Meticillin-resistant *Staphylococcus aureus* infection in diabetic mice enhanced inflammation and coagulation

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BALB/cA mice were used to study the interaction of diabetes and meticillin-resistant *Staphylococcus aureus* (MRSA) infection on pathogen distribution, cytokine profile and inflammatory and endothelial-injury markers, as well as coagulation and anticoagulation factors. Meticillin-susceptible *S. aureus* (MSSA) infection did not cause death within the experimental period. MRSA-infected nondiabetic and diabetic mice died on 19.1 ± 1.4 and 10.6 ± 0.7 days post-infection (p.i.), respectively. MRSA and MSSA infection in diabetic mice did not result in symptomatic bacteraemia; however, MRSA infection in diabetic mice significantly reduced glucose levels ($P < 0.05$). Diabetic mice showed significantly higher levels of C-reactive protein, fibrinogen, fibronectin and von Willebrand factor than nondiabetic mice ($P < 0.05$), and MRSA infection further elevated the plasma levels of these inflammatory and endothelial markers ($P < 0.05$). Before infection, diabetic mice had significantly higher plasminogen activator inhibitor-1 (PAI-1) activity, lower antithrombin III (AT-III) and protein C activities ($P < 0.05$), and MRSA infection significantly increased PAI-1 activity further and reduced the activity of AT-III and protein C ($P < 0.05$). MRSA infection increased the production of three Th1 cytokines, interleukin 2 (IL-2), tumour necrosis factor alpha and gamma interferon, in diabetic mice ($P < 0.05$); however, three Th2 cytokines, IL-4, IL-6, IL-10, were elevated at 2 and 4 days p.i., and then dropped gradually. MRSA infection in diabetic mice accelerated the inflammation process, endothelial injury and blood coagulation in diabetic mice. Therefore, the development of proper infection diagnosis and timely use of effective treatments for MRSA-infected diabetic individuals is important and necessary.

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INTRODUCTION

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a common nosocomial pathogen in Taiwan and other countries (Mattila *et al.*, 1998; Jang *et al.*, 1999). Healthcare-associated MRSA strains are resistant to many antibiotics (Hiramatsu *et al.*, 1997; Chang *et al.*, 2000), and MRSA infection markedly increases the morbidity and mortality in hospitalized patients. In our previous study we found that MRSA infection in nondiabetic BALB/cA mice resulted in MRSA distribution in the blood and organs, and elevated plasma levels of interleukin-6 (IL-6), an inflammation marker, and fibronectin, an endothelial injury marker (Tsao *et al.*, 2003). However, there is a lack of *in vivo* information regarding the influence of MRSA infection on other indicators for inflammation or endothelial injury, such as C-reactive protein,

fibrinogen and von Willebrand factor (VWF), in nondiabetic and diabetic mice.

Type I diabetes is an immunoinflammatory disease. Several studies have indicated that the balance of Th1 type cytokines, such as gamma interferon (IFN- γ) and tumour necrosis factor alpha (TNF- α), and Th2 type cytokines, such as IL-4 and IL-10, is altered in diabetics, and that both cytokine types are involved in the development of type I diabetes (Shimada *et al.*, 1996; Muller *et al.*, 2002). However, it has been documented that infection interfered with the balance of Th1/Th2 cytokines in nondiabetic mice also (Geiger *et al.*, 2001; Gonzalez *et al.*, 2003). It remains unclear whether infection, especially MRSA infection, in diabetic individuals affects the regulation of Th1 and Th2 cytokines. Furthermore, the blood coagulation system in diabetic individuals is predominant over the anticoagulation system (Juhan-Vague *et al.*, 2002; Kain *et al.*, 2003; Lapolla *et al.*, 2003). It is also known that infection is associated with a risk of thromboembolic complications (Lowry, 1998; Leinonen & Saikku, 2000). Therefore, there is a need for *in vivo* evidence to help

Abbreviations: IFN- γ , gamma interferon; IL, interleukin; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-susceptible *Staphylococcus aureus*; p.i., post-infection; PVL, Panton–Valentine leukocidin; TNF- α , tumour necrosis factor alpha; VWF, von Willebrand factor.

elucidate the influence of the diabetes and MRSA infection interaction on haemostatic balance.

