2012, 180:104–112; DOI: 10.1016/j.ajpath.2011.09.026)

Type 1 diabetes is an autoimmune disorder that results in the destruction of the insulin-producing β cells in the pancreas, reducing or eliminating the body's ability to produce insulin. More than 25.8 million people in the United States (8.3% of the population) have been diagnosed with diabetes mellitus.¹ Diabetic patients have increased incidences of atherosclerosis, coronary artery disease, and myocardial infarction.^{2,3} Diabetic patients also have increased mortality, worse long-term prognosis after myocardial infarction, and a higher risk of cardiomyopathy compared to patients without diabetes.^{3–5}

Similarly, acute hyperglycemia is reported in many critical care settings and is occasionally referred to as stress diabetes or stress hyperglycemia.^{6,7} Acute hyperglycemia was originally regarded as nonproblematic and potentially beneficial during the fight-or-flight response in critically ill adult patients, ensuring an adequate supply of glucose to the brain and immune system. However, recent data suggest an association between high glucose levels at the time of admission and adverse outcomes.⁸ The question of whether acute hyperglycemia in critically ill patients is simply related to disease severity or is an independent risk factor for morbidity and mortality remains to be fully elucidated.

Complement can be activated by three separate pathways: classical, alternative, and lectin (Figure 1). Complement activation plays a role in diabetic cardiovascular complications.^{9–11} Diabetic patients have a glycosylated

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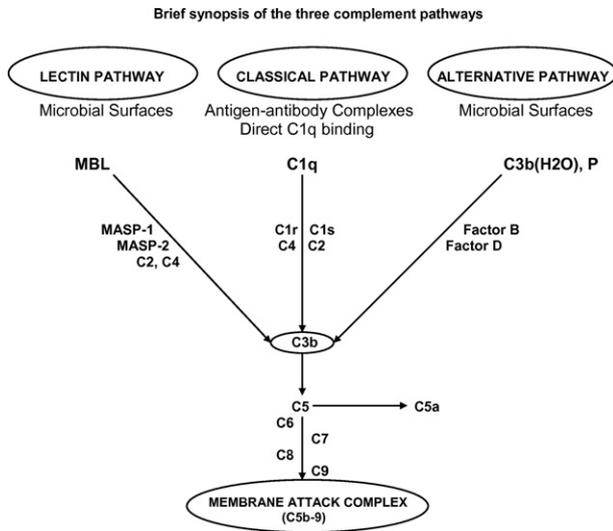


Figure 1. The lectin pathway is C1q, C1r, C1s, and antibody independent. P, properdin.

form of CD59, which renders this complement regulator inactive.¹² CD59 glycation might lead to micro- and macrovascular pathology, but the molecular mechanisms for complement activation in hyperglycemic states are unknown. Hansen and colleagues have identified higher mannose-binding lectin (MBL) levels in patients with type 1 diabetes and its association with vascular complications.^{13–15} Interestingly, C1 inhibitor (which also inhibits MBL-associated serine protease-2) reduces diabetic macular edema.¹⁶

Our group recently demonstrated decreased dilated cardiomyopathy, hypertrophic remodeling, and loss of cardiac progenitor cells in MBL-null mice compared to WT mice after 14 days of acute hyperglycemia.⁹ Further, MBL binds to fructosamines, advanced glycation end products.¹⁷ These findings suggest a role for lectin complement pathway activation and/or the MBL complex in diabetic cardiomyopathy. The purpose of the present study was to further characterize the role of MBL and complement activation in acute hyperglycemia-induced cardio- and vasculopathy.

Materials and Methods

All procedures were reviewed and conducted according to the institute's Animal Care and Use Committee. All experiments were performed under the standards and principles set forth in the Guide for Care and Use of Laboratory Animals, published by the NIH (publication no. 85-23, revised 1996).

Animals

C57BL/6 (WT) mice (8 to 12 weeks old; Taconic Farms, Hudson, NY) were used as background controls for genetically modified animals with specific complement component deletions [MBL-null and C2/factor (f) B-null mice], as described previously.^{18,19} The following groups

were studied: i) WT; ii) MBL-null; iii) WT after 4, 7, or 14 days' hyperglycemia; iv) MBL-null after 4, 7, or 14 days' hyperglycemia; v) WT + anti-C5 monoclonal antibody (BB5.1, 20 mg/kg²⁰) + 4 days' hyperglycemia; vi) MBL null + recombinant human (rh) MBL (75 μg i.p. daily^{19,21}) + 4 days' hyperglycemia; and vii) C2/fB null + 4 days' hyperglycemia. Mice were housed four per cage and had unlimited access to water and standard mouse chow.

