Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY ORIGINAL ARTICLE

Delayed activation of host innate immune pathways in streptozotocin-induced diabetic hosts leads to more severe disease during infection with *Burkholderia pseudomallei*

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doi:10.1111/j.1365-2567.2011.03544.x
Received 6 June 2011; revised 7 November 2011; accepted 28 November 2011.
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Summary

Diabetes mellitus is a predisposing factor of melioidosis, contributing to higher mortality rates in diabetics infected with *Burkholderia pseudomallei*. To investigate how diabetes alters the inflammatory response, we established a streptozotocin (STZ) -induced diabetic murine acute-phase melioidosis model. Viable *B. pseudomallei* cells were consistently detected in the blood, liver and spleen during the 42-hr course of infection but the hyperglycaemic environment did not increase the bacterial burden. However, after 24 hr, granulocyte counts increased in response to infection, whereas blood glucose concentrations decreased over the course of infection. A genome-wide expression analysis of the STZ-diabetic murine acute melioidosis liver identified ~ 1000 genes whose expression was altered in the STZ-diabetic mice. The STZ-diabetic host transcriptional response was compared with the normoglycaemic host transcriptional response recently reported by our group. The microarray data suggest that the presence of elevated glucose levels impairs the host innate immune system by delaying the identification and recognition of *B. pseudomallei* surface structures. Consequently, the host is unable to activate the appropriate innate immune response over time, which may explain the increased susceptibility to melioidosis in the STZ-diabetic host. Nevertheless, a general 'alarm signal' of infection as well as defence programmes are still triggered by the STZ-diabetic host, although only 24 hr after infection. In summary, this study demonstrates that in the face of a *B. pseudomallei* acute infection, poor glycaemic control impaired innate responses during the early stages of *B. pseudomallei* infection, contributing to the increased susceptibility of STZ-induced diabetics to this fatal disease.

Keywords: acute melioidosis; *Burkholderia pseudomallei*; diabetes; hyperglycaemia; innate immunity

Introduction

In 2010, diabetes mellitus (DM) affected 284 million people worldwide with a concomitant dramatic impact on health in terms of morbidity and mortality of affected individuals.[1] The projection for 2030 indicates a prevalence of 439 million individuals comprising ~ 7·7% of the world population.[1] Diabetes has been identified as an important risk factor for infection, particularly Gram-negative infections,[2–4] including melioidosis, an infection caused by the soil bacterium *Burkholderia pseudomallei* that is endemic in Southeast Asia and Northern Australia.[5,6] The increased susceptibility of diabetics to infection has been suggested to be the result of defects in immunity such as impaired chemotaxis, phagocytosis, oxidative burst and killing activity, as well as of increased microbial adherence to diabetic cells.[4,7] Little is known about the basis for the increased susceptibility of diabetic patients to *B. pseudomallei* infection but impaired neutrophil function is believed to be one of the possible causes of this increased prevalence of infection.[6]

Patients with DM (up to 60% are type 2 diabetes) present with a high incidence of melioidosis.[6] Although insulin is thought to have a direct effect on the growth of *B. pseudomallei*,[8] subsequent studies have attributed the

inhibitory effect to a preservative used with insulin.[9,10] Recently, Pongcharoen *et al.*[11] reported that patients with DM have defective interleukin-17 (IL-17) production in response to *B. pseudomallei* infection, whereas Chanchamroen *et al.*[12] reported that defective polymorphonuclear neutrophils (PMN) of diabetic subjects in the early phase of the inflammatory reaction against *B. pseudomallei* may contribute to increased susceptibility to melioidosis. Despite such clinical observations, little is known about how diabetes impairs protective immunity. Moreover, an intensive study on the immune response of diabetic hosts with respect to this bacterium is still not available. As prevalence of diabetes is expected to increase rapidly worldwide, there is potential for an increased number of individuals at risk of severe infection with *B. pseudomallei*. Hence, establishing a diabetes infection model is a logical first step to investigate the mechanism of impaired host defence in diabetes.

Diabetic mice established by multiple low-dose treatments with streptozotocin (STZ), a pancreatic islet β-cell toxin, have been widely used to study a number of infections.[3,13] The multiple low-dose STZ treatments induce an autoimmune insulitis that leads to insulin insufficiency and diabetes that mimics several of the aetiological events that occur in the development of human type 1 diabetes.[3] Although both type 1 and type 2 diabetes have different aetiologies, they share common clinical symptoms of hyperglycaemia, glucose intolerance, poor wound healing, nephropathy, vascular abnormalities and increased risk of infection.[2,4,14] A study on *Porphyromonas gingivalis* infection demonstrated that the inflammatory response to this infection is not dependent upon the type of diabetes, but rather is the consequence of hyperglycaemia.[4]

The present study was conceived to investigate the exquisite interplay between the STZ-diabetic host response and *B. pseudomallei*. We established a systemic acute melioidosis infection of STZ-induced diabetic mice and performed transcriptional analysis of the liver and spleen isolated from diabetic mice infected over a 42-hr time period. Our study is the first to report on a global STZ-diabetic host–*B. pseudomallei* interaction by whole transcriptome analysis.

Materials and methods

Bacteria

The *B. pseudomallei* clinical isolate, referred to herein as *Bp*D286, was obtained from the Pathogen Laboratory, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Malaysia, and was previously characterized based on biochemical tests as well as by 16S rRNA sequencing.[15] Bacteria were grown in brain–heart infusion broth overnight at 37°. The cells were centrifuged at 10 000 ***g***, suspended in brain–heart infusion broth containing 20% glycerol, frozen immediately in aliquots of 10^9 colony-forming units (CFU)/ml and stored at −80°.[16]

Mice

BALB/c male mice (5 to 7 weeks old) were purchased from the Institute for Medical Research, Malaysia. They were housed in High Temperature Polysufone (Tecniplast, Buguggiate, Italy) cages with a bedding of wood shavings, subjected to a 12-hr light/dark cycle and were fed on a diet of commercial pellets and distilled water *ad libitum*. All animal experiments were performed in accordance with the Universiti Kebangsaan Malaysia animal ethics guidelines and approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee.

STZ-induced diabetes in BALB/c mice

Diabetic mice were established using the Low-Dose STZ Induction Protocol by Animal Models of Diabetic Complication Consortium as described elsewhere with minor modifications.[3,13,17] BALB/c mice were rendered diabetic by treatment with STZ (50 mg/kg body weight) in 10 mM sodium citrate buffer (pH 4·5) by intra-peritoneal injection daily for 5 days. Mice were considered to be diabetic when blood glucose levels exceeded 14 mmol/l. Mice remained diabetic for 3–5 days before inoculation with bacteria. At the time the experiments were initiated, blood glucose levels ranged from 14·1 to 33·3 mmol/l. A second group of mice not treated with STZ (normoglycaemic mice) were used as non-diabetic controls. These mice had blood glucose levels that ranged from 5 to 8 mmol/l. Tail vein blood glucose levels were assessed with the glucometer Accu-Chek Active (Roche Diagnostics, Mannheim, Germany).

Development and characterization of acute melioidosis STZ-diabetic model

Infection experiments, determination of bacterial loads and leucocyte differential counts were performed as previously described.[18] Mice were monitored for a period of 10 days based on the accepted protocol for observing mortality in *B. pseudomallei* acute-stage infection.[19–21]

Gene expression analyses and microarray data analysis

Microarray experiments were performed using the SentrixMouseRef-8 Expression BeadChips (Illumina, San Diego, CA), containing over 24 000 probes according to the instructions provided and as described previously.[18] BeadStudio version 1.0 (Illumina) software was used to generate signal intensity values from the scans according to the standard procedure within the software. In brief, the sample intensities are scaled by a factor equal to the

ratio of average intensity of virtual sample to the average intensity of the given sample. Background is subtracted before the scaling. The normalized data were analysed by GeneSpring GX7.3.1 Expression Analysis (Agilent Technologies, Santa Clara, CA) as previously described for acute normoglycaemic microarray data.[18] In brief, normalization was applied in two steps: 'per chip normalization', by which each measurement was divided by the 50th percentile of all measurements in its array; and 'per gene normalization', by which all the samples were normalized against the median of the control samples (uninfected STZ-diabetic control tissues). The expression of each gene was reported as the ratio of the value obtained for each condition relative to the control condition after normalization of the data as previously described.[18] The normalized data were grouped on the basis of the experimental conditions (organs and infection time-points). The Volcano Plot with parametric test was performed to determine differentially expressed genes. Differentially expressed genes were defined as those having a *P*-value $\leq 0{\cdot}05$ and an absolute change greater than twofold for *B. pseudomallei*-infected tissue at 16, 24 or 42 hr relative to the uninfected STZ-diabetic control tissue. The data discussed in this publication have been deposited in the NCBI Gene Expression Omnibus and are accessible through the GEO Series accession number GSE28683 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE28683).

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed in the Mastercycler® ep realplex (Eppendorf, Harburg, Germany) to quantify the expression of *TLR2*, *TLR4*, *TLR5*, *IFNg*, *CXCL1* and *CCL7* genes as previously described.[18]

Results

Melioidosis susceptibility of STZ-induced diabetic mice

Diabetes was successfully induced by multiple low-dose treatments with STZ. An average of 70% of the STZ-treated mice (21 of 30 mice) had blood glucose levels > 14 mmol/l and were considered diabetic.[3] These STZ-diabetic mice exhibited signs of polydipsia and polyuria. Susceptibility to *B. pseudomallei* infection between STZ-diabetic and non-diabetic control mice was compared. Both groups of mice were infected with $1{\cdot}2 \times 10^3$ CFU/ml *B. pseudomallei* D286 via the intravenous route and survival was monitored over 10 days post-infection. *Burkholderia pseudomallei*-infected STZ-diabetic mice and *B. pseudomallei*-infected normoglycaemic mice had similar survival percentages over the first 48-hr post-infection (p.i.) (Fig. 1). However, the *B. pseudomallei*-infected STZ-diabetic mice were more susceptible to melioidosis than the *B. pseudomallei*-infected normoglycaemic mice at the later stage of infection (Fig. 1). Mortality was first observed for the *B. pseudomallei*-infected STZ-diabetic mice on day 3 p.i. with a median survival of 7 days, whereas the *B. pseudomallei*-infected normoglycaemic mice had a longer median survival (9 days). By day 10 p.i., mortality of *B. pseudomallei*-infected STZ-diabetic mice was 75% (six of eight mice) compared with 50% for normoglycaemic mice (two of four mice) (Fig. 1). However, we postulate that the *B. pseudomallei*-infected STZ-diabetic group will continue to succumb to melioidosis based upon the day-10 post-mortem observation of abscesses on the spleens of surviving diabetic mice.

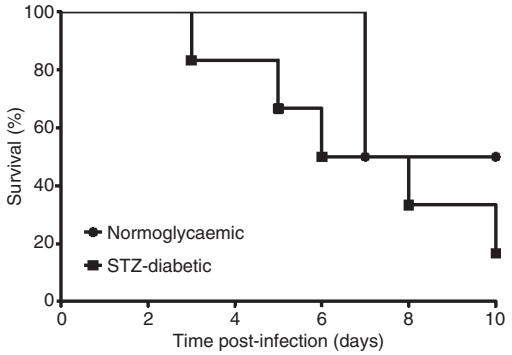

Figure 1. Melioidosis susceptibility of mice with streptozotocin (STZ)-induced diabetes. Mortality of STZ-diabetic mice ($n = 8$ mice) compared with normoglycaemic mice ($n = 4$ mice) following *Burkholderia pseudomallei* infection [Logrank (Mantel–Cox) test, *P*-value = 0·4483]. Animals were observed daily up to 10 days and the percentage survival was plotted against time. A representative of two independent experiments is shown. Mice were infected with $1{\cdot}2 \times 10^3$ colony-forming units (CFU)/ml *B. pseudomallei* via the intravenous route.