The purposes of this study were to investigate the influence of MRSA infection on pathogen distribution, inflammation, endothelial injury, cytokine profile and coagulation in diabetic mice. Meticillin-susceptible *S. aureus* (MSSA) was used for comparison. The production of haemolysins and genes encoding potential virulence factors, such as Pantone–Valentine leukocidin (PVL) and enterotoxin, were also compared between MRSA and MSSA. The difference in these clinical signs between diabetic and nondiabetic BALB/cA mice was monitored for several time periods in order to provide novel information for further clinical application.

METHODS

Animals. Male BALB/cA mice (8–9 weeks old) were obtained from the National Laboratory Animal Center (Science Council, Taipei City, Taiwan). Mice with bodyweight at 24.3 ± 0.6 g were used in this study. Mice were housed on a 12 h light/12 h dark schedule, and fed with rat and mouse standard diet no. 1120 and water *ad libitum*. Use of the mice was reviewed and approved by the Chung Shan Medical University Animal Care Committee. To induce diabetes, the mice were treated with streptozotocin [40 mg (kg bodyweight)⁻¹ in 0.1 M citrate buffer, pH 4.5] by intraperitoneal administration for 5 consecutive days. Blood glucose levels were monitored on days 2, 5 and 10 from the tail vein by using a one-touch blood glucose meter. Mice with fasting blood glucose levels over 300 mg dl⁻¹ were defined as diabetic mice and used for this study.

Strains and survival rate. A total of 11 clinical MSSA isolates and 11 clinical MRSA isolates were obtained from patients with diabetes and renal dysfunction at Chung Shan Medical University Hospital from October 2003 to April 2004. All isolates were identified by Vitek (Vitek AMS; bioMérieux Vitek, USA) and API 20E (API-bioMérieux, France). Antibiotic resistance profiles using vancomycin, meticillin, penicillin, cefotaxime and tetracycline were determined by disc diffusion. The discs with antibiotics were purchased from Sigma. Discs were placed on the surface of Mueller–Hinton agar plates supplemented with 2% NaCl and seeded with MSSA or MRSA. Inhibition zones were measured after 24 h incubation at 35 °C. Interpretation of resistance was based on the National Committee for Clinical Laboratory Standards criteria (National Committee for Clinical Laboratory Standards, 1999). The 11 MRSA isolates were susceptible to vancomycin and resistant to the other four test antibiotics. All cultures were routinely maintained on Mueller–Hinton agar plates at 25 °C until used. Prior to infection, MSSA and MRSA were diluted with PBS to 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} and 10^{12} c.f.u. ml⁻¹, which could give approximately 10^6 – 10^{11} c.f.u. per mouse in a volume of 200 µl. Twenty mice were used for each MSSA or MRSA concentration. The survival rate of nondiabetic and diabetic mice was measured at 2 days post-infection (p.i.).

Detection of genes for PVL (*lukPV-1*), enterotoxins (*sea*, *seb*) and haemolysins. Genomic DNA was extracted from MSSA and MRSA cultures grown on agar plates and used as a template for amplification. The presence of genes *lukPV-1*, *sea* and *seb* was confirmed by PCR (Jaffe *et al.*, 2000). Oligonucleotide primers were designed according to the published sequences of *lukPV-1*, *sea* and *seb* genes (GenBank accession nos X72700, M18970 and M11118). The primers for these three genes were 5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCCA-3', 5'-GAA AAA AGT CTG AAT TGC AGG GAA CA-3' and 5'-ATT CTA TTA AGG ACA CTA AGT TAG

GGA-3', respectively. The production of haemolysins (α or β) was confirmed by the method described by Nilsson *et al.* (1999). Briefly, MRSA and MSSA were cultivated in Columbia broth (Difco) at 37 °C for 10 h. After centrifuging at 3000 g, the supernatant was collected and tested against sheep erythrocytes.