Agents and Chemicals

Streptozotocin (STZ) and N-(methylnitrosocarbamoyl)-α-D-glucosamine were purchased (Sigma-Aldrich, St. Louis, MO). Citrated saline was purchased from Alexis (Lausen, Switzerland). The rhMBL was a gift from Dr. Kazue Takahashi.

Hyperglycemia Induction

Hyperglycemia was induced by a single injection of freshly prepared STZ solution (200 mg/kg body weight, i.p.) in citrated saline, pH 4.2. Urinary glucose was tested on day 4 after injection and again before experimentation. Mice with urinary glucose levels greater than 500 mg/dL (measured with Glucostix; Diastix, Bayer, Elkhart, IN) in both tests were considered hyperglycemic.

Glucose and Insulin Measurements

Serum samples were collected from nonfasted WT and MBL-null mice at 4, 7, and 14 days after STZ injection and from WT normoglycemic controls. Serum glucose was measured with a Glucose Assay Kit (Cayman Chemical, Ann Arbor, MI) per the manufacturer's protocol. Serum insulin levels were measured in the same groups using an Ultra-Sensitive Mouse Insulin enzyme-linked immunosorbent assay kit (Crystal Chem Inc., Downers Grove, IL) as per the manufacturer's protocol.

Endothelium-Dependent Relaxation

Anesthesia was induced and maintained with isoflurane. After a midline laparotomy, the intestine was removed *en bloc* with mesenteric vessels and placed in cold Krebs-Henseleit buffer (Sigma-Aldrich). Mesenteric arterioles with an internal diameter of 100 to 300 μm were isolated, attached to micropipettes, pressurized to 54 cm H₂O, and superfused with Krebs-Henseleit buffer (gassed with 95% O₂ and 5% CO₂ at 37°C) as previously described.^{22–24} After equilibration for 45 minutes, vessels were constricted to 50% to 70% of baseline with norepinephrine (0.1 μmol/L), and then endothelium-dependent relaxation was induced by cumulative additions of acetylcholine (ACh; 0.001 to 10 μmol/L). Vessels that failed to relax to baseline were subjected to sodium nitroprusside (0.1 μmol/L) to verify functional vascular smooth muscle. All data were continuously recorded via VCR, and vessel relaxation expressed as percentage relaxation from constricted baseline as previously described.²²

Echocardiography

Transthoracic echocardiography was used to evaluate cardiac functional parameters of the mice after 4 and 14 days of hyperglycemia. Echocardiography (Philips Sonos 5500, Andover, MA; 7 to 15 MHz probe) was performed as described measuring long and short axis area during systole and diastole and used to calculate ejection fraction (EF).^{9,19,25}

Histology and Immunohistochemistry

Hearts were collected and fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO). Samples were embedded in paraffin and sectioned (5 μ m) from apex to midmyocardium for H&E and macrophage (Iba-1, Mac-2, YM-1) and T-cell (CD3) evaluation. Slides were stained for macrophages using a Trilogy (Cell Marque, Rocklin, CA) steaming protocol. Briefly, slides were steamed in two changes of Trilogy for a total of 1 hour. After steaming, slides were cooled, rinsed with H₂O, and incubated in methanol/30% H₂O₂ for 20 minutes. Slides were rinsed and blocked using Protein Block (Dako, Carpinteria, CA) for 10 minutes. Sections were then incubated with Mac-2 (Cedarlane Laboratories, Burlington, NC), YM-1 (Stem-cell Technologies, Vancouver, Canada), Iba-1 (Wako Chemicals, Richmond, VA), or CD3 (Abcam, Cambridge, MA) diluted with AB Diluent (Dako) for 1 to 2 hours at room temperature. Mac-2 and YM-1 were visualized using the Vectastain Elite ABC kit (Vector, Burlingame, CA) and Iba-1 or CD3 using the SuperPicture Kit (Invitrogen, Carlsbad, CA). For collagen and elastin fibers identification, hearts were collected in OCT (Sakura Finetek USA Inc., Torrance, CA), frozen, and sectioned (5 μ m) from apex to midmyocardium. Collagen staining was identified by a Picrosirius Red Stain Kit (Polysciences Inc., Warrington, PA) according to the manufacturer's protocol. Elastin fibers were identified with Verhoeff's Elastin Stain Kit (IMEB Inc., San Marcos, CA) according to the manufacturer's protocol.