Development and characterization of acute melioidosis in an STZ-diabetic mouse model

To characterize acute melioidosis in an STZ-diabetic mouse model, we monitored mouse blood glucose levels, bacterial loads in various organs and leucocyte differential counts during the course of infection in STZ-induced diabetic BALB/c mice infected with $9{\cdot}36 \times 10^3$ CFU/ml *B. pseudomallei* D286. Non-fasting blood glucose levels were measured 2 weeks after STZ treatment (before infection). Mice with confirmed elevated blood glucose levels (14–33 mmol/l) were then injected with *B. pseudomallei*. Blood glucose levels were again measured 1 hr p.i. and at the conclusion of each time-point: 16, 24 and 42 hr p.i. (Fig. 2). Blood glucose levels of STZ-treated mice (mean = 24·51 mmol/l) remained > 14 mmol/l at 1 hr p.i., confirming that the infected mice from all the experimental groups were hyperglycaemic at the time of infection (Fig. 2). The mean blood glucose concentrations for

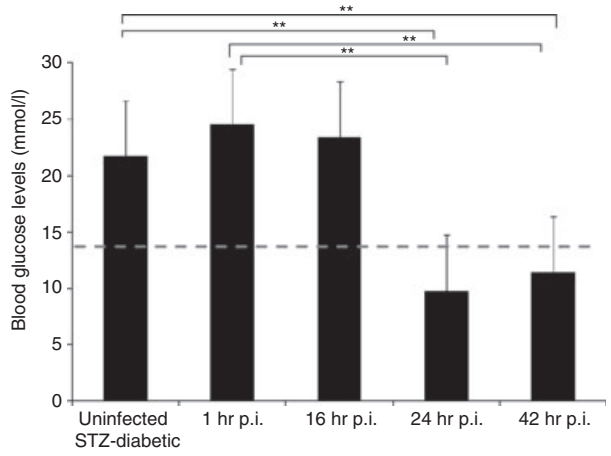

Figure 2. Mouse blood glucose concentrations. Non-fasting blood glucose levels of mice ($n = 5$ mice/group) were measured 2 weeks after streptozotocin (STZ) treatments, 1 hr post-infection (p.i.) and at the conclusion of each time-point: 16, 24 and 42 hr p.i., respectively. Diabetic mice had blood glucose levels > 14 mmol/l (above the dashed line). Data are mean ± standard deviation of five mice per group. Significance was determined using the Student's *t*-test (**P-value < 0·01).

B. pseudomallei-infected mice at 16, 24 and 42 hr p.i., were 23·36, 9·76 and 11·4 mmol/l, respectively. Blood glucose levels of *B. pseudomallei*-infected STZ-diabetic mice decreased gradually to hypoglycaemic levels before the mice succumbed to infection.

The bacterial loads in liver, spleen and blood at 42 hr p.i. were significantly higher than bacterial loads at 16 hr p.i. (Fig. 3a–c), indicating propagation of intracellular bacteria in the infected host. The presence of high numbers of *B. pseudomallei* in the organs and blood confirms that systemic acute septicaemic melioidosis was successfully developed in STZ-diabetic BALB/c mice. No significant differences were observed in liver and spleen weights at all infection time-points (data not shown). To further characterize the STZ-diabetic host innate immune response to acute melioidosis, leucocyte counts and composition of blood samples taken at 16, 24 and 42 hr time-points during infection of STZ-diabetic mice were analysed. Analysis of the differential blood film after infection with $9{\cdot}36 \times 10^3$ CFU/ml *B. pseudomallei* D286 revealed no changes in the neutrophil and leucocyte counts at 16 hr p.i. compared with the uninfected STZ-diabetic mice (Fig. 3d). Nonetheless, neutrophilia was observed at 24 hr p.i. onwards (Fig. 3d), indicating a delay in triggering the STZ-diabetic host innate immune response to acute *B. pseudomallei* infection compared with the infected normoglycaemic mice.[18] Moreover, several blood cell abnormalities, including monocyte vacuolization, Pseudo Pelger, hypersegmentation of neutrophil nuclei and Rouleaux formation frequently seen in serious bacterial infections[22,23] were also seen in the blood samples at 42 hr p.i. (Fig. 3e). This observation was not seen in normoglycaemic *B. pseudomallei*-infected mice, or in uninfected non-diabetic control mice, and has not been reported previously in any of the melioidosis cases. Peripheral blood cell morphology provides additional unique diagnostic information on *B. pseudomallei* infection.

Global transcriptional responses to acute-stage melioidosis in STZ-diabetic mice

STZ-diabetic BALB/c mice were infected with *B. pseudomallei* D286 intravenously. Gene expression profiles were obtained from a comparison of the transcriptome of

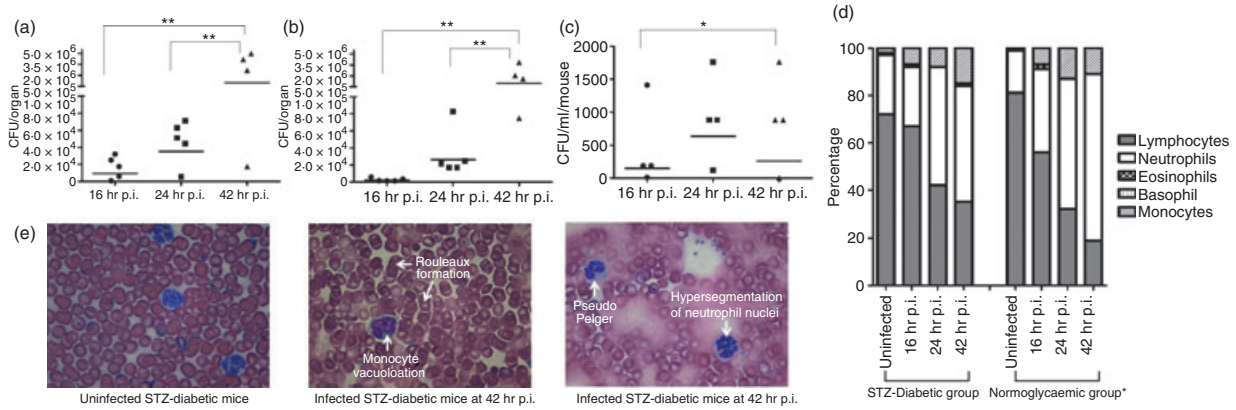

Figure 3. Characterization of streptozotocin (STZ) -induced diabetic acute melioidosis model. The bacterial loads in (a) liver; (b) spleen and (c) blood of STZ-induced diabetic BALB/c mice ($n = 5$ mice/group) at 16, 24 and 42 hr after intravenous infection with $9{\cdot}36 \times 10^3$ colony-forming units (CFU)/ml *Burkholderia pseudomallei.* Each symbol represents one mouse; bar indicates geometric mean. Significance was determined using the Student's *t*-test (*P-value < 0·05; **P-value < 0·01). The control mice are not represented because no colonies were observed by plating. (d) Changes in differential leucocyte counts for both acute STZ-diabetic and acute normoglycaemic mice. Values are an average of pooled blood from three to five infected mice at a particular time-point. Data for the acute normoglycaemic infection model is adopted from Chin *et al.*[18] (e) blood cell abnormalities.

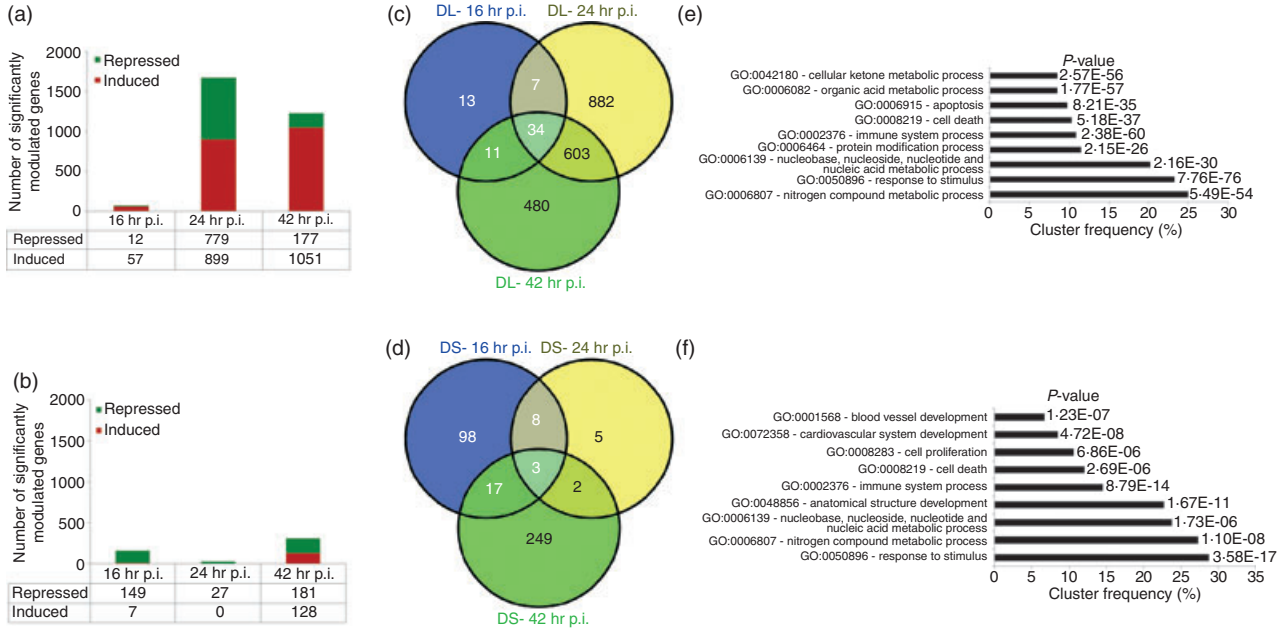

Figure 4. Differential gene expression of an acute melioidosis streptozotocin (STZ) -diabetic model over 42 hr relative to uninfected diabetic mice. Number of genes modulated during acute *Burkholderia pseudomallei* D286 infection in STZ-induced diabetic BALB/c mice at 16, 24 and 42 hr post-infection (p.i.) in both (a) liver and (b) spleen identified by Volcano plots with the cut-off of twofold change and *P*-value < 0·05; Venn diagrams demonstrating the overlap between different experimental conditions in both (c) STZ-diabetic liver (DL) and (d) STZ-diabetic spleen (DS) as determined by VENNY; Major biological processes modulated in acute diabetic model in (e) liver and (f) spleen as determined by GOTerm Finder analysis.

infected STZ-diabetic liver and STZ-diabetic spleen with uninfected STZ-diabetic mice organs. We noted that an acute *B. pseudomallei* infection in STZ-diabetic mice results in more differentially expressed genes in the liver, particularly at 24 hr p.i. onwards (Fig. 4a), compared with the spleen. Surprisingly, very few genes were modulated in the STZ-diabetic spleen throughout the infection period (Fig. 4b). Analyses of the identified genes were further represented by Venn diagrams demonstrating the overlap between different experimental conditions in both liver (Fig. 4c) and spleen (Fig. 4d). There were only 34 and three common genes whose expression is consistently differentially modulated throughout the course of infection in STZ-diabetic liver and spleen, respectively. These expression profiles suggest that common responses, particularly the immune response to acute *B. pseudomallei* infection, are not well modulated when the diabetic host initially encounters the bacterium.