Experimental design. Mice infected with MSSA or MRSA at 10^7 – 10^8 c.f.u. ml⁻¹ showed a 100% survival rate in both nondiabetic and diabetic groups. Thus, MSSA or MRSA at a concentration of 10^8 c.f.u. ml⁻¹ was used for the following infection experiments. Diabetic and nondiabetic mice were infected by injecting 200 µl MSSA- or MRSA-PBS solution via the tail vein. After infection, mice were kept under standard laboratory conditions with free access to food and water. On the day before infection (defined as day 0), and on 2, 4, 7, 9, 11, 14 and 17 days p.i., 10 mice from each group were killed with carbon dioxide. Blood, liver, kidney and spleen were collected from each mouse. Plasma was separated from erythrocytes immediately after blood collection. Each 200 mg of tissue was homogenized with 2 ml PBS (pH 7.2) in a motor-driven Teflon-glass homogenizer (Glas-Col). The filtrate was used for further analyses. Plasma glucose concentration (mg dl⁻¹) was determined by a glucose HK kit (Sigma).

Culture. Serial dilutions from plasma and the filtrate from each organ at 100 µl were cultured on Mueller–Hinton agar plates supplemented with 2% NaCl. After incubation for 24 h at 35 °C, colonies were counted and calculated as log₁₀ c.f.u. ml⁻¹ or log₁₀ c.f.u. g⁻¹.

Measurement of inflammation and endothelial injury markers. Plasma levels of C-reactive protein, fibrinogen, fibronectin and VWF were measured as inflammation and endothelial injury markers. C-reactive protein level (µg ml⁻¹) was determined with a commercial ELISA kit (Anogen). Fibrinogen level (g l⁻¹) was assayed by using a commercial kit (Iatroset Fbg; Iatron Laboratory) based on the principle of salting out. Fibronectin (mg ml⁻¹) was assayed by rabbit anti-rat fibronectin antibody and quantified by solid phase immunoenzymic ELISA. VWF antigen levels were measured by an ELISA, using a rabbit anti-rat VWF polyclonal antibody (DAKO). The VWF level was expressed as relative percentages compared to normal pooled plasma.

Cytokine measurement. Plasma levels of IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ before infection and during the course of the infection were detected by ELISA using Cytoscreen immunoassay kits (BioSource International). Samples were run in duplicates according to the manufacturer's instructions. The sensitivity of the assay with the lowest limit was 5 pg ml⁻¹ for IL-2, IL-4, IL-6 and IL-10, and 10 pg ml⁻¹ for TNF- α , IFN- γ .

Measurement of coagulation and anticoagulation factors. Coagulation factor, plasminogen activator inhibitor-1 (PAI-1), and anticoagulation factors, antithrombin III (AT-III) and protein C, were measured in this study. Blood samples were anticoagulated using sodium citrate according to the protocols provided by the manufacturers of the kits used. PAI-1 activity (U ml⁻¹) was assayed by a commercial kit (Trinity Biotech). The activity (%) of AT-III and protein C in plasma was determined by chromogenic assays according to the manufacturer's instructions using commercial AT-III and protein C kits (Sigma), and was shown as the ratio of those in normal human plasma.

Statistical analysis. A total of 11 clinical MSSA and MRSA isolates obtained from infected patients were used in this study. Data were expressed as mean $\pm$ SD of 11 experiments ($n=11$). Data were treated by one-way analysis of variance (ANOVA) and computed using the SAS general linear model procedure (SAS, 1990). Statistical significance was assayed by Student's *t*-test for unpaired data and differences were considered to be significant at $P<0.05$.

Table 1. Clinical features of mice before infection, and survival rate of mice infected with MSSA or MRSA at various concentrationsData are means $\pm$ SD ($n=20$).