Heart Lysate and Western Blot Test

Additional cardiac tissue was collected, weighed, and finely diced in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors, as per the manufacturer's recommendations (Santa Cruz, Santa Cruz, CA). The tissue was homogenized using a Next

Advance Bullet Blender and then centrifuged at 10,000 \times g for 10 minutes at 4°C. The resulting supernatant contained the total cardiac cell lysate.

Protein concentrations were determined using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA). Western blot testing was performed Bio-Rad 10% BioCast gels, transferred onto nitrocellulose membranes, and blocked. Membranes were incubated with a primary pAb against collagen type IV (United States Biological, Swampscott, MA), washed, incubated with a secondary goat anti-rabbit Ig near infrared fluorochrome (LI-COR IR700 nm; LI-COR Biosciences, Lincoln, NE), and scanned using the Odyssey system (LI-COR). Protein loading equivalence was verified by probing for glyceraldehyde 3-phosphate dehydrogenase.

TBARS

Overall oxidative stress was evaluated in the plasma of WT and MBL-null mice and after 14 days of hyperglycemia. Lipid peroxidation [malondialdehyde (MDA)-TBARS] was measured in the plasma using the methods provided by the manufacturer of OXltek (ZeptoMetrix, Buffalo, NY).

TGF- β 1 Enzyme-Linked Immunosorbent Assay

Transforming growth factor (TGF)- β 1 was determined from mouse serum using the Quantikine Mouse TGF- β 1 Immunoassay Kit (R&D Systems, Minneapolis, MN).

Statistical Analysis

Data are presented as means \pm SEM. Comparisons between paired sets of data were made by Student's *t*-test. Analyses of variance were used for statistical comparisons of multiple groups followed by a Student-Newman-Keuls test with *P* < 0.05 being considered statistically significant.

Results

STZ injection resulted in acute hyperglycemia and hypoinsulinemia within 4 days after injection compared to normoglycemic mice (Table 1). No significant differences in serum glucose or insulin concentrations were observed between WT and MBL-null mice at any time point after STZ injection throughout the study (Table 1).

Table 1. Serum Glucose and Insulin in WT and MBL Mice

Measurement	WT		WT			MBL null		
	Normoglycemic	MBL Normoglycemic	4 days STZ	7 days STZ	14 days STZ	4 days STZ	7 days STZ	14 days STZ
Glucose (mg/dL)	46.9 \pm 0.1	50.2 \pm 0.1	95.0 \pm 0.1*	122.9 \pm 0.1*	113.1 \pm 0.2*	123.3 \pm 0.1*	109.2 \pm 0.1*	123.6 \pm 0.1*
Insulin (pg/mL)	250 \pm 31	259 \pm 69	ND	ND	ND	ND	ND	ND

A significant increase and decrease in serum glucose and insulin levels were observed after administration of STZ in both groups, respectively. No significant differences are observed between hyperglycemic WT and MBL-null serum glucose or insulin levels at any of the experimental time points. All data are mean \pm SEM of six animals per group.

**P* < 0.01.

ND, results were below the detection limit of 100 pg/mL.

Cardiovascular Remodeling Resulting from Hyperglycemia Is Significantly Decreased in MBL-Deficient Mice

We previously observed echocardiographic evidence of dilated cardiomyopathy after 14 days of hyperglycemia.⁹ In the present study, echocardiographic evidence of hypertrophic myocardial remodeling appeared to be initiated within 4 days of acute hyperglycemia (Figure 2). Echocardiographic findings suggest that left ventricle systolic function is impaired in hyperglycemic WT mice at 4 days with significantly increased short-axis area measurements in systole compared to MBL-null mice. By day 14, we observed increased area measurements in the short and long axis during systole in WT hearts compared to MBL-null hearts. In diastole, on the other hand, we observed decreased long-axis area measurements at 4 days of hyperglycemia, which we hypothesize is a result of ventricular stiffening. In MBL-null mice, we observed a significant decrease in short-axis area measurements in diastole after 14 days of hyperglycemia.