The microarray expression profile revealed differentially up-regulated genes (24 hr p.i. onwards) were clustered as those involved in immune response and cellular metabolism (Fig. 4e,f). The cytokine–cytokine receptor interaction, Jak-STAT signalling pathway, Toll-like receptor signalling pathway, chemokine signalling pathway, apoptosis, antigen processing and presentation were up-regulated as shown in Table 1. Concomitantly, the major down-regulated Kyoto Encyclopaedia of Genes and Genomes pathways include drug metabolism, cytochrome P450, glycine, serine and threonine metabolism, tryptophan metabolism, fatty acid metabolism and tricarboxylic acid cycle (Table 1). The identified genes were further categorized according to functional categories and the fold change relative to the uninfected diabetic control mice is presented as a heat map (Fig. 5 and Table 2). Kinetic profiles of the expression of host genes modulated by *B. pseudomallei* infection in normoglycaemic models[18] is also included in Table 2 for comparison between the transcriptional expression responses in both models. As a result of the large number of significantly differentiated genes modulated during the infection, only data related to genes that have some functional information are shown and discussed below.

Delayed activation of host defence responses to *B. pseudomallei* infection in STZ-diabetic mice correlates with the delayed Toll-like receptor2 signature

Our recent genome-wide expression study of *B. pseudomallei* infected normoglycaemic mice revealed that the Toll-like receptor (TLR2) -mediated signalling pathway is

Table 1. Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways regulated in *Burkholderia pseudomallei*-infected streptozotocin-induced diabetic liver analysed by GeneTrail

Categories	KEGG pathways	*P*-value
Up-regulated	Cytokine–cytokine receptor interaction	1·86698e−05
	Jak-signal transducer and activator of transcription signalling pathway	1·92797e−05
	Adipocytokine signalling pathway	0·0131694
	Toll-like receptor signalling pathway	3·63952e−09
	Apoptosis	1·37271e−05
	Chemokine signalling pathway	1·37271e−05
	Antigen processing and presentation	3·38894e−05
	Mitogen-activated protein kinase signalling pathway	0·000283262
	Systemic lupus erythematosus	8·11437e−05
	Cell adhesion molecules	0·000293927
Down-regulated	Drug metabolism – cytochrome P450	1·81645e−12
	Glycine, serine and threonine metabolism	2·68347e−06
	Tryptophan metabolism	2·76687e−06
	Methane metabolism	2·22093e−05
	Alanine, aspartate and glutamate metabolism	2·42568e−05
	ABC transporters	0·00317722
	Nitrogen metabolism	0·0039838
	PPAR signalling pathway	0·0171655
	Fatty acid metabolism	0·00368175
	Tricarboxylic acid cycle	0·0933579

responsible for recognition and initiation of the inflammatory response, leading to the elevation of a broad range of innate immune mechanisms, including the 'core host immune response' genes commonly seen in general inflammatory infections.[18] To unravel the susceptibility of DM patients to melioidosis, we compared the STZ-diabetic host transcriptional response, particularly the innate immune response, to the normoglycaemic host transcriptional response.[18] Figure 6 shows the fold change levels of TLR2 and several major transcription factors [interferon-γ (IFN-γ), tumour necrosis factor (TNF), nuclear factor-κB1 (NF-κB1), interferon regulatory factor 1 (IRF-1), IRF-7, signal transducer and activator of transcription 1 (STAT1) and STAT2] that are responsible for regulating various defence mechanisms in both STZ-diabetic and normoglycaemic infected mice relative to the uninfected mice, respectively. In response to acute *B. pseudomallei* infection, these genes were induced in normoglycaemic mice as early as 16 hr p.i. but were only induced 24 hr p.i. in the STZ-diabetic host. Concomitantly, induction of 'common host immune response' genes representing a general 'alarm signal' for inflammatory infections by several different human pathogens was delayed (after 24 hr) in the STZ-diabetic liver (Fig. 7) compared with normoglycaemic mice. This cluster of genes includes the pro-inflammatory mediators [*TNF*, *IL1b*, colony stimulating factor 1 (*CSF1*) and *CSF3*], the chemokines (*CCL3*, *CCL4*, *CXCL1*, *CXCL2*, *CXCL3*), IFN-stimulated genes (ISGs) (*OAS*) and the IFN-inducible chemokine genes (*CCL9*, *CXCL10*, *CXCL11*) (Fig. 5 and Table 2). However, many of these defence genes [intercellular adhesion molecule 1 (*ICAM1*), TNF-α-induced protein 3 (*TNFAIP3*), macrophage inflammatory protein 1a (*MIP1a*), *MIP1b*, dual specificity phosphatase (*DUSP*) 8, *CXCL1*, *CXCL2*, *CXCL10*, *CSF3*, NF-κB family members (*Nfkbia*, *Nfkbib*, *Nfkbie*), *IFNg*, *TNF*, *IL1b* and mitogen-activated protein kinase kinase kinase 8 (*MAP3K8*)] were suppressed at 42 hr p.i. in the infected STZ-diabetic liver (Fig. 5 and Table 2). On the other hand, in the STZ-diabetic spleen, a small number of immune response genes (*CXCL1*, *CXCL2*, *CXCL10*, *CASPASE7*, *OAS1g*, *OASL1* and *OASL2*) were mildly elevated at 42 hr p.i. (Fig. 5 and Table 2), including the 'common host immune response' genes (Fig. 8). The relative expression of selected differentially regulated host-cell genes was analysed by qRT-PCR on the same samples as those analysed by microarray analysis. The samples from both STZ-diabetic and normoglycaemic mice were verified by qRT-PCR as up-regulated or down-regulated, albeit with magnitudes different from those recorded by the microarray analysis (see Supplementary material, Data S2).

Discussion

Diabetes mellitus has a dramatic impact on health; its complications cause a high degree of morbidity and mortality[3] and it is an important predisposing factor for melioidosis.[6] The mechanism for the increased susceptibility of patients with DM to melioidosis still remains to be fully understood. In the present study, we addressed this using an animal model of DM, the first mouse model of

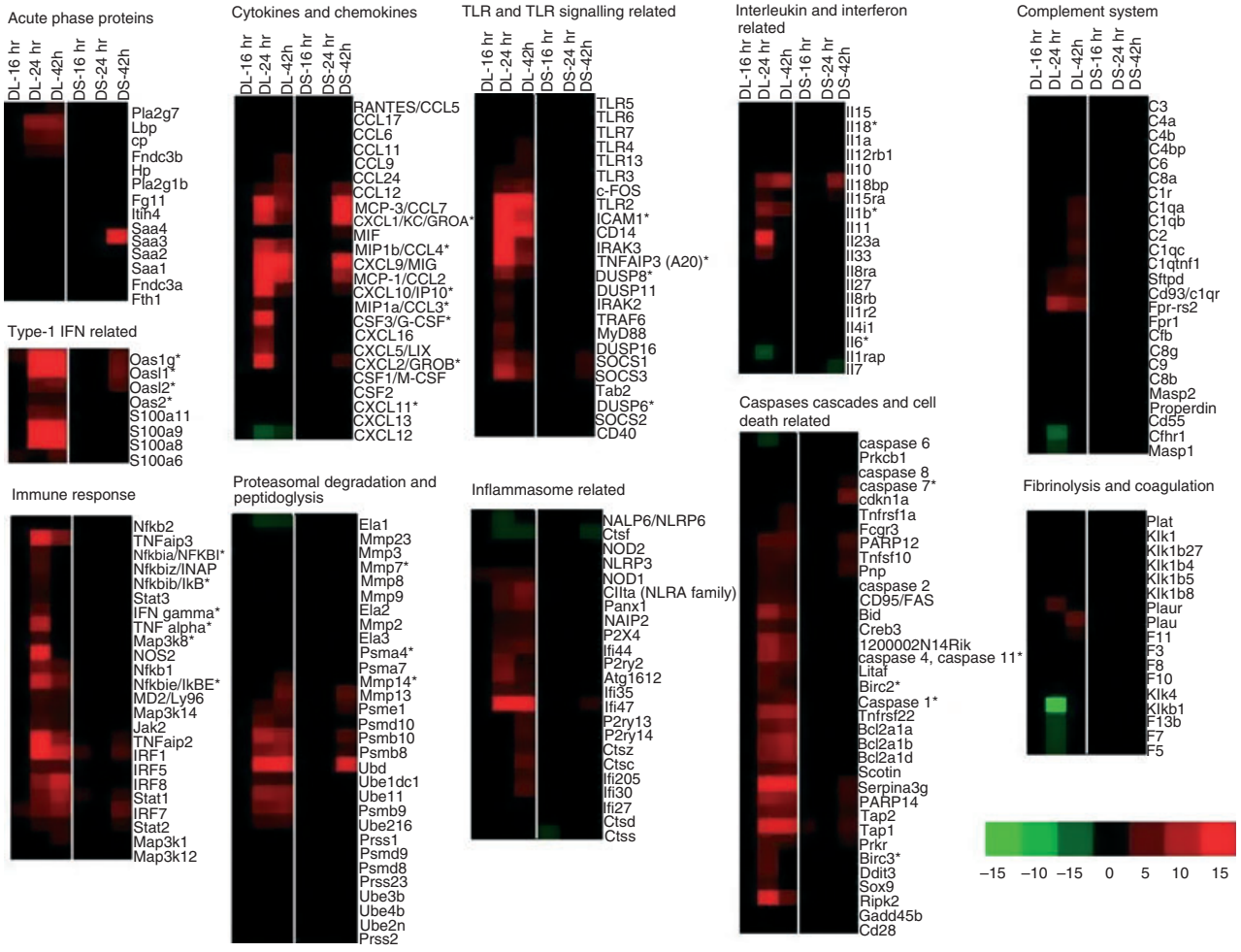

Figure 5. Transcriptional responses to acute *Burkholderia pseudomallei* infection in the mice with streptozotocin (STZ) -induced diabetes relative to uninfected diabetic mice. Hierarchical clustering of the expression profile of STZ-diabetic liver (DL) and STZ-diabetic spleen (DS) infected with *B. pseudomallei* at 16, 24 and 42 hr post-infection (p.i.) according to functional categories. The heat maps indicate the fold change in STZ-diabetic liver or STZ-diabetic spleen gene expression greater than (red) or less than (green) twofold at least once during the time course. Genes whose expression did not change are coloured in black. *Immune-related genes known to be associated with the general bacterial infection.

DM for acute melioidosis in understanding the STZ-diabetic host response to infection, globally.

Our study first described how blood glucose levels of the STZ-diabetic hosts were influenced by *B. pseudomallei* infection (Fig. 2). The STZ-diabetic mice continued to be hypoglycaemic (< 5 mmol/l) before succumbing to *B. pseudomallei* infection, suggesting that hypoglycaemia is associated with a prognosis of severe acute illness. In addition, high blood glucose levels (> 30 mmol/l) were associated with high mortality in *B. pseudomallei* infection (unpublished data). A recent cohort study by Peralta *et al.*[24] reported that alteration of the blood glucose concentration is associated with risk of death among patients with community-acquired gram-negative rod bacteraemia. Patients with blood glucose concentrations between 7·77 and 9·43 mmol/l appeared to have low mortality rates of 3·3%, whereas patients with blood glucose concentrations < 5·16 or > 12·06 mmol/l had the highest mortality (12·05%), suggesting a direct relationship between sepsis-related mortality with high or low blood glucose concentrations.[24] Hence, alteration of blood glucose levels is potentially detrimental, although the precise relationship with the eventual outcome in melioidosis patients has yet to be determined.