	Nondiabetic	Diabetic
Clinical features		
Bodyweight (g)	26.3 $\pm$ 1.6	21.0 $\pm$ 1.2*
Liver weight (g)	0.731 $\pm$ 0.027	0.706 $\pm$ 0.031
Kidney weight (g)	0.142 $\pm$ 0.012	0.155 $\pm$ 0.021
Spleen weight (g)	0.096 $\pm$ 0.008	0.084 $\pm$ 0.014
Fasting glucose level (mg dl ⁻¹)	105 $\pm$ 13	423 $\pm$ 28*
Survival rate for MSSA		
10 ¹¹	95 %	90 %
10 ¹⁰	100 %	95 %
10 ⁹	100 %	100 %
10 ⁷ –10 ⁸	100 %	100 %
Survival rate for MRSA		
10 ¹⁰	90 %	35 %*
10 ⁹	100 %	75 %*
10 ⁷ –10 ⁸	100 %	100 %

*Values are significantly different ($P<0.05$) from nondiabetic group.

RESULTS

The clinical signs and survival rate of mice infected with various MSSA and MRSA concentrations are shown in Table 1. Diabetic mice had significantly lower bodyweights and higher fasting glucose levels than nondiabetic mice before infection ($P<0.05$). The expressed rate of PVL (*lukPV-1*), enterotoxins (*sea*, *seb*) and haemolysins (α , β) in MSSA and MRSA strains are presented in Table 2. The test MRSAs expressed more *lukPV*, *sea* and *seb* genes than MSSA. Similarly, the test MRSAs also produced more haemolysins (α , β or α and β) than the 11 test MSSAs.

The distribution of MSSA and MRSA in plasma and organs is presented in Fig. 1. MSSA infection did not cause death during the entire experimental period, and the level of this pathogen in plasma or organs reduced gradually after 11 days infection. MRSA-infected nondiabetic and diabetic

Table 2. Expression of genes encoding PVL (*lukPV-1*), enterotoxins (*sea*, *seb*) and haemolysins (α , β) in 11 MSSA and 11 MRSA strains

Gene	MSSA	MRSA
<i>lukPV-1</i>	2/11	9/11
<i>sea</i> only	2/11	3/11
<i>seb</i> only	3/11	1/11
<i>sea</i> and <i>seb</i>	0/11	7/11
Haemolysin α only	1/11	3/11
Haemolysin β only	2/11	3/11
Haemolysin α and β	0/11	5/11

Fig. 1. MSSA or MRSA level in plasma (log₁₀ c.f.u. ml⁻¹), and in liver, kidney and spleen (log₁₀ c.f.u. g⁻¹) from nondiabetic (NDM) and diabetic (DM) mice. Data are means $\pm$ SD ($n=11$). ◇, Plasma; ◆, liver; ■, kidney; X, spleen.

mice died on 19.1 $\pm$ 1.4 and 10.6 $\pm$ 0.7 days p.i., respectively. In nondiabetic mice, MSSA or MRSA was distributed in plasma, kidney, liver and spleen; however, the level of either MSSA or MRSA in diabetic mice was significantly increased only in kidney ($P<0.05$). Plasma glucose levels for diabetic or nondiabetic mice infected with MSSA or MRSA are presented in Fig. 2. MSSA infection did not significantly affect glucose level ($P>0.05$); however, MRSA infection in diabetic mice caused significant reduction in glucose level ($P<0.05$).

Fig. 2. Glucose level (mg dl⁻¹) in plasma from nondiabetic (○) and diabetic (●) mice before (day 0) and after MSSA (dotted lines) or MRSA (continuous lines) infection. Data are means ± SD (*n* = 11).