Cardiac EF was significantly impaired in hyperglycemic WT hearts as early as 4 days after hyperglycemia induction (Figure 3). By day 14, we observed a further and significant reduction in EF in hyperglycemic WT mice compared to MBL-null mice, similar to what we observed previously.⁹

MBL-Deficient Mice Are Protected from Hyperglycemia-Induced Vasculopathy

Diabetes mellitus results in decreased nitric oxide (NO)-mediated vascular relaxation,²⁶ which has been ascribed to increased oxidative stress in the diabetic state.²⁷ We investigated the oxidative state in our model by looking at plasma MDA-TBARS levels after 14 days of hyperglycemia. As shown in Figure 4A, we observed a significant increase in plasma MDA after 14 days of hyperglycemia in WT mice compared to normoglycemic WT mice. Fur-

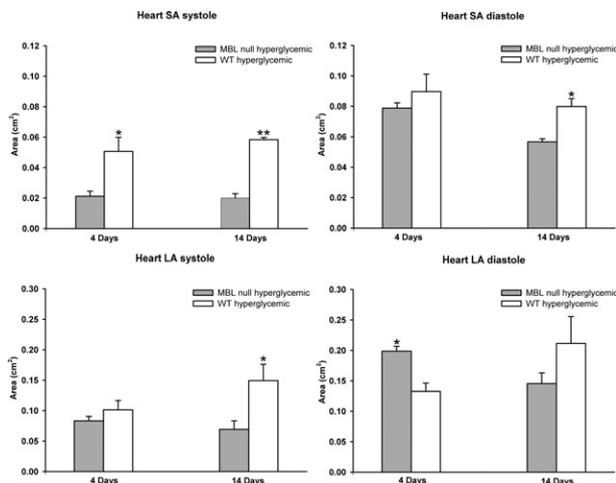


Figure 2. Myocardial short (SA) and long (LA) axis area measurements. Systolic and diastolic areas (cm²) were measured at 4 and 14 days of hyperglycemia. All data are mean ± SEM of three animals per group. **P* < 0.05; ***P* < 0.02.

EF in hyperglycemic mice (%)

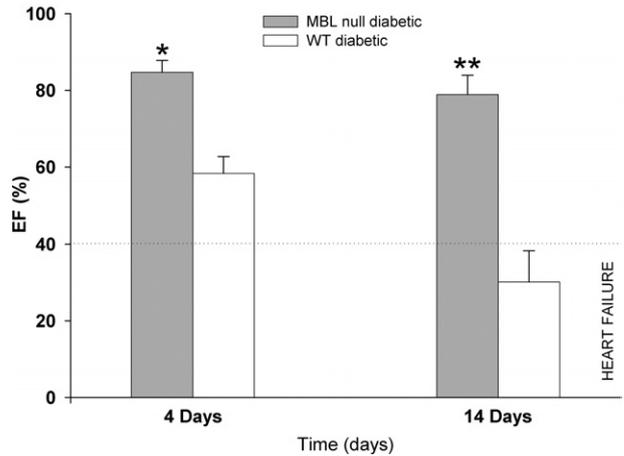


Figure 3. Myocardial EF measurements. EF was measured at 4 and 14 days of hyperglycemia. All data are mean ± SEM of three animals per group. **P* < 0.05; ***P* < 0.02.

thermore, MBL-null mice displayed the same magnitude of oxidative stress as the WT mice after induction of hyperglycemia. Thus, the oxidative stress level is not significantly different in WT and MBL-null mice after STZ-induced hyperglycemia.

After 4 days of hyperglycemia, WT mesenteric arterioles had a significant loss of ACh-induced relaxation compared to WT normoglycemic vessels (Figure 4B). Further, ACh induced relaxation of arterioles from hyperglycemic MBL-null mice were not significantly different from normoglycemic WT or MBL-null vessels. The EC₅₀ for ACh-induced relaxation was increased (three orders of magnitude) for hyperglycemic WT (5.0 ± 2.9 μmol/L) compared to normoglycemic MBL-null (12 ± 5 nmol/L), normoglycemic WT (6 ± 2 nmol/L), or MBL-null hyperglycemic (11 ± 3 nmol/L) mice. Reconstitution of hyperglycemic MBL-null mice with rhMBL (75 μg/mouse/day, i.p.) restored hyperglycemia-induced vasculopathy (EC₅₀ = 2 ± 1 μmol/L) to WT hyperglycemic mice levels. These data suggest that the loss of MBL alone is responsible for the loss of NO-mediated relaxation after acute hyperglycemia.