Some microorganisms become more virulent in a hyperglycaemic environment[4] and this could explain the increased susceptibility to infections in diabetic patients. Geerlings *et al.*[25] reported that glucosuria enhances the growth of different *Escherichia coli* strains, leading to increased incidence of urinary tract infections in diabetic patients. However, bacterial loads were not increased over the first 24 hr following *B. pseudomallei* infection in our STZ-diabetic model (Fig. 3a–c) when compared with the acute normoglycaemic model (see Supplementary material,

Table 2. Kinetic profiles of host genes expression modulated by *Burkholderia pseudomallei* infection in streptozotocin (STZ) -induced diabetes and normoglycaemic models for both liver and spleen

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
Toll-like receptor (TLR) and TLR-related													
TLR2	NM_011905	107	72	25	7	8	3	–	93	23	–	–	–
TLR3	NM_126166	3	2	3	–	–	–	–	2	2	–	–	–
TLR4	NM_021297	–	–	2	–	–	3	–	–	2	–	–	–
TLR5	NM_016928	−5	−2	−5	–	–	–	–	–	–	–	–	–
TLR6	NM_011604	–	–	–	–	3	–	–	–	–	–	–	–
TLR7	NM_133211	–	–	–	3	5	3	–	–	–	–	–	–
TLR13	NM_205820	–	–	4	–	2	4	–	–	3	–	–	–
c-FOS	NM_010234	5	4	3	7	8	3	–	4	6	–	–	–
ICAM1	NM_010493	28	45	18	5	3	3	–	51	12	–	–	–
TRAF6	NM_009424	4	3	–	–	–	–	–	2	–	–	–	–
IRAK2	NM_172161	3	–	–	–	–	–	–	4	–	–	–	–
IRAK3	NM_028679	30	39	28	6	8	–	–	30	6	–	–	–
DUSP11	NM_028099	2	2	–	–	–	–	–	2	–	–	–	–
DUSP16	NM_130447	2	2	2	5	5	2	–	4	–	–	–	–
DUSP6	NM_026268	−3	–	–	–	–	–	–	–	–	–	–	–
DUSP8	NM_008748	8	4	2	–	–	–	–	8	3	–	–	–
CD14	NM_009841	106	259	151	10	20	5	–	171	48	–	–	–
MyD88	NM_010851	8	4	3	4	3	3	–	5	–	–	–	–
CD40	NM_170704	3	–	–	–	2	–	–	–	–	–	–	–
Tab2	NM_138667	–	–	2	–	–	–	–	–	–	–	–	–
SOCS1	NM_009896	12	9	6	10	8	5	–	8	4	–	–	3
SOCS2	NM_007706	–	0	5	–	4	–	–	–	–	–	–	–
SOCS3	NM_007707	11	5	–	13	11	–	–	11	6	–	–	3
TNFAIP3 (A20)	NM_009397	92	63	31	–	–	–	–	61	8	–	–	–
Cyokines and chemokines													
MCP-1/CCL2	NM_011333	28	69	39	39	51	11	–	90	13	–	–	10
MIP1a/CCL3	NM_011337	3	5	–	39	48	12	–	4	–	–	–	–
MIP1b/CCL4	NM_013652	20	30	27	39	35	10	–	12	8	–	–	–
RANTES/CCL5	NM_013653	–	11	14	–	3	–	–	–	–	–	–	–
MCP-3/CCL7	NM_013654	17	11	12	88	107	40	–	15	7	–	–	19
CCL9	NM_011338	–	2	4	–	–	4	–	–	4	–	–	–
CCL12	NM_011331	3	4	13	18	25	27	–	3	6	–	–	6
CCL6	NM_009139	–	–	5	–	–	–	–	–	–	–	–	–
CCL24	NM_019577	–	–	4	–	–	–	–	–	4	–	–	–
CCL11	NM_011330	2	4	–	13	16	2	–	–	–	–	–	–
CCL17	NM_011332	–	–	–	3	6	–	–	–	–	–	–	–
CXCL1/KC/GROA	NM_008176	27	18	16	103	113	26	–	13	5	–	–	27
CXCL10/IP10	NM_021274	11	16	–	17	10	–	–	17	4	–	–	3
CXCL11	NM_019494	–	–	–	3	3	–	–	–	–	–	–	–
CXCL12	NM_013655	−3	−3	–	–	–	–	–	−4	−2	–	–	–
CXCL13	NM_018866	–	2	–	–	–	4	–	–	–	–	–	–
CXCL16	NM_023158	–	7	8	–	4	4	–	6	–	–	–	–
CXCL2/GROB	NM_009140	7	11	–	36	41	6	–	24	–	–	–	4
CXCL5/LIX	NM_009141	3	11	–	20	20	9	–	8	–	–	–	–
CXCL9/MIG	NM_008599	67	67	57	20	22	–	–	41	38	–	–	16
MIF	XM_147409	–	–	–	–	3	3	–	–	–	–	–	3
CSF1/M-CSF	NM_007778	2	3	5	2	2	–	–	–	–	–	–	–
CSF2	NM_009969	–	2	–	7	5	2	–	–	–	–	–	–
CSF3/G-CSF	NM_009971	9	15	–	29	62	–	–	24	–	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
Immune response													
MD2/Ly96	NM_016923	–	2	–	–	–	–	–	3	3	–	–	–
Nfkb1	NM_008689	4	4	3	–	–	–	–	7	4	–	–	–
Nfkb2	NM_019408	3	3	3	–	–	–	–	–	–	–	–	–
Nfkbia/NFKBI	NM_010907	6	5	–	–	6	–	–	4	–	–	–	–
Nfkbib/IkB	NM_010908	3	3	–	–	–	–	–	3	–	–	–	–
Nfkbie/IkBE	NM_008690	3	13	13	–	–	–	–	13	6	–	–	–
Nfkbiz/INAP	NM_030612	24	5	7	–	8	–	–	4	–	–	–	–
IFNg	NM_008337	6	4	–	148	112	10	–	4	–	–	–	–
TNFa	NM_013693	3	6	2	9	5	3	–	11	–	–	–	–
TNFaip2	NM_009396	8	17	6	–	–	–	–	31	7	–	–	2
TNFaip3	NM_009397	92	63	31	–	–	–	–	61	8	–	–	–
NOS2	NM_010927	3	11	3	–	3	–	–	27	–	–	–	–
IRF1	NM_008390	14	19	9	4	4	4	–	27	12	3	–	4
IRF5	NM_012057	–	3	2	–	–	–	–	4	4	–	–	–
IRF7	NM_016850	5	6	5	7	10	7	3	5	8	–	–	6
IRF8	NM_008320	8	6	6	–	–	–	–	8	13	–	–	–
Jak2	NM_008413	2	2	3	–	2	–	–	4	3	–	–	–
Stat1	NM_009283	11	6	9	4	5	5	–	6	10	2	–	3
Stat2	NM_019963	–	–	2	4	5	4	–	3	3	–	–	2
Stat3	NM_011486	6	3	3	3	3	3	–	2	–	–	–	–
Map3k1	NM_011945	3	2	5	–	–	–	–	–	3	–	–	–
Map3k12	NM_009582	–	–	–	−2	–	–	–	–	–	–	–	–
Map3k14	NM_016896	3	2	–	–	–	–	–	3	2	–	–	–
Map3k8	NM_007746	4	3	2	–	6	–	–	3	–	–	–	–
Interleukin and interleukin-related													
Il10	NM_010548	–	–	–	2	7	4	–	–	–	–	–	–
Il11	NM_008350	–	3	–	2	–	–	–	3	–	–	–	–
Il12rb1	NM_008353	–	–	–	3	4	–	–	–	–	–	–	–
Il15	NM_008357	–	–	–	5	4	2	–	–	–	–	–	–
Il15ra	NM_133836	–	–	–	4	7	4	–	3	–	–	–	2
Il18	NM_008360	–	−3	–	–	–	–	–	–	–	–	–	–
Il18bp	NM_010531	–	14	21	6	12	14	–	7	11	–	–	8
Il1a	NM_010554	–	–	–	8	9	–	–	–	–	–	–	–
Il1b	NM_008361	7	8	7	10	9	–	–	8	5	–	–	–
Il1r2	NM_010555	2	3	3	10	17	10	–	–	–	–	–	–
Il1rap	NM_008364	–	−6	−5	–	–	–	–	−4	–	–	–	–
Il23a	NM_031252	–	11	–	2	–	–	–	20	–	–	–	–
Il27	NM_145636	–	–	–	4	4	–	–	–	–	–	–	–
Il33	NM_133775	3	5	–	6	7	5	–	6	–	–	–	–
Il4i1	NM_010215	–	15	15	–	–	–	–	–	–	–	–	–
Il6	NM_031168	–	–	–	83	75	6	–	–	–	–	–	–
Il7	NM_008371	4	–	–	−3	−3	–	–	–	–	–	–	−2
Il8ra	NM_178241	–	–	–	2	2	5	–	–	–	–	–	–
Il8rb	NM_009909	–	–	–	3	5	5	–	–	–	–	–	–
Caspases cascades and cell death													
caspase 1	NM_009807	2	3	5	–	–	–	–	3	5	–	–	–
caspase 2	NM_007610	2	–	3	–	–	–	–	3	2	–	–	–
caspase 4, caspase 11	NM_007609	7	9	12	5	11	4	–	9	6	–	–	–
caspase 6	NM_009811	–	−4	–	−2	–	–	–	−3	–	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
caspase 7	NM_007611	–	2	–	–	2	2	–	–	–	–	–	2
caspase 8	NM_009812	–	–	–	–	2	–	–	–	–	–	–	–
PARP12	NM_172893	6	5	4	8	8	4	–	5	5	–	–	4
PARP14	NM_145481	13	10	8	6	6	4	–	9	8	–	–	3
Creb3	NM_013497	3	3	–	–	–	–	–	4	2	–	–	–
Prkcb1	NM_008855	–	–	2	–	–	–	–	–	–	–	–	–
Bid	NM_007544	4	8	4	–	–	–	–	10	5	–	–	–
Tnfrsf1a	NM_011609	–	–	–	–	2	3	–	–	3	–	–	–
CD95/FAS	NM_007987	–	4	4	4	5	–	–	3	3	–	–	–
Tnfrsf22	NM_023680	3	6	12	–	–	–	–	10	10	–	–	–
Tnfsf10	NM_009425	2	4	3	4	4	2	–	3	4	–	–	3
1200002N14Rik	NM_027878	10	22	23	4	5	5	–	9	6	–	–	–
Bcl2a1a	NM_009742	8	6	8	–	–	–	–	5	7	–	–	–
Bcl2a1b	NM_007534	11	9	12	–	–	–	–	8	10	–	–	–
Bcl2a1d	NM_007536	12	10	13	–	–	–	–	7	9	–	–	–
Birc2	NM_007465	5	5	6	–	–	–	–	5	2	–	–	–
Birc3	NM_007464	5	4	–	–	–	–	–	5	–	–	–	–
Cd28	NM_007642	–	–	6	–	–	–	–	–	–	–	–	–
Cdkn1a	NM_007669	29	12	12	11	13	5	–	–	–	–	–	7
Ddit3	NM_007837	–	5	6	–	–	–	–	5	–	–	–	–
Fcgr3	NM_010188	–	2	5	–	–	–	–	–	3	–	–	–
Gadd45b	NM_008655	4	4	2	9	9	4	–	–	–	–	–	–
Litaf	NM_019980	6	3	3	3	4	3	–	5	3	–	–	–
Pnp	NM_013632	4	3	3	5	5	3	–	3	2	–	–	3
Prkr	NM_011163	8	4	3	7	6	4	–	5	3	–	–	2
Ripk2	NM_138952	31	17	6	3	–	–	–	38	5	–	–	–
Scotin	NM_025858	3	5	5	–	–	–	–	4	5	–	–	–
Serpina3g	XM_354694	39	30	28	7	8	8	–	24	30	–	–	3
Sox9	NM_011448	6	4	19	–	–	–	–	3	–	–	–	–
Tap1	NM_013683	7	7	6	3	3	–	–	14	14	2	–	3
Tap2	NM_011530	6	7	8	–	–	–	–	6	6	–	–	2
Inflammasome-related													
NAIP2	NM_010872	–	2	4	–	–	–	–	2	2	–	–	–
NLRP3	NM_145827	–	–	–	2	–	–	–	–	–	–	–	–
CIIta (NLRA family)	NM_007575	–	4	4	–	–	–	–	4	7	–	–	–
NALP6/NLRP6	NM_133946	–	−4	−4	–	–	–	–	−2	–	–	–	–
NOD1	NM_172729	3	2	3	–	3	4	2	3	3	–	–	–
NOD2	NM_145857	–	–	–	3	3	2	–	–	–	–	–	–
P2X4	NM_011026	–	2	2	–	–	–	–	3	3	–	–	–
P2ry13	NM_028808	2	2	3	–	–	–	–	–	3	–	–	–
P2ry14	NM_133200	2	3	4	–	–	–	–	–	4	–	–	–
P2ry2	NM_008773	3	3	–	4	5	3	–	6	2	–	–	–
Panx1	NM_019482	–	6	6	–	–	–	–	4	6	–	–	–
Ctsz	NM_022325	–	–	–	–	–	3	–	–	3	–	–	–
Ctss	NM_021281	–	–	4	–	–	–	–	–	–	−2	–	–
Ctsc	NM_009982	–	–	5	–	–	3	–	–	5	–	–	–
Ctsf	NM_019861	–	−3	−3	–	–	–	–	−2	−2	–	–	−2
Ctsd	NM_009983	–	–	–	–	–	3	–	–	–	–	–	–
Atg16l2	XM_133655	6	6	5	–	–	–	–	7	3	–	–	–
Ifi205	NM_172648	4	2	3	3	3	–	–	–	3	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
Ifi27	NM_029803	–	2	5	–	2	3	–	–	–	–	–	–
Ifi30	NM_023065	–	–	4	–	–	–	–	–	4	–	–	–
Ifi35	NM_027320	3	3	–	–	4	2	–	3	3	–	–	–
Ifi44	NM_133871	5	5	4	3	–	2	–	4	5	–	–	–
Ifi47	NM_008330	28	18	15	–	–	–	–	31	20	–	–	3
Type 1 interferon (IFN) related													
Oas1g	NM_011852	8	22	10	–	9	6	2	16	17	–	–	6
Oas2	NM_145227	2	3	2	9	10	6	–	2	2	–	–	–
Oasl1	NM_145209	13	22	12	19	18	6	–	15	15	–	–	7
Oasl2	NM_011854	3	4	4	6	7	5	–	5	6	–	–	5
S100a9	NM_009114	27	31	55	–	–	–	–	31	24	–	–	–
S100a8	NM_013650	26	37	60	–	–	–	–	33	27	–	–	–
S100a6	NM_011313	–	–	6	–	–	–	2	–	4	–	–	–