The blood markers of inflammation and endothelial injury are shown in Table 3. Diabetic mice showed significantly higher levels of C-reactive protein, fibrinogen, fibronectin and VWF than nondiabetic mice (*P* < 0.05). MSSA infection resulted in a slight, but not significant, elevation in these markers in both nondiabetic and diabetic mice (*P* > 0.05); however, MRSA infection caused a significant increase in these markers, especially in diabetic mice (*P* < 0.05). The influence of diabetes and MRSA infection on coagulation and anticoagulation factors is presented in Table 4. Before infection, diabetic mice had significantly lower AT-III and protein C activities, and a higher PAI-1 activity (*P* < 0.05) than nondiabetic mice. MSSA infection did not further affect these coagulation and anticoagulation factors (*P* > 0.05); however, MRSA infection in diabetic mice significantly reduced the activity of AT-III and protein C (*P* < 0.05) further, as well as significantly increasing PAI-1 activity (*P* < 0.05).

Plasma levels of Th1 and Th2 cytokines are presented in Figs 3 and 4. MRSA infection significantly increased the levels of three Th1 cytokines, IL-2, TNF-α and IFN-γ, and three Th2 cytokines, IL-4, IL-6 and IL-10, in diabetic mice (*P* < 0.05); however, the levels of Th2 cytokines dropped at 7 and 9 days p.i.

Table 4. Activity of AT-III (%), protein C (%) and PAI-1 (U ml⁻¹) in plasma from nondiabetic (NDM) and diabetic (DM) mice at day 0 (before infection) and days 4, 9, 14, 17 after MSSA or MRSA infection

Data are mean ± SD (*n* = 11).

	MSSA		MRSA	
	NDM	DM	NDM	DM
AT-III (%)				
Day 0	125 ± 12	97 ± 5	129 ± 10	98 ± 8*
Day 4	120 ± 10	94 ± 7	85 ± 6	51 ± 7*
Day 9	118 ± 13	95 ± 4	78 ± 4	21 ± 3*
Day 14	117 ± 8	96 ± 8	72 ± 6	–
Day 17	122 ± 14	95 ± 5	69 ± 5	–
Protein C (%)				
Day 0	96 ± 8	82 ± 6	94 ± 6	80 ± 7*
Day 4	92 ± 6	85 ± 3	70 ± 7	44 ± 5*
Day 9	95 ± 7	83 ± 5	59 ± 9	16 ± 6*
Day 14	93 ± 4	82 ± 7	52 ± 5	–
Day 17	93 ± 5	85 ± 4	42 ± 6	–
PAI-1 (U ml⁻¹)				
Day 0	7.2 ± 0.6	8.6 ± 0.8	7.0 ± 1.1	17.6 ± 1.0*
Day 4	7.3 ± 0.5	8.3 ± 1.0	7.5 ± 1.4	18.2 ± 1.4*
Day 9	7.5 ± 0.7	8.7 ± 0.7	8.7 ± 0.9	23.9 ± 2.1*
Day 14	7.1 ± 0.5	8.1 ± 0.9	9.5 ± 0.7	–
Day 17	7.6 ± 0.4	8.4 ± 1.1	10.4 ± 1.2	–

*Values are significantly different (*P* < 0.05) from MRSA-infected nondiabetic group.

DISCUSSION

The 11 MRSA isolates used produced markedly more haemolysins and harboured more genes encoding enterotoxins and PVL, a synergohymenotropic toxin. Thus, it is reasonable to observe severe infection, inflammation and coagulation in these MRSA-infected mice. Based on the lower survival rate and shorter survival length, diabetic mice were susceptible to MRSA infection. However, both MRSA and MSSA infection did not result in marked symptomatic bacteraemia in diabetic mice. Thus, cultures from blood

Table 3. Plasma levels of C-reactive protein, fibrinogen, fibronectin and VWF in nondiabetic (NDM) and diabetic (DM) mice at day 0 (before infection) and day 9 after MSSA or MRSA infection

Data are mean ± SD (*n* = 11).