Complement activation, particularly formation of C5b-9, leads to loss of NO-mediated relaxation of arteries and arterioles.^{22,28} To characterize the role of complement activation in the loss of NO-mediated relaxation after acute hyperglycemia, we evaluated ACh-induced relaxation of arterioles from hyperglycemic WT mice treated with anti-C5 monoclonal antibody (BB5.1), as well as from hyperglycemic C2/fB-deficient mice. As shown in Figure 4C, genetic inhibition of complement activation (eg, C2/fB-deficient mice) or immunotherapeutic inhibition of C5 cleavage (eg, BB5.1) attenuated the loss of ACh-induced relaxation. The EC₅₀ values for both of these complement inhibited groups were located between that of hyperglycemic MBL-null and hyperglycemic WT mice. Collectively, these data suggest that complement activation contributes to the loss of NO-mediated relaxation, which supports our previous findings.^{22,28} However, the data also suggest the MBL complex con-

tributes to the loss of ACh-induced relaxation of arterioles after acute hyperglycemia because oxidative stress levels between the two genotypes are not significantly different (Figure 4A).

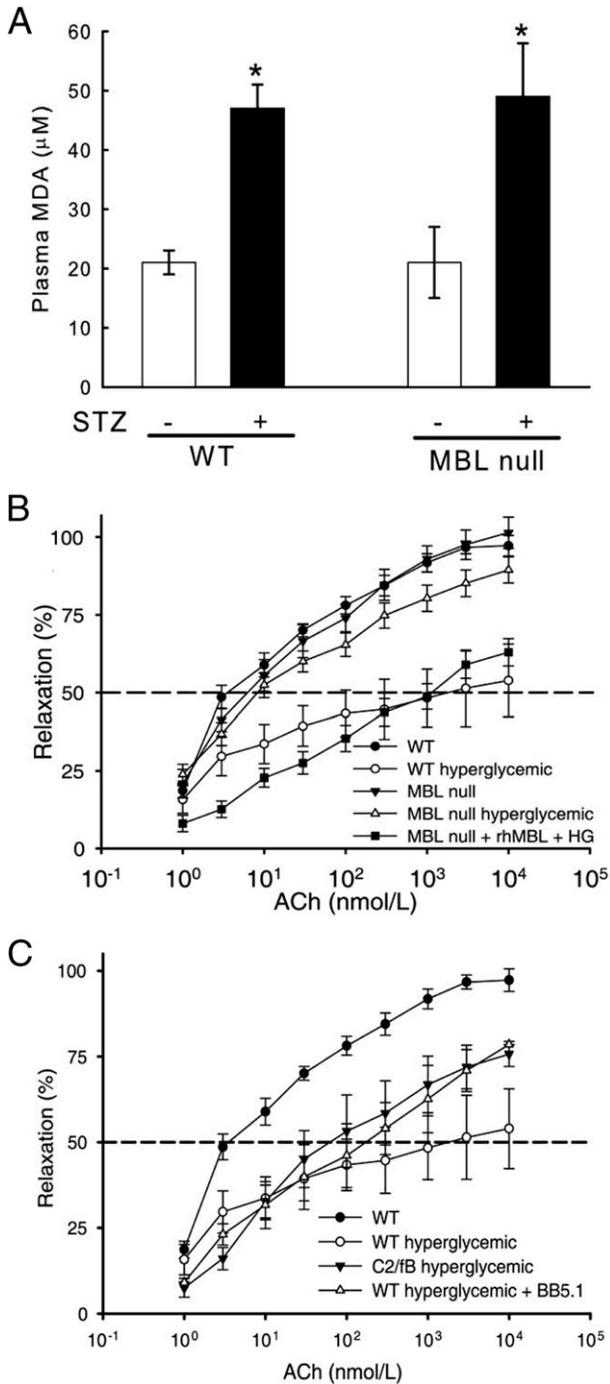


Figure 4. **A:** Thiobarbituric acid reactive substances (MDA) in plasma after 14 days of hyperglycemia (STZ⁺) versus normoglycemic (STZ⁻) WT and MBL-null mice; *N* = 4 per group. **P* < 0.05 compared to respective normoglycemic group. **B:** Microvascular endothelium-dependent relaxation of mesenteric arterioles after 4 days of hyperglycemia. The EC₅₀ for ACh-induced relaxation of contracted arterioles; *P* < 0.01. Hyperglycemic WT arterioles have a significantly reduced capacity to relax to the endothelium dependent reagent, ACh. C, control groups. Sodium nitroprusside relaxed all vessels normally. *N* = 6 per group.