S100a11	NM_016740	–	5	5	–	–	2	–	5	5	–	–	–
Acute-phase proteins													
Fgl1	NM_145594	3	3	3	–	–	–	–	–	–	–	–	–
Itih4	NM_018746	–	2	–	–	–	–	–	–	–	–	–	–
Cp	NM_007752	5	4	6	2	3	4	–	5	6	–	–	–
Hp	NM_017370	–	5	5	–	7	5	–	–	–	–	–	–
Pla2g7	NM_013737	–	2	3	–	–	–	–	–	2	–	–	–
Pla2g1b	NM_011107	–	–	–	25	29	–	–	–	–	–	–	–
Lbp	NM_008489	7	6	8	–	–	–	–	8	8	–	–	–
Saa1	NM_009117	13	13	13	–	–	–	–	–	–	–	–	–
Saa2	NM_011314	92	86	74	–	–	–	–	–	–	–	–	–
Saa3	NM_011315	124	121	112	36	91	94	–	–	–	–	–	56
Saa4	NM_011316	3	2	–	–	–	–	–	–	–	–	–	–
Fndc3b	NM_173182	6	3	5	–	3	–	–	2	3	–	–	–
Fndc3a	NM_207636	–	–	–	3	3	–	–	–	–	–	–	–
Fth1	NM_010239	–	–	–	–	2	2	–	–	–	–	–	–
Proteasomal degradation and peptidoglysis													
Mmp13	NM_008607	3	–	6	49	26	10	–	–	6	–	–	6
Mmp14	NM_008608	–	–	6	2	4	3	–	–	3	–	–	–
Mmp2	NM_008610	−2	–	–	−4	−3	–	–	–	–	–	–	–
Mmp23	NM_011985	–	–	–	−3	−3	–	–	–	–	–	–	–
Mmp3	NM_010809	–	–	–	8	41	35	–	–	–	–	–	–
Mmp7	NM_010810	–	2	3	–	–	–	–	–	–	–	–	–
Mmp8	NM_008611	–	–	3	3	10	13	–	–	–	–	–	–
Mmp9	NM_013599	–	2	4	–	3	5	–	–	–	–	–	–
Ela2	NM_007919	–	–	–	20	21	–	–	–	–	–	–	–
Ela1	NM_033612	−7	−12	–	14	14	–	–	−2	−2	–	–	–
Ela3	NM_026419	–	–	–	34	57	–	–	–	–	–	–	–
Ubd	NM_023137	137	192	203	28	33	30	–	262	218	–	–	14
Psma4	NM_011966	–	–	–	–	2	2	–	–	–	–	–	–
Psma7	NM_011969	–	–	–	–	3	2	–	–	–	–	–	–
Psmb10	NM_013640	7	10	7	3	4	2	–	11	9	–	–	5
Psmb8	NM_010724	4	9	8	–	–	–	–	7	8	–	–	2
Psmb9	NM_013585	7	7	8	–	–	–	–	6	7	–	–	–
Psmd10	NM_016883	4	5	5	–	–	–	–	5	3	–	–	–
Psmd8	NM_026545	–	2	2	–	–	–	–	–	–	–	–	–
Psmd9	NM_026000	–	−2	–	–	–	–	–	–	–	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
Psme1	NM_011189	–	4	3	–	3	3	–	3	4	–	–	3
Prss1	NM_053243	–	–	–	45	67	–	–	–	–	–	–	–
Prss2	NM_009430	–	–	–	21	28	–	–	–	–	–	–	–
Prss23	NM_029614	–	–	–	–	3	2	–	–	–	–	–	–
Ube1dc1	NM_025692	2	2	–	–	–	–	–	3	3	–	–	–
Ube1l	NM_023738	6	11	8	3	3	2	–	9	8	–	–	–
Ube2l6	NM_019949	–	3	3	–	–	–	–	3	3	–	–	–
Ube2n	NM_080560	–	−2	–	–	–	–	–	–	–	–	–	–
Ube3b	NM_054093	–	−2	–	–	–	–	–	–	–	–	–	–
Ube4b	NM_022022	2	–	–	–	–	–	–	–	–	–	–	–
Complement system													
C1r	NM_023143	–	–	–	–	2	–	–	–	–	–	–	–
C1qa	NM_007572	–	–	–	–	–	3	–	–	3	–	–	–
C1qb	NM_009777	–	–	–	–	–	3	–	–	4	–	–	–
C1qc	NM_007574	–	–	–	–	–	3	–	–	4	–	–	–
C1qtnf1	NM_019959	–	–	–	–	–	2	–	–	2	–	–	–
C2	NM_013484	–	2	2	–	4	5	–	–	2	–	–	–
C3	NM_009778	–	–	–	–	2	3	–	–	–	–	–	–
C4a	NM_011413	–	–	–	–	–	3	–	–	–	–	–	–
C4b	NM_009780	–	–	–	–	–	3	–	–	–	–	–	–
C4bp	NM_007576	–	–	2	–	–	–	–	–	–	–	–	–
C6	NM_016704	–	–	−4	–	–	–	–	–	–	–	–	–
C8a	NM_146148	–	−3	–	–	–	–	–	–	–	–	–	–
C8b	NM_133882	–	−3	−6	–	–	–	–	–	–	–	–	–
C9	NM_013485	–	−3	−4	–	–	–	–	–	–	–	–	–
C8g	XM_130127	–	−2	−4	–	–	–	–	–	–	–	–	–
Cfb	NM_008198	–	–	–	6	9	9	–	–	–	–	–	–
Cfhr1	NM_015780	–	−3	−2	–	–	–	–	−6	–	–	–	–
Masp1	NM_008555	–	−4	−3	–	–	–	–	−3	–	–	–	–
Masp2	XM_358353	–	−2	–	–	–	–	–	–	–	–	–	–
Properdin	XM_135820	–	–	2	–	–	–	–	–	–	–	–	–
Cd55	NM_010016	–	–	3	–	–	–	–	–	–	–	–	–
Cd93/c1qr	NM_010740	–	2	4	–	4	3	–	3	3	–	–	–
Sftpd	NM_009160	2	3	9	–	–	2	–	3	4	–	–	–
Fpr-rs2	NM_008039	13	12	17	7	10	10	–	10	7	–	–	–
Fpr1	NM_013521	–	–	2	–	6	5	–	–	–	–	–	–
Fibrinolysis and coagulation													
Plau	NM_008873	–	–	–	–	–	5	–	–	6	–	–	–
Plaur	NM_011113	6	3	–	12	9	4	–	4	–	–	–	–
Plat	NM_008872	–	–	–	3	9	3	–	–	–	–	–	–
Klkb1	NM_008455	−3	−28	−5	–	–	–	–	−15	–	–	–	–
Klk1	NM_010639	–	–	–	–	27	–	–	–	–	–	–	–
Klk1b27	NM_020268	–	–	–	9	22	–	–	–	–	–	–	–
Klk1b4	NM_010915	–	–	−3	10	21	–	–	–	–	–	–	–
Klk1b5	NM_008456	–	–	–	14	26	–	–	–	–	–	–	–
Klk1b8	NM_008457	–	–	–	–	3	–	–	–	–	–	–	–
Klk4	NM_019928	–	–	–	–	3	–	–	–	–	–	–	–
F10	NM_007972	–	−2	–	3	6	4	–	–	–	–	–	–
F11	NM_028066	–	–	2	–	–	–	–	–	2	–	–	–
F13b	NM_031164	–	−7	−4	–	–	–	–	−3	–	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
F3	NM_010171	–	−2	–	6	5	–	–	–	–	–	–	–
F5	NM_007976	–	−3	–	–	–	–	–	−3	–	–	–	–
F7	NM_010172	–	−2	–	–	–	–	–	−3	–	–	–	–
F8	NM_007977	–	−2	–	–	–	–	–	–	–	–	–	–
Oxidative and anti-oxidative													
Gpx2	NM_030677	–	–	6	–	–	–	–	–	3	–	–	–
Gpx3	NM_008161	–	2	4	–	–	5	–	3	–	–	–	–
Gpx7	NM_024198	–	–	2	–	–	–	–	–	2	–	–	–
Gsta4	NM_010357	–	−3	−2	–	–	–	–	–	–	–	–	–
Gstm1	NM_010358	–	−3	–	–	–	–	–	−2	–	–	–	–
Gstm2	NM_008183	–	–	–	–	–	2	–	–	–	–	–	–
Gstm3	NM_010359	–	−2	–	–	–	–	–	−2	–	–	–	–
Gstm4	NM_026764	−3	−5	−3	–	–	–	–	−4	–	–	–	–
Gstm6	NM_008184	–	−4	−2	–	–	–	–	−3	–	–	–	–
Gstm7	XM_359308	–	−5	–	–	–	–	–	−3	–	–	–	–
Gsto1	NM_010362	–	−3	–	–	–	–	–	−2	–	–	–	–
Gstt1	NM_008185	–	−5	−6	–	–	–	–	–	−3	–	–	–
Gstt2	NM_010361	−2	−3	–	–	–	–	–	–	–	–	–	–
Gstt3	NM_133994	–	−6	−2	–	–	–	–	–	–	–	–	–
Gstz1	NM_010363	–	−3	–	–	–	–	–	−2	–	–	–	–
Mgst3	NM_025569	–	−3	−3	–	−11	–	–	–	–	–	–	–
Gsr	NM_010344	–	–	–	–	–	2	–	–	–	–	–	–
Sod2	NM_013671	–	2	–	5	6	4	–	2	–	–	–	–
Tgm2	NM_009373	6	7	7	3	5	5	–	9	9	–	–	5
Noxo1	NM_027988	–	13	6	–	–	–	–	6	#N/A	–	–	–
Vnn3	NM_011979	13	25	10	2	–	–	–	27	11	–	–	–
Glycolysis/gluconeogenesis													
Hk2	NM_013820	7	4	6	5	6	4	–	6	4	–	–	–
Hk3	NM_001033245	3	3	4	–	4	6	–	4	7	–	–	–
Khk	NM_008439	−2	–	−9	–	−2	–	–	–	–	–	–	–
Pfkl	NM_008826	–	–	–	–	3	–	–	–	–	–	–	–
Pfkp	NM_019703	–	–	–	–	3	3	–	–	3	–	–	–
Pgk1	NM_008828	–	–	–	–	4	3	–	–	–	–	–	–
Pfkm	NM_021514	−4	−4	−3	–	–	–	–	−3	–	–	–	–
Pgam1	NM_023418	–	−2	–	–	–	–	–	–	–	–	–	–
Eno1	NM_023119	–	−2	–	–	–	–	2	–	–	–	–	–
Eno2	NM_013509	–	–	–	–	3	–	–	–	–	–	–	–
Aldoa	NM_007438	−4	−3	−3	–	–	3	–	–	–	–	–	–
Aldoc	NM_009657	−2	−4	−5	–	–	–	–	–	–	–	–	–
Gapdh	NM_008084	–	–	–	–	2	–	–	–	–	–	–	2
Pkm2	NM_011099	–	5	7	–	2	3	–	6	11	–	–	–
Ldha	NM_010699	–	–	–	–	2	–	–	–	–	–	–	2
Pdk4	NM_013743	–	–	–	–	3	–	–	–	–	–	–	–
Pdhb	NM_024221	–	−2	−3	–	–	–	–	–	–	–	–	–
Pdk1	NM_172665	−3	−4	−3	–	–	–	–	−4	–	–	–	–
Pdk3	NM_145630	–	–	2	–	–	–	–	2	2	–	–	–
Pdk2	NM_133667	−2	−3	–	–	–	–	–	−3	–	–	–	–
Glycogen breakdown													
Pygl	NM_133198	–	−10	−4	–	–	3	–	–	–	–	–	–
Pgm2	NM_028132	–	−2	–	–	4	–	–	–	–	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
Glycogen synthesis													
Ugp2	NM_139297	−4	−7	−3	–	–	–	–	−6	–	–	–	–
Ugt2b1	NM_152811	–	−9	−41	–	–	–	–	−5	–	–	–	–
Ugt2b34	NM_153598	–	−6	−2	–	–	–	–	−6	–	–	–	–
Ugt3a2	NM_144845	–	−6	−10	–	–	–	–	−3	–	–	–	–
Ugt1a7c	NM_201410	–	–	–	−2	–	–	–	−4	–	–	–	–
Tricarboxylic acid cycle													
Aco1	NM_007386	–	−3	–	–	–	–	–	−3	–	–	–	–
Idh1	NM_010497	–	–	−3	–	–	–	–	−3	–	–	–	–
Idh3b	NM_130884	–	−2	−2	–	–	–	–	–	–	–	–	–
Idh3a	NM_029573	−2	–	–	–	–	–	–	–	–	–	–	–
Sdhb	NM_023374	–	−3	−4	–	–	–	–	–	−2	–	–	–
Sdhc	NM_025321	–	−2	–	–	–	–	–	−2	–	–	–	–
Sdhd	NM_025848	–	−3	–	–	–	–	–	−3	–	–	–	–
Mdh1	NM_008618	–	−4	−3	–	–	–	–	−3	–	–	–	–
Fatty acid metabolism													
Ehhadh	NM_023737	–	−6	–	–	–	–	–	–	–	–	–	–
Hadh	NM_008212	–	−3	−2	–	–	–	–	−2	–	–	–	–
Acat1	NM_144784	–	−6	−7	–	–	–	–	−3	−4	–	–	–
Acat2	NM_009338	–	−2	−3	–	–	–	–	–	–	–	–	–
Acat3	NM_153151	–	–	2	–	–	–	–	–	–	–	–	–
Valine, leucine and Isoleucine degradation													
Hmgcs1	NM_145942	–	–	−2	–	–	–	–	–	–	–	–	–
Hmgcs2	NM_008256	–	−7	–	−3	–	–	–	–	–	–	–	–
Aldh1a1	NM_013467	–	−7	−2	–	–	–	–	−4	–	–	–	–
Aldh1a7	NM_011921	–	−2	–	–	–	–	–	–	–	–	–	–
Aldh1b1	NM_028270	−3	−9	–	–	–	–	–	−5	–	–	–	–
Aldh1l1	NM_027406	–	−3	−4	–	–	–	–	–	–	–	–	–
Aldh3b1	NM_026316	–	3	5	–	–	–	–	–	3	–	–	–
Aldh4a1	NM_175438	−2	−14	−6	–	–	–	–	−5	–	–	–	–
Aldh5a1	NM_172532	–	−2	–	–	–	–	–	–	–	–	–	–
Aldh6a1	NM_134042	–	−3	–	–	–	–	–	−2	–	–	–	–
Aldh8a1	NM_178713	−5	−8	–	–	–	–	–	−3	–	–	–	–
Cytochrome *b*													
Cyp17a1	NM_007809	–	−3	–	–	–	–	–	–	–	–	–	–
Cyp1b1	NM_009994	–	–	–	–	5	5	–	3	–	–	–	–
Cyp1a2	NM_009993	−3	−11	−88	–	–	–	–	−7	−14	–	–	–
Cyp27a1	NM_024264	–	−2	−5	−2	−2	–	–	–	–	–	–	–
Cyp2a12	NM_133657	–	−3	–	–	–	–	–	–	–	–	–	–
Cyp2a4	NM_009997	−4	−5	−3	–	–	–	–	−6	–	–	–	–
Cyp2a5	NM_007812	−8	–	−10	–	–	–	–	−8	−4	–	–	–
Cyp2b10	NM_009999	–	–	2	–	–	–	–	–	–	–	–	–
Cyp2c29	NM_007815	–	−6	−49	–	–	–	–	−3	–	–	–	–
Cyp2c37	NM_010001	–	−8	−112	–	–	–	–	−4	–	–	–	–
Cyp2c39	NM_010003	–	−9	–	–	–	–	–	–	–	–	–	–
Cyp2c50	NM_134144	–	−11	−22	–	–	–	–	−3	−3	–	–	–
Cyp2c54	NM_206537	–	−4	−5	–	–	–	–	−5	−10	–	–	–
Cyp2c55	NM_028089	–	–	−3	–	–	–	–	–	–	–	–	–
Cyp2d10	NM_010005	–	−3	−3	–	–	–	–	–	–	–	–	–
Cyp2d13	NM_133695	−3	−5	−6	–	–	–	–	−3	–	–	–	–

