Introduction

Melioidosis is a serious infectious disease caused by the environmental Gram-negative bacillus *Burkholderia pseudomallei*. The organism is distributed in soil across much of southeast Asia and northern Australia. *B. pseudomallei* is a soil saprophyte endemic to Southeast Asia and northern Australia. The clinical presentation of melioidosis may mimic tuberculosis (both cause chronic suppurative lesions unresponsive to conventional antibiotics and both commonly affect the lungs). The two diseases have overlapping risk profiles (e.g., diabetes, corticosteroid use, and both *B. pseudomallei* and *Mycobacterium tuberculosis* are intracellular pathogens). There are however important differences: the majority of melioidosis cases are acute, not chronic, and present with severe sepsis and a mortality rate that approaches 50% despite appropriate antimicrobial therapy. By contrast, tuberculosis is characteristically a chronic illness with mortality <2% with appropriate antituberculosis chemotherapy. We examined the gene expression profiles of total peripheral leukocytes in two cohorts of patients, one with acute melioidosis (30 patients and 30 controls) and another with tuberculosis (20 patients and 24 controls). Interferon-mediated responses dominate the host response to both infections, and both type 1 and type 2 interferon responses are important. An 86-gene signature previously thought to be specific for tuberculosis is also found in melioidosis. We conclude that the host responses to melioidosis and tuberculosis are similar: both are dominated by interferon-signalling pathways and this similarity means gene expression signatures from whole blood do not distinguish between these two diseases.
important risk factor for tuberculosis, but there is no established association between HIV and melioidosis [6]. The taxonomic relationship between *B. pseudomallei* and *M. tuberculosis* is distant (they are in different phyla: Proteobacteria and Actinobacteria, respectively). Their cell surfaces also present different pathogen-associated molecular patterns (PAMP) to the host immune system, and it seems reasonable to expect the host to respond differently to challenge by different PAMPs.

In this study, we sought differences in host response between acute melioidosis and tuberculosis using whole genome arrays to compare gene expression in circulating peripheral blood leukocytes collected from two cohorts of patients, one with melioidosis and one with tuberculosis. We also sought to define whether whole blood gene expression profiling distinguishes between melioidosis and tuberculosis.

### Materials and Methods

The melioidosis data were taken from a previously published cohort of 30 patients and 30 healthy controls, frequency-matched for diabetes and glibenclamide use (an oral hypoglycaemic drug used to treat diabetes mellitus) [5]. Each group contained 10 non-diabetics and 20 diabetics. Diabetics were divided into 10 taking glibenclamide (= glyburide) and 10 not taking any sulphonylurea diabetics and 20 diabetics. Diabetics were divided into 10 taking glibenclamide (Gb) and 10 not taking any sulphonylurea alone). We adjusted for diabetes and glibenclamide because two-thirds of all melioidosis patients have diabetes, diabetes is itself a pro-inflammatory condition, and because glibenclamide is anti-inflammatory [5]. The tuberculosis cohort has been published previously and consists of 20 patients with pulmonary tuberculosis and 24 healthy controls [7]. That study did not control for the effect of confounders such as diabetes. Inclusion and exclusion criteria for both studies have been published previously [5,7]. Eligible cases for both studies were persons aged between 18 and 75 years. In the melioidosis cohort, diabetes was defined as an abnormal Hb A1c at enrolment or a previous diagnosis of diabetes. The tuberculosis cohort excluded patients with diabetes. Both studies excluded patients who were pregnant or immunosuppressed.

### Melioidosis Microarrays

The methods used in the melioidosis cohort have been reported previously [3] and the data is deposited at ArrayExpress, EMBL-EBI (accession number E-TABM-852-n). In brief, a 3 ml blood sample was collected from each study subject in a PaxGene™ Blood RNA tube (PreAnalytiX, GmbH) and stored at −70°C. RNA was extracted using the PaxGene™ Blood RNA Purification Kit (PreAnalytiX) according to the manufacturer’s instructions. The RNA was amplified using the Illumina® TotalPrep RNA Amplification Kit (Applied Biosystems) and assayed using the Illumina® HumanWG-6 v3.0 Expression BeadChip (Illumina®), which probes 48,803 transcripts from across the human genome. Quantitative PCR verification of these microarrays has been reported previously [5].