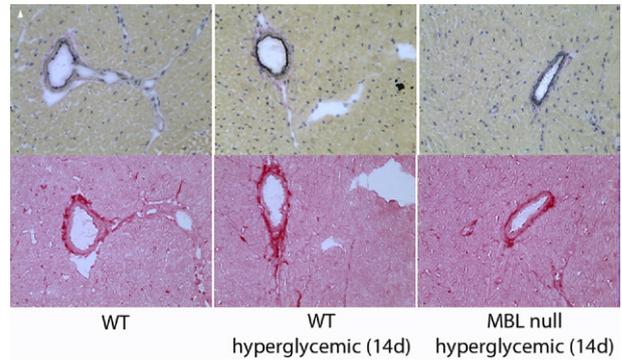


Figure 5. Cardiac elastin and collagen deposition are significantly increased in hyperglycemic WT mice compared to normoglycemic WT or hyperglycemic MBL-null mice. **A:** Representative photomicrographs of elastin-stained (Verhoeff's elastin stain) hearts obtained on day 14 of hyperglycemia compared to normoglycemic WT mice. Significant elastin deposition was noted in WT mice after 14 days of acute hyperglycemia (black, elastic fibers; red, collagen; blue to black, nuclei). Original magnification, ×40. **B:** Increased extracellular matrix and collagen deposition (Picrosirius red stain) in WT mice compared to MBL-null mice after 14 days of acute hyperglycemia (red, collagen). Original magnification, ×40.

MBL Deficiency Results in Decreased Matrix Deposition and Profibrotic Signaling

One of the hallmarks of hyperglycemia is vascular and myocardial remodeling, where myocardial fibrosis is an early manifestation.²⁹ To examine whether MBL played a role in myocardial remodeling, hyperglycemic hearts were examined by Picrosirius red, Verhoeff's elastin staining and Western blot test to observe and quantify extracellular matrix deposition. We found that cardiac elastin and collagen deposition are increased in WT hearts after 14 days of hyperglycemia compared to hyperglycemic MBL-null hearts (Figure 5). Western blot analysis confirmed the histological observations and demonstrated an increase in collagen type IVα1 in hyperglycemic WT hearts compared to hyperglycemic MBL-null hearts (Figure 6). Extracellular matrix deposition is regulated through various signaling pathways, including the profibrotic cytokine TGF-β1.³⁰ Serum TGF-β1 levels were significantly decreased in hyperglycemic MBL-null mice compared to hyperglycemic WT mice (Figure 7). These data suggest that hyperglycemic MBL-deficient mice are protected from myocardial remodeling, likely involving TGF-β1 signaling, at least in part.

Significant Myocardial Morphological Changes in Hyperglycemic WT Mice

WT hearts demonstrated a moderate reduction in cardiac muscle cross striations and increased hypereosinophilic

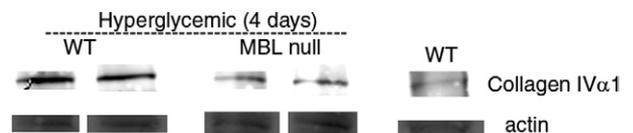


Figure 6. Cardiac collagen deposition is significantly decreased in MBL-null mice. Cardiac supernatants probed for collagen indicate a decrease in collagen type IV in MBL-null hearts compared to WT hyperglycemic mice after 4 days of hyperglycemia.

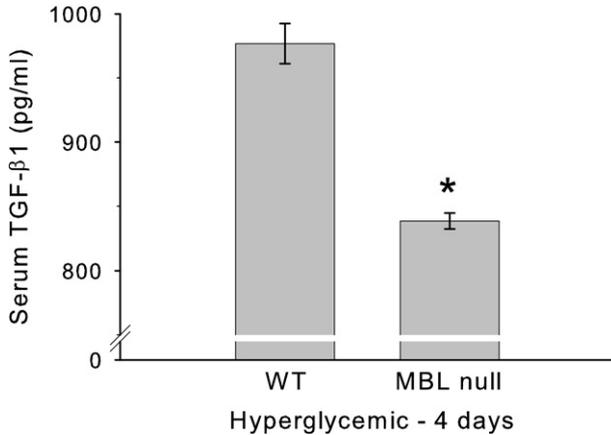


Figure 7. Serum TGF-β1 levels after 4 days of hyperglycemia in WT and MBL-null mice. *N* = 4 per group; **P* < 0.05.

cytoplasm after hyperglycemia compared to MBL-null mice (Figure 8). Infiltration of myocardial inflammatory cells in hyperglycemic WT hearts after 7 days of hyperglycemia were confirmed to be macrophages by staining (Iba-1, Mac-2) (Figure 9). A significant decrease in Mac-2 staining was observed in hyperglycemic MBL-null compared to WT mice at 7 and 14 days after induction of hyperglycemia. Interestingly, few YM-1-positive cells were observed in either group after 14 days of hyperglycemia (data not shown), suggesting that most of the macrophages were Mac-2⁺ M1 cells.³¹ CD3 staining was not different between the groups (data not shown). Thus, MBL deficiency may lead to an anti-inflammatory state within the myocardium during acute hyperglycemia.