Table 2. (*Continued*)

		Fold change in transcript at hour post-infection[1]											
		Normoglycaemic[2]						STZ-diabetic					
		Liver			Spleen			Liver			Spleen		
Gene symbol	GenBank	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr	16 hr	24 hr	42 hr
Cyp2d22	NM_019823	–	−4	−3	–	–	2	–	−2	−3	–	–	–
Cyp2d26	NM_029562	–	−4	−2	–	–	–	–	−3	–	–	–	–
Cyp2d9	NM_010006	–	−3	−3	–	–	–	–	–	–	–	–	–
Cyp2e1	NM_021282	–	−3	−12	–	–	–	–	−2	–	–	–	–
Cyp2f2	NM_007817	–	−6	−11	–	–	–	–	–	–	–	–	–
Cyp2g1	NM_013809	−3	−3	−3	–	–	–	–	−3	–	–	–	–
Cyp2j9	NM_028979	–	−3	–	–	–	–	–	−3	–	–	–	–
Cyp3a11	NM_007818	–	−6	−5	–	–	–	–	−3	–	–	–	–
Cyp3a25	NM_019792	–	−7	−3	–	–	–	–	−3	–	–	–	–
Cyp4a10	NM_010011	–	−7	–	–	–	–	–	–	–	–	–	–
Cyp4a12	NM_177406	–	−2	−2	–	–	–	–	–	–	–	–	–
Cyp4a12b	NM_172306	–	−4	−4	–	–	–	–	–	–	–	–	–
Cyp4b1	NM_007823	−2	–	–	–	−3	–	–	–	–	–	–	–
Cyp4f14	NM_022434	−2	−12	−50	–	–	–	–	−4	–	–	–	–
Cyp4f15	NM_134127	−5	−14	−4	–	–	–	–	−12	−4	–	–	–
Cyp4v3	NM_133969	−3	−6	−3	–	–	2	–	−3	−3	–	–	–
Cyp51	NM_020010	–	–	−3	–	–	–	–	–	–	–	–	–
Cyp7a1	NM_007824	−176	−228	−211	–	–	–	–	−98	−39	–	–	–
Cyp7b1	NM_007825	–	–	–	–	3	4	–	–	–	–	–	–
Cyp8b1	NM_010012	–	−154	−137	–	–	–	–	−68	–	–	–	–
Miscellaneous													
Faah	NM_010173	−3	−36	−5	–	–	–	–	−10	–	–	–	–
Car1	NM_009799	−2	−4	−5	–	–	−6	−2	−6	−7	–	–	–
Car13	NM_024495	4	7	8	5	8	4	–	4	3	–	–	–
Car14	NM_011797	−4	−13	−10	–	–	–	–	−3	−4	–	–	–
Car2	NM_009801	–	–	–	–	–	−11	–	–	–	–	–	–
Car3	NM_007606	–	−6	−263	–	–	–	–	−4	−34	–	–	–
Car4	NM_007607	2	12	–	–	5	2	–	–	–	–	–	–
Car5a	NM_007608	−7	−26	−11	–	–	–	–	−19	–	–	–	–
Akr1b3	NM_009658	–	–	3	–	–	–	–	–	–	–	–	–
Akr1b7	NM_009731	–	–	4	–	–	–	–	–	–	–	–	–
Akr1c14	NM_134072	−8	−23	−12	–	–	–	–	−26	–	–	–	–
Akr1c19	NM_001013785	–	−5	−8	–	–	–	–	−7	−6	–	–	–
Akr1c6	NM_030611	–	−17	−24	–	–	–	–	−5	–	–	–	–
Akr1e1	NM_018859	–	−3	–	–	–	–	–	−2	–	–	–	–
Akr7a5	NM_025337	–	−4	−2	–	–	–	–	–	–	–	–	–
Ddc	NM_016672	−14	−31	−4	–	–	–	–	−13	–	–	–	–
Apoa2	NM_013474	−3	−6	–	–	–	–	–	–	–	–	–	–
Apoa5	NM_080434	–	−24	–	–	–	–	–	−11	–	–	–	–
Aqp1	NM_007472	−3	–	–	–	−4	−12	–	–	–	–	–	–
Aqp11	NM_175105	−5	−5	−2	–	–	–	–	−3	−2	–	–	–
Aqp4	NM_009700	–	−3	−2	–	–	–	–	−2	–	–	–	–
Aqp8	NM_007474	−6	–	−6	–	–	–	–	–	–	–	–	–
Aqp9	NM_022026	−5	−24	−4	–	–	−4	–	−8	–	–	–	–
Arg1	NM_007482	–	−5	–	–	3	5	–	−4	–	–	–	–
Arg2	NM_009705	–	19	11	4	6	3	–	12	–	–	–	–

[1]Modulated transcripts are classified according to functional groups. Only genes with a more than twofold change in *B. pseudomallei*-infected liver or spleen versus uninfected control tissue are shown; '–', no significant change in gene expression.