### Tuberculosis Microarrays

The methods used in the tuberculosis cohort have been published elsewhere previously [7]. In brief, a 3 ml blood sample was collected into Tempus tubes (Applied Biosystems, California) and stored at −20 to −80°C. RNA was extracted using the PerfectPure RNA Blood Kit (5 PRIME) according to the manufacturer’s instructions. The RNA was then amplified using the Illumina CustomPrep RNA amplification kit (Applied Biosystems) and assayed using the Illumina Human HT-12 v3 BeadChip array (Illumina®), which uses the same probe set as the HumanWG-6 v3.0. Raw data was downloaded from a publicly available repository (NCBI GEO accession number GSE19491) and consists of tuberculosis patients with controls recruited in London. The study also included data from a cohort of South African tuberculosis patients, but that cohort was excluded from this analysis because it does not contain uninfected controls, which made it impossible to normalize across the cohorts. The original study was analysed using GeneSpring, but we reanalysed the raw data using Bioconductor for the sake of comparability.

### Ethics

Approval for the melioidosis study was obtained from the Oxford Tropical Research Ethics Committee (OXTREC 018-07) and the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM 2008-001-01) [5]. Approval for the tuberculosis study was obtained from the Research Ethics Committee at St Mary’s Hospital, London, UK (REC 06/Q0403/128) [7]. Written informed consent was obtained from all subjects.

### Statistical Methods

Differential expression analyses were performed using Bioconductor [8] version 2.12.1 running on R 2.13.0 [9]. Pre-processing was performed using the beadarray 2.2.0 package [10,11], and background correction was performed using normexp [12,13]. Fluorescence intensities were quantile-normalized between arrays within each cohort and non-expressed probes were removed (detection p-value >0.05). Differential expression was performed
using limma 3.8.1 [14]. For the melioidosis cohort, the linear model fit was $\log e = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_1 x_1 x_2$, where $e$ is expression, $x_1$ is melioidosis, $x_2$ is diabetes and $x_3$ is glibenclamide treatment. The expression values for melioidosis, $\beta_1$, are therefore adjusted for diabetes and glibenclamide treatment. For the tuberculosis data, the model was $\log e = \beta_0 + \beta_1 x_1$, where $x_1$ is tuberculosis. The $p$-value cut-off of 0.01 was set following visual inspection of the histogram of unadjusted $p$-values for $\beta_1$, as
**Table 2.** Genes upregulated in melioidosis and tuberculosis, arranged by pathway.