Discussion

Whereas poor glycemic control has well-established ties to microvascular and macrovascular disease, the link with hard cardiovascular events is less clear. Recent evidence suggests that hyperglycemic patients have the same cardiovascular risk as patients who have had a myocardial infarction.³² Further, acute hyperglycemic disturbances are a potent predictor of mortality in patients without previously known diabetes mellitus.³³

Complement is known to play a role in diabetes mellitus. Diabetic patients have a glycosylated form of CD59, which renders this complement inhibitor inactive.¹² CD59

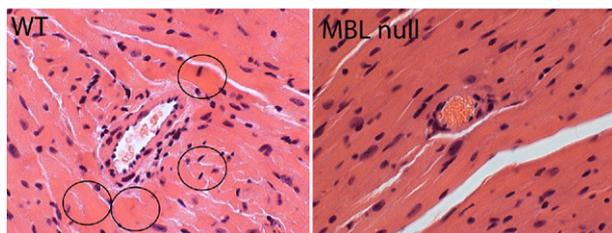


Figure 8. Representative H&E-stained myocardial sections after 7 days of hyperglycemia. Each panel represents one individual mouse heart. In WT mice myocardium, cross striations are partially lost, together with hyper-eosinophilic cytoplasm (circles). In MBL-null mice myocardium, clear cross striations may be observed. Original magnification, ×40.

Figure 9. **A:** Representative immunohistochemistry of myocardial sections after 7 days of hyperglycemia for macrophages (Mac-2). Original magnification, ×40 (right); ×20 (left). **B:** Myocardial Mac-2-positive cells after 7 or 14 days of hyperglycemia in WT or MBL-null mice. **P* < 0.05 at 7 days, ***P* < 0.002 at 14 days of hyperglycemia compared to WT group.

glycation is thought to lead to micro- and macrovascular pathology in diabetic patients.³⁴ Loss of CD59 function may be important in protecting against loss of NO-induced relaxation, as we previously demonstrated.²⁸ Hansen and colleagues identified higher MBL levels in patients with type 1 diabetes and an association with vascular complications.^{13–15} High serum MBL levels are predictive for development of micro- or macroalbuminuria.³⁵ Recently, MBL was demonstrated to bind to fructosamines, thereby establishing a potential ligand for MBL binding and complement activation in acute hyperglycemia.¹⁷ Thus, MBL levels appear to be associated with vascular complications in the diabetic patient.

In the present study, several important findings were observed relating MBL deficiency and acute hyperglycemia induced tissue injury, thus extending our previous observations.⁹ First, hyperglycemic MBL-null mice had significantly better cardiac ejection fractions compared to hyperglycemic WT mice, especially after 14 days of hyperglycemia. We previously demonstrated that MBL-null mice were protected from myocardial injury after 2 weeks of uncontrolled hyperglycemia and were similar to insulin-treated hyperglycemic mice.⁹ We extended those previous findings by characterizing an earlier phase of acute hyperglycemia. We observed significant left ventricular systolic and diastolic dysfunction after only 4 days of hyperglycemia in WT mice. These early differences ultimately lead to a significant reduction in EF after 14 days of hyperglycemia in WT compared to the MBL-null mice. These changes may be due, in part, to a loss of

cardiac progenitor cells in the hyperglycemic WT heart, leading to impairments in myocyte regeneration.^{9,36,37}