[2]Data adopted from our recent work on genome-wide expression profiling of a murine acute melioidosis model.[18]

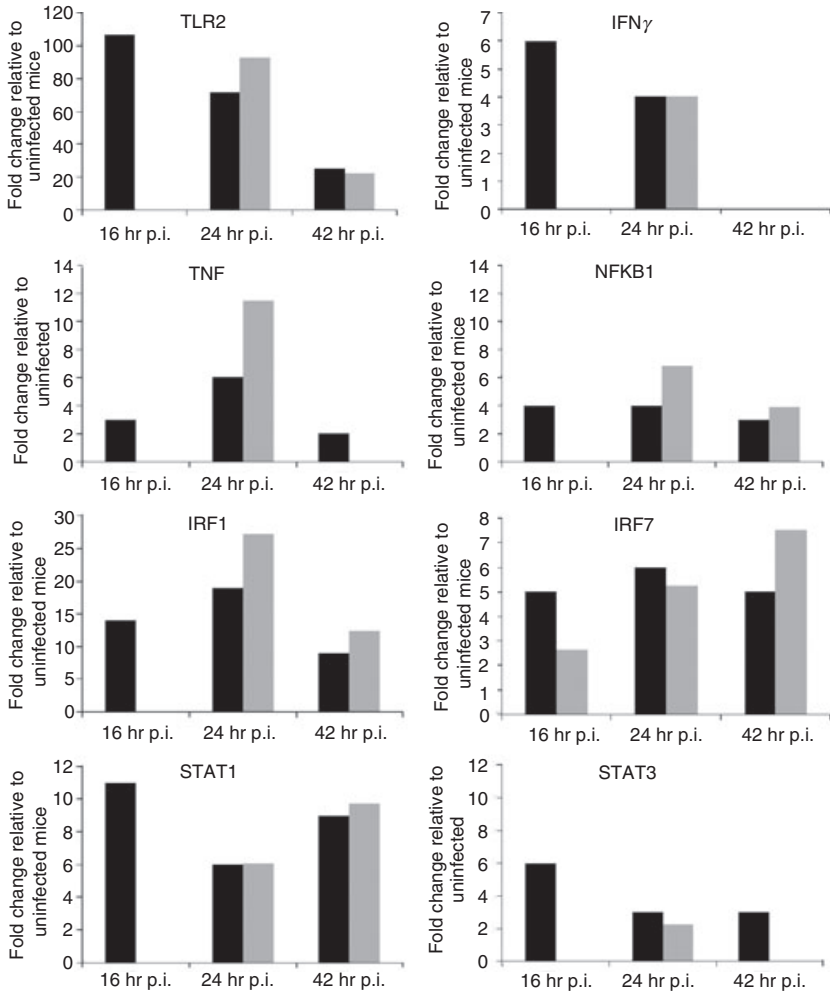

Figure 6. Expression profiles of Toll-like receptors and transcription factors. Toll-like receptor 2 and various transcription factors expression profiles (fold change relative to uninfected mice) during acute melioidosis infection in liver for both the streptozotocin (STZ) -induced diabetes (grey bars) and normoglycaemic infection models (black bars), respectively. Data for the acute normoglycaemic infection model is adopted from Chin *et al.*[18]

Data S1). As there was no correlation between blood glucose levels and bacterial replication, the hyperglycaemic environment most likely does not promote *B. pseudomallei* growth at this early phase of infection. Martens *et al.*[17] reported that hyperglycaemia *per se* does not directly promote *Mycobacterium tuberculosis* growth in the STZ-diabetic mouse with acute tuberculosis. Nevertheless, the effect of hyperglycaemia on cellular and metabolic functions of intracellular *B. pseudomallei* remains to be elucidated.

During bacterial infections, these pathogens intimately engage the defence response by inducing various inflammatory responses; this observation is also true for an acute melioidosis infection. Surprisingly, this genome-wide study clearly demonstrated that the STZ-diabetic host does not initiate the innate immune system at the early onset of infection as a means to eliminate *B. pseudomallei*. Nevertheless, this transcriptional study on the infected STZ-diabetic host is consistent with previous *B. pseudomallei*-infected *in vivo* and *in vitro* studies.[26,27] This transcriptional analysis strongly suggests that TLR2 is also responsible for the initiation of the STZ-diabetic host defence response to *B. pseudomallei* infection as previously seen in the acute normoglycaemic model.[18]

Very few immune response genes were modulated in the STZ-diabetic spleen (Figs 5 and 8), indicating a possible malfunction of the STZ-diabetic spleen with a limited ability to respond to *B. pseudomallei* infection over a 42-hr period of infection. Dysfunction of the STZ-diabetic spleen correlates with uncontrolled spread of intracellular bacteria in multiple organs (Fig. 3a–c) and ultimately, increased the presentation of susceptibility to infection in diabetics. In addition, several 'common host immune response' genes [*IL6*, *IL18*, *CXCL11*, matrix metallopeptidase 7 (*MMP7*), proteosome (prosome, macropain) subunit alpha (*PSMA4*) and *DUSP6*] were not modulated in this study. Among these, *IL6* is the chief stimulator of most acute-phase proteins (APPs)[28] and we noted that production of APPs was strongly induced in the acute normoglycaemic mice infected with *B. pseudomallei*.[18] These proteins are believed to be the cause of the severe tissue damage commonly seen in acute melioidosis as a result of an overwhelmed inflammatory response.[18] The expression profiles demonstrate delayed

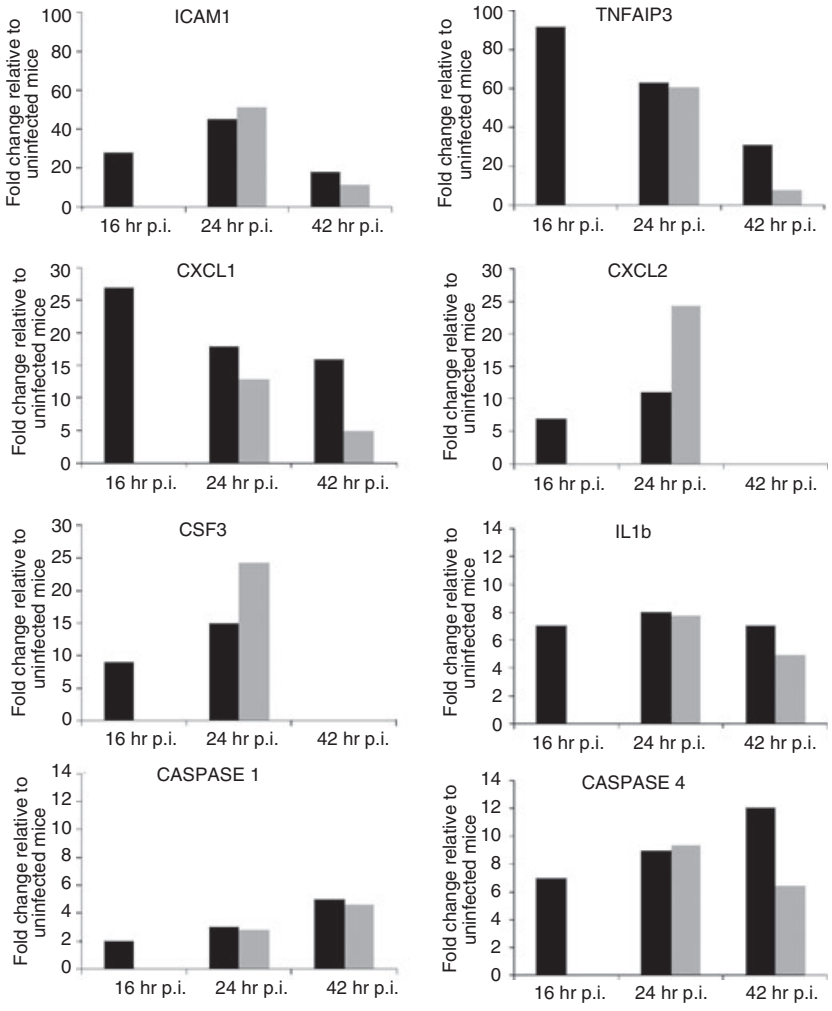

Figure 7. Expression profiles of 'core immune response' genes in the *Burkholderia pseudomallei*-infected liver. Several 'common core immune response' gene expression profiles (fold change relative to uninfected mice) during acute melioidosis infection in liver for the streptozotocin (STZ) -induced diabetes (grey bars) and normoglycaemic infection models (black bars), respectively. Data for the acute normoglycaemic infection model is adopted from Chin *et al.*[18]

activation of appropriate immune response-related genes at the early stage of infection, as well as suppression of potent inflammation-related genes (Fig. 4), contributing to intracellular bacterial propagation and dissemination (Fig. 3).

Acute forms of melioidosis that lead to sepsis, multiple organ failure and death are thought to result from an uncontrolled inflammatory reaction that ultimately may lead to excessive inflammation[29] and eventually tissue injury in the *B. pseudomallei*-infected host. Recently, Koh *et al.*[30] reported reduced mortality in diabetic patients with melioidosis who were treated with glyburide, a drug prescribed for diabetes that acts via an anti-inflammatory route. These findings support our data demonstrating an overwhelmed inflammatory response that is contributing to increased mortality in acute melioidosis. Acute inflammation studies have focused on the liver, the centre of the acute-phase response and the major target site for pro-inflammatory cytokines.[31] The APPs are commonly used as an early indicator to diagnose occurrence of inflammation and disease.[31] They are important in providing protective functions at sites of tissue injury,[28] neutralizing the pathogens, preventing further pathogen entry while minimizing tissue damage and promoting repair processes. This then permits host homeostatic mechanisms to rapidly restore normal physiological functions.[32] We previously reported that prolonged expression of APP may lead to tissue injury, as numerous APPs [ceruloplasmin (CP), lipopolysaccharide binding protein (LBP), haptoglobin, platelet-activating factor acetylhydrolase, serum amyloid A (SAA) and fibronectin type III domain containing 3B (FNDC3B)] were induced in response to the *B. pseudomallei* acute infection in normoglycaemic mice. Both SAA2 and SAA3 were highly induced throughout the infection period.[18] However, in this study, only CP, LBP and FNDC3B were elevated 24 hr p.i. with expression levels similar to that in normoglycaemic infected mice (Fig. 5 and Table 2). This suggests that CP, LBP and FNDC3B are specific signatures of an acute *B. pseudomallei* infection regardless of host metabolism. Expression profiles of these APP genes, the acute-phase responses factor genes (*STAT3* and *IL6*) as well as proteasomal degra-