	NDM MSSA, day 0/day 9	DM MSSA, day 0/day 9	NDM MRSA, day 0/day 9	DM MRSA, day 0/day 9
C-reactive protein (μg ml ⁻¹)	19.5 ± 1.7/22.3 ± 2.2	29.8 ± 2.7/33.5 ± 4.1	20.2 ± 1.5/39.6 ± 3.4*	31.0 ± 2.5/66.4 ± 5.8*
Fibrinogen (g l ⁻¹)	2.25 ± 0.33/2.31 ± 0.51	4.11 ± 0.50/4.47 ± 0.61	2.17 ± 0.25/2.94 ± 0.30*	4.18 ± 0.44/7.62 ± 0.72*
Fibronectin (mg ml ⁻¹)	2.42 ± 0.28/2.78 ± 0.30	4.08 ± 0.35/4.66 ± 0.44	2.45 ± 0.31/3.42 ± 0.28*	4.15 ± 0.29/5.96 ± 0.52*
VWF (%)	34.7 ± 5.7/38.9 ± 6.1	57.3 ± 6.3/64.2 ± 7.8	33.6 ± 4.9/73.9 ± 8.2*	55.9 ± 7.0/127.2 ± 10.2*

*Values are significantly different (*P* < 0.05) from the same group at day 0.

Fig. 3. Th1 cytokines (IL-2, TNF- α , IFN- γ) in plasma from non-diabetic ($\diamond$) and diabetic ($\blacklozenge$) mice after MSSA (dotted lines) or MRSA (continuous lines) infection. Data are means $\pm$ SD ($n=11$).

(plasma or serum) may not be appropriate for detecting MRSA or MSSA infection in diabetic individuals. Therefore, other clinical diagnoses should be used for examining early infection in these individuals. Another novel finding from this study was that MRSA infection markedly decreased plasma glucose in diabetic mice. It has been reported that the TNF- α overproduction could cause hypoglycaemia in mice (Endo, 1991; Vogel *et al.*, 1991). In our present study, a high plasma level of TNF- α was present in MRSA-infected diabetic mice, which may contribute to the observed hypoglycaemia.

Type I diabetes patients are characterized by elevated levels of C-reactive protein, fibrinogen, fibronectin and VWF; these endothelial-associated proteins are commonly used as indicators for inflammation and endothelial injury (Ciarla *et al.*, 2001; Gomes *et al.*, 2003; Aso *et al.*, 2004). The results of our study agree with those of previous studies: diabetic induction increased the levels of these markers. Furthermore, our study found that MRSA infection in diabetic mice exacerbated the inflammation process and vascular-endothelial dysfunction. Besides acting as inflammation or endothelial-injury markers, fibrinogen, fibronectin and VWF are also important adhesive and procoagulant proteins because these factors participate in atherosclerotic plaque thrombus formation and are involved in platelet

Fig. 4. Th2 cytokines (IL-4, IL-6, IL-10) in plasma from nondiabetic ($\diamond$) and diabetic mice ($\blacklozenge$) after MSSA (dotted lines) or MRSA (continuous lines) infection. Data are means $\pm$ SD ($n=11$).

dysfunction (Bennet & Kolodziej, 1992; Mohamed-Ali *et al.*, 2001). Thus, we believe that the increase of these blood markers in MRSA-infected diabetic mice accelerated coagulation progression. However, we also found the interaction of diabetes and MRSA infection resulted in markedly up-regulated PAI-1 activity and down-regulated AT-III and protein C activities. It is known that PAI-1 is the primary physiological inhibitor of fibrinolysis (Urano *et al.*, 2000), and AT-III and protein C are anticoagulation factors, because AT-III could inhibit the activity of a number of proteases in the coagulation cascade (Asakawa *et al.*, 2000), and protein C could inactivate coagulation factors such as factors Va and VIIIa (Shen *et al.*, 1997). Thus, these results also suggest that this interaction of diabetes and MRSA infection strongly impaired anticoagulation and fibrinolysis systems, and favoured the development of thrombosis and atherosclerosis. The affected haemostatic balance in these MRSA-infected mice may be partially due to the changed cytokine profiles. It has been shown that IFN- γ and TNF- α could induce the synthesis of PAI-1, which up-regulated lymphoid-cell-associated procoagulant activity (Sakamoto *et al.*, 1999; Urano *et al.*, 2000), and IL-4 could induce the expression of anticoagulant protein S (Smiley *et al.*, 1997). In our study, both an elevation in IFN- γ and TNF- α , and a reduction in IL-4 were observed in MRSA-infected diabetic mice, which might consequently enhance lymphoid-cell-associated