Second, WT arterioles had significantly decreased ability to relax to the endothelium-dependent relaxing agent, ACh, after only 4 days of hyperglycemia compared to arterioles from hyperglycemic MBL-null mice. Both MBL-null and WT mice displayed similar significant increases in plasma MDA levels after 14 days of hyperglycemia, suggesting that the amount of oxidative stress was similar and that the generation of reactive oxygen species alone cannot be responsible for the difference in vascular reactivity observed in this study. Previous studies have linked diabetes mellitus to impaired NO signaling and enhanced oxidative stress, resulting in endothelial dysfunction, inflammation, and arterial remodeling.^{26,27} Our data suggest that either the MBL complex or complement activation plays an important role in the loss of vascular reactivity. We previously demonstrated an important role of C5b-9 in mediating the loss of NO-mediated relaxation in porcine and rat vessels.^{22,28} Immunotherapeutic inhibition of murine C5 (eg, monoclonal antibody BB5.1) or genetic depletion of C2 and fB (ie, inhibition of complement activation) demonstrated an intermediate level of protection from hyperglycemia-induced loss of vascular reactivity compared to MBL-null mice. Because MBL complexes are still present and functional in anti-C5 monoclonal antibody treated WT or C2/fB-null mice, these findings suggest complement activation plays a partial role in disease progression. Importantly, these data also indicate an essential role for earlier complement components, including MBL and its associated serine proteases, to establish the observed vasculopathy. Furthermore, reconstitution of the lectin pathway with rhMBL in MBL-null mice demonstrated that NO-mediated relaxation is partially MBL complex dependent. Reactive oxygen species are increased in acute hyperglycemia.^{38,39} We previously demonstrated the important role of oxidative stress to up-regulate endothelial cell MBL ligands, MBL complex activation, and ultimately lectin complement pathway activation.^{40–42} Our present findings support the hypothesis that oxidative stress alone is not solely responsible for loss of NO-mediated relaxation of arterioles and extends an important role for MBL in cardiovascular complications and endothelial dysfunction after acute hyperglycemia.

One of the consequences of endothelial cell dysfunction and NO loss is induction of an inflammatory state. Type 1 diabetes is associated with a subclinical, chronic inflammation, which is at least partly correlated to the ensuing ischemia.⁴³ Hyperglycemic MBL-null mice had significantly less cardiac Mac-2⁺ (M1) infiltration compared to WT mice, whereas YM-1⁺ cells (M2) were few in number in both groups. It is still poorly understood whether the presence of inflammation is the consequence of or a marker for vascular damage. However, studies suggest that a chronic inflammatory state likely precedes and contributes to the pathogenesis and development of micro- and macrovascular disorders in diabetes.^{43,44} Our data suggest that MBL complexes and lectin pathway activation play a significant role in enhancing diabetes-induced inflammation, similar to that observed after myocardial infarction.^{9,19}

Finally, profibrotic molecule production and extracellular matrix deposition were significantly increased in hyperglycemic WT compared to MBL-null mice. Myocardial fibrosis is an early manifestation of hypertrophic cardiomyopathy.²⁹ High glucose levels, leading to advanced glycation end products formation, are associated with TGF- β 1/Smad induction, collagen cross-linking, impaired NO signaling, and enhanced oxidative stress.^{45,46} TGF- β 1 plays an important role in cardiac fibrogenesis, endothelial-to-mesenchymal transition, and other advanced glycation end product-mediated diabetic complications.^{47,48} TGF- β 1 increases connective tissue growth factor expression, which induces monocyte/macrophage migration, and cytokine and acute-phase protein production.⁴⁹ TGF- β 1 expression and inflammatory cell infiltrates were significantly decreased in hyperglycemic MBL-null mice compared to hyperglycemic WT mice. Diabetic cardiomyopathy pathogenesis is likely multifactorial, with excess extracellular matrix and interstitial inflammation being hallmarks of the disease.^{50,51} Myocardial fibrosis and collagen deposition was observed in hyperglycemic WT hearts as significant collagen staining within myocardial and perivascular tissue. Precisely how MBL deficiency is protective against structural remodeling in diabetes is unclear and will be investigated further by our laboratory in future studies. However, we hypothesize that reduced inflammation and TGF- β 1 signaling likely play important roles.

In conclusion, a pathophysiological role for MBL in hyperglycemia-induced cardiac fibrosis, systolic and diastolic dysfunction, and vascular reactivity was observed in a model of acute hyperglycemia. Because 10% to 15% of the population is functionally MBL deficient, it would be of clinical interest to investigate whether functional MBL serum levels correlate to cardiovascular complications associated with hyperglycemia compared to patients under adequate glycemic control. High-throughput screening for functional MBL levels is now possible, making this relatively easy and testable hypothesis once an adequate sample repository and database are available.⁵² A more clinically relevant issue needs to be addressed in future studies: does reduction of MBL levels improve outcomes after establishment of the disease? This question can only be addressed currently in humans until murine MBL inhibitors are developed.⁴¹

Acknowledgments

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