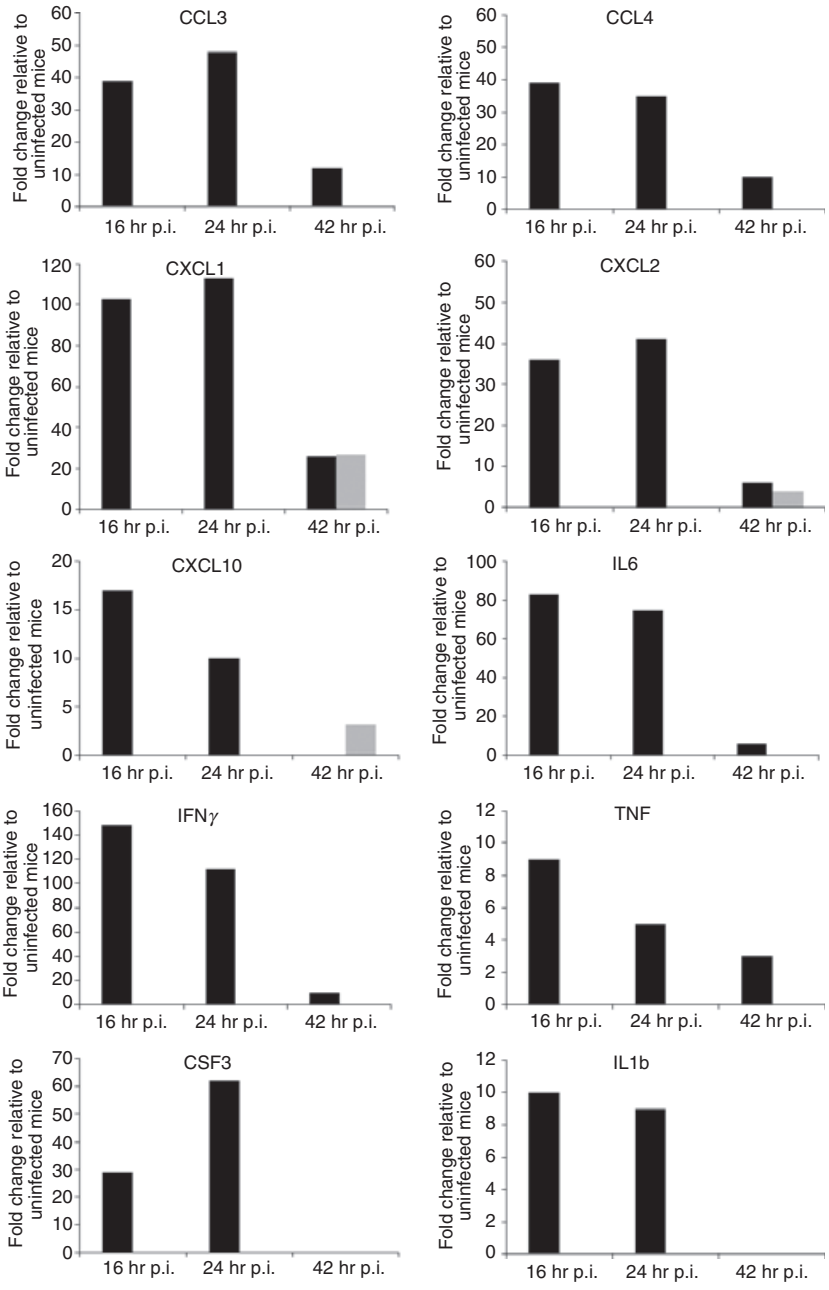

Figure 8. Expression profiles of 'core immune response' genes in the *Burkholderia pseudomallei*-infected spleen. Several 'common core immune response' gene expression profiles (fold change relative to uninfected mice) during acute melioidosis infection in spleen for the streptozotocin (STZ) -diabetic (grey bars) and normoglycaemic infection models (black bars), respectively. Data for the acute normoglycaemic infection model is adopted from Chin *et al.*[18]

dation and proteolysis-related genes suggest that the *B. pseudomallei*-infected STZ-diabetic host does not encounter consequences attributed by an overwhelming inflammatory response as noted in the normoglycaemic infection model.

Neutrophils are the host frontline defence system, essential in initiating the responses to pathogens and orchestrating later immune system by releasing cytokines and chemokines, which attract other cells to the site of infection.[33] The reduction in bactericidal activity, impaired phagocytosis, reduced production of reactive oxygen species and decreased release of lysosomal enzymes contribute to the high susceptibility to and severity of infections in DM.[34] Previously, diabetic PMNs exhibited impaired *B. pseudomallei* phagocytosis and reduced migration in response to IL8, a major chemokine responsible for this function.[12] In this study, CXCL1, CXCL2 and CSF3, which play an important role in neutrophil migration and mobilization were elevated 24 hr p.i. in the diabetic host (Figs 7 and 8). These expression profiles were consistent with increased production of neutrophils 24 hr p.i. (Fig. 3d) in the *B. pseudomallei*-infected STZ-diabetic mice. Zinc deficiency disturbs the lymphocyte response and impaired chemotaxis of diabetic PMN and patients with type 1 and type 2 DM are known to have low plasma zinc levels.[4,35] This may explain the

static leucocyte counts in the diabetic mice following *B. pseudomallei* acute infection at the early stage (16 hr p.i.) (Fig. 3d). These results revealed that the STZ-diabetic host fails to activate potent chemokines in time, leading to delayed PMN infiltration, which is pivotal for removing intracellular pathogens by phagocytosis, and eventually favours bacterial survival at the later stage of infection (Fig. 3). In addition, neutrophil counts for *B. pseudomallei*-infected STZ-diabetic mice were lower when compared with infected normoglycaemic mice, particularly at 42 hr p.i. (Fig. 3d), suggesting that inadequate neutrophil production increases the severity of infection. Taken together, the expression profiles suggest that elevated glucose levels impair the STZ-diabetic host innate immune system by delaying the identification and recognition of the *B. pseudomallei* surface structure. Consequently, the host is unable to activate the appropriate innate immune response over time, hence, increasing susceptibility to melioidosis in an STZ-diabetic host.

Cytokine expression as a result of an infection in diabetics has been the subject of considerable controversy; some reports indicated diminished inflammatory cytokine expression, while others reported enhanced expression upon bacterial infections.[3,4,13] Our study on diabetes and *B. pseudomallei* infection suggests an impaired immune response at an early stage, similar to a report on reduced interleukin-17 expression in PMN of diabetics infected with *B. pseudomallei* and *B. thailandensis*.[11] Furthermore, Williams *et al.*[36] recently reported that diabetic mice with an extended period of uncontrolled hyperglycaemia (chronic diabetic) have impaired innate immune responses to *B. pseudomallei*. The decreased expression of *IL12* and *IL18* by bone marrow-derived dendritic cells isolated from chronic diabetic compared with non-diabetic-derived bone marrow-derived dendritic cells suggested inadequate stimulation of T helper type 1 protective responses during a *B. pseudomallei* infection.[36] Yamashiro *et al.*[13] reported that production of inducible nitric oxide synthase (iNOS), IL-12 and IFN-γ were lower in diabetic mice in response to *Mycobacterium tuberculosis* infection when compared with normal mice. However, in this study, expression of iNOS was highly induced (27-fold change) at 24 hr p.i. in the STZ-diabetic liver when compared with the normoglycaemic infected mice (11-fold change) at the same time-point (Table 2).[18] In contrast, IFN-γ production was completely attenuated in the *B. pseudomallei*-infected STZ-diabetic spleen (Fig. 8) when compared with the infected-normoglycaemic mice, whereas the expression of *IL12* was not elevated in either acute diabetic or acute normoglycaemic studies.

The complement system of the vertebrate host forms a powerful immune barrier for invading microbes, and many pathogens have used multiple evasion strategies to interfere with and to inactivate the complement attack.[37] Our previous work also described for the first time that suboptimal activation and function of the downstream complement system promotes uncontrolled spread of *B. pseudomallei*.[18] In this diabetes study, the complement-related genes were not modulated over the course of infection (Fig. 5 and Table 2), suggesting that the membrane-attack complex formation may fail to remove the intracellular pathogen. It has been shown that the capsular polysaccharide renders *B. pseudomallei* resistant to *in vitro* phagocytosis by reducing C3b deposition on the

Table 3. Immune responses towards acute *Burkholderia pseudomallei* infection: acute streptozotocin (STZ) -induced diabetes model versus acute normoglycaemic model

Immune responses towards *B. pseudomallei* infection	Acute melioidosis infection (within 42-hr infection period)	
	STZ-diabetic model (this study)	Normoglycaemic model[18]
The TLR2 is responsible for recognition and initiation of defence response	TLR2 and several transcription factors were elevated 24 hr p.i. in the diabetic liver	Rapid induction of TLR2. Several transcription factors were elevated as early as 16 hr p.i. in both liver and spleen
Induction of various immune response genes, including the 'core immune response' genes to general inflammation infections	Most of the inflammatory genes were elevated only 24 hr p.i. in the diabetic liver, but were mildly elevated after 42 hr p.i. in the diabetic spleen	Rapid and overwhelmed inflammatory response throughout the infection period. Several potent chemokines were suppressed at 42 hr p.i.
Activation of APPs lead to occurrence of tissue damage	Mild elevation of some APPs and proteasomal degradation-related genes after 24 hr p.i. the liver	High induction of many APPs in the liver. Peptidoglysis and proteasomal degradation-related genes were elevated throughout the infection period in both liver and spleen
Activation of complement pathway	Not activated in response to infection	Complement pathway-related genes were mildly elevated after 24 hr p.i. but some key genes of membrane attack complex formation were suppressed
Activation of various cell death mechanisms	Caspase and cell death-related genes were elevated in the liver 24 hr p.i.	Caspase and inflammasome-related genes were elevated in both liver and spleen as early as 16 hr p.i.

APP, acute-phase protein; p.i., post-infection; TLR, Toll-like receptor.

bacterial surfaces upon infection.[12] Hence, this study suggests that poor glycaemic control impairs the complement system of an STZ-diabetic host rendering it unable to eliminate intracellular bacteria, hence increasing the susceptibility of diabetics to infection. Nonetheless, the *B. pseudomallei*-infected STZ-diabetic host over-expressed many cell death-related, inflammasone-related and proteasomal degradation genes 24 hr p.i. in the STZ-diabetic liver (Fig. 5 and Table 2). These expression profiles are similar to our previous work on the acute normoglycaemic model[18] although activation is delayed. Hence, the *B. pseudomallei*-infected host most likely triggers cell death programmes and proteolysis to limit a favourable niche for the intracellular pathogen regardless of host metabolic conditions.

In conclusion, we have provided the first genome-wide expression profile on an STZ-diabetic mouse model in response to acute *B. pseudomallei* infection. The STZ-diabetic and normoglycaemic host immune response to acute *B. pseudomallei* infection is summarized in Table 3. Our transcriptional analysis suggests that pattern recognition receptors of the STZ-diabetic host are defective in sensing pathogens during early infection (16 hr) leading to delayed activation of an appropriate innate immune response. Nonetheless, various inflammatory and immune responses as well as the general 'alarm signal' of infection were still elevated 24 hr p.i. and were mainly triggered via the TLR2 pathway, as seen in the acute normoglycaemic host. Malfunction of the immune response of the STZ-diabetic spleen also correlates with uncontrolled spread of intracellular bacteria in multiple organs. We believe that the impaired innate immunity in diabetics during early *B. pseudomallei* infection contributes to their increased susceptibility to this fatal disease.

Acknowledgements

We would like to thank Mohd. Nor Hasan for his technical assistance in animal handling. We are grateful to the Animal House of Universiti Kebangsaan Malaysia for the animal facilities. This study was supported by the Malaysia Genome Institute–Stanford University International Research Grant awarded to S.N. by the Ministry of Science, Technology and Innovation, Malaysia. C.Y.C. was supported by the National Science Fellowship from the Ministry of Science, Technology and Innovation, Malaysia.

Disclosures

There is no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Bacterial loads in *Burkholderia pseudomallei*-infected mice with streptozotocin (STZ) -induced diabetes and normoglycaemic mice.

Data S2. Quantitative real-time PCR analysis of host genes from mice with streptozotocin (STZ) -induced diabetes and normoglycaemic mice that were found to be differentially expressed by microarray analysis.

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