coagulation by increasing PAI-1 synthesis, diminishing the protein S-related anticoagulant activity and impairing fibrinolytic activity.

The diabetes induced by low doses of streptozotocin is a T lymphocyte-dependent model. Several studies have indicated that both Th1 and Th2 cytokines are overexpressed during diabetic development in this model (Herold *et al.*, 1996; Muller *et al.*, 2002). In our present study, we also observed that diabetic induction caused a marked increase in both Th1 and Th2 cytokines (data not shown). Furthermore, we found MRSA infection further elevated Th1 cytokines in diabetic mice. These results suggest the interaction of MRSA infection and diabetes strongly stimulated the expression of Th1 cytokines. MRSA infection also up-regulated Th2 cytokines in diabetic mice; however, this up-regulation appeared at the early stage of infection (2 and 4 days p.i.) only. The dramatic decline of Th2 cytokines at the late stage was found in MRSA-infected diabetic mice only; thus, we believe it was definitely due to the interaction of MRSA infection and diabetes. This finding suggested that the concomitant rise of Th1 cytokines with the decline of Th2 cytokines in MRSA-infected diabetic individuals seemingly represented a deteriorative development. This information might be helpful for developing a clinical diagnosis to examine infection in the diabetic individual. However, MRSA infection in nondiabetic mice caused the overproduction of Th1 and Th2 cytokines, which definitely contributed to the increased release of proteins responsible for inflammation, endothelial injury and coagulation. Thus, MRSA-infected nondiabetic mice were also in danger of atherosclerosis and thrombus complications. However, without the impact of diabetes, these MRSA-infected nondiabetic mice had a longer survival time.

It has been indicated that proinflammatory cytokines, IL-6 and TNF- α , were central mediators for the regulation of several biomarkers such as C-reactive protein and VWF (McCarty, 1999; Mohamed-Ali *et al.*, 2001; Tomita *et al.*, 2004), which consequently contributed to the progression of inflammation, endothelial dysfunction and coagulation. In our study, the interaction of MRSA infection and diabetes definitely caused an elevation of IL-6 and TNF- α in the early stages (2 and 4 days p.i.); thus, the observed up-regulation of coagulation factors and markers for inflammation and endothelial injury at this stage could be explained. However, the IL-6 level decreased while TNF- α and adhesive and procoagulant proteins still increased at the late stage (after 4 days p.i.). Does this result suggest IL-6 is less important than TNF- α in regulating these endothelial-associated proteins? Or is IL-6 an inducer for the acute-phase response only? The other question is why does increased TNF- α not cause IL-6 elevation, since TNF- α could stimulate several tissues to release IL-6 (McCarty, 1999)? It seems that other factors and/or conditions are involved in the action of IL-6/TNF- α in regulating endothelial-associated proteins in MRSA-infected diabetic individuals. Further studies are necessary to elucidate the related factors and their relationships.

In conclusion, MRSA infection did not result in marked symptomatic bacteraemia in diabetic mice. The interaction of diabetic pathogenesis and MRSA infection in BALB/cA mice changed the Th1 and Th2 cytokine profile, which further impaired glycaemic regulation. Furthermore, this interaction markedly enhanced the production of several adhesive and procoagulant proteins, which accelerated the inflammation process, endothelial injury and blood coagulation in diabetic mice. Therefore, the development of proper infection diagnosis and timely use of effective treatments for MRSA-infected diabetic individuals is important and necessary.

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