| Pathway                                      | Melioidosis (total = 651) | Tuberculosis (total = 847) |
|----------------------------------------------|---------------------------|---------------------------|
| **IFN-γ pathway**                            |                           |                           |
| Pathway Genes List                          | p-value                   | Pathway Genes List        | p-value                   |
| IFN-γ pathway                                |                           |                           |
| MEF2A, SLC3A2, IL4R, LYN, SLC3A2, TXN, PML, MKNK1, CDKN2B, RALB, IL1B, JUNB, DDIT3, COL1A2, FGR, TF3E, VDR, ATF6, CDKN1A, FES, MAX, CASP1, PPP2R2A, CEBPB, HCK, JAK2, JAK3, STAT5A, STAT5B, LAMC1, PPARG, PTEN, GADD45G, GADD45B, GADD45A, MAP3K11, FKBP1A, ZBTB17, BCL6, CSNK1A1, YWHAB, STAT1, MAPK13, MAPK14, SOCS3, SOCS1, GRB2, ITG8, RUNX1, PTPN6, PTPN2, EIF4E, SAP30, IFNGR1, PRKCD, TNF, XIAP, MAP3K3, IL2RG, IRF7, IRF1 | <0.0001 | MEF2A, MAP3K7, MAP3K8, MAPK11, MAPK12, DUSP10, ARQ1, LYN, SLC3A2, TXN, GSK3B, RALB, IL1B, ARRBD2, ATF6, CDKN1A, MAX, CASP1, PKN1, CEBPB, PAPR14, PPARG, PTEN, RBBP4, RAB5A, YWHAZ, FKBPA1, BCL6, CSNK1A1, YWHAB, YWHAH, DYNL1B1, GRB2, IGF3, RAP1A, PRKCD, MAP3K3, CAMK2D, NUP153, IRF7, IRF1, CTNNB1, TGFBR2, PML, PPARCA, SOCS1, SOCS3, PPP2CA, PPP2CB, DAB2, JUNB, DDIT3, FGR, VDR, TRAF6, ZFYVE16, NCOA2, PPM1A, NR3C1, NEDD4L, JAK2, LAMC1, CREBBP, RHEB, GADD45G, GADD45B, GADD45A, HBP1, STAT1, MAPK14, SOCS3, SOCS1, ELK4, PTPN2, SAP30, PAR2, IFNGR1, EGR3, EGR2, DAPK1, PPP1R15A | <0.0001 |
| **Glypican network**                        |                           |                           |
| Pathway Genes List                          | p-value                   | Pathway Genes List        | p-value                   |
| Glypican network                             |                           |                           |
| MEF2A, NFKBIB, NFKBIA, ARF1, SLC9A1, NOD2, LYN, SLC3A2, TXN, PML, MKNK1, DAPP1, CDKN2B, RALB, RELB, JUNB, DDIT3, COL1A2, ARFGAP1, FGR, TF3E, MDK, VDR, VAV1, ATF6, CDKN1A, CDC42, MAX, CASP9, PPP2R2A, REL, CEBPB, HCK, LAMC1, ASAP1, GOSR2, PPARG, NFKB2, PTEN, GADD45G, GADD45B, GADD45A, FKBP1A, ZBTB17, CSNK1A1, YWHAB, MAPK13, MAPK14, PAG1, ERC1, GRB2, RUNX1, PTPN6, EIF4E, SAP30, IFNGR1, PRKCD, TNF, XIAP, MAP3K3, IL2RG, IRF7 | <0.0001 | APP, ARRB2, ASAP1, ATF6, BCL10, BCL3, BIRC2, CASP9, CDC42, CDKN1A, CEBPB, CLT, CREBBP, CSNK1A1, CTNNB1, DAB2, DAPP1, DDIT3, DUSP10, DYNL1B1, EGR2, EGR3, ELK4, FBXW11, FGR, FBKP1A, FZD1, GADD45A, GADD45B, GADD45G, GOSR2, GRB2, GSK3B, HBP1, HSPOA1A, IRF7, JUNB, LAMC1, LYN, MACF1, MAP3K3, MAP3K7, MAP3K8, MAPK1, MAPK14, MAPK3, MAX, MEF2A, NCOA2, NEDD4L, NFKBIA, NFKBIB, NR3C1, NRAS, NUP153, PAG1, PAK2, PKN1, PML, PPARG, PPM1A, PPP1R15A, PPP2CA, PPP2CB, PRKCD, PTEN, PTPRC, RAB5A, RALB, RAP1A, RBBP4, RELB, RHEB, RIPK2, SAP30, SLC3A2, SOS1, TGFBR2, TRAF6, TNX, UBE2D3, VDR, YWHAZ, YWHAH, YWHAZ, ZFYVE16 | <0.0001 |
| **TRAIL (TNF superfamily, member 10) signalling** |                           |                           |
| Pathway Genes List                          | p-value                   | Pathway Genes List        | p-value                   |
| TRAIL (TNF superfamily, member 10) signalling |                           |                           |
| NFKBIB, NFKBIA, ARF1, ASAH1, NOD2, LYN, SLC3A2, MKNK1, DAPP1, RELB, FADD, JUNB, ARFGAP1, FGR, VAV1, CDKN1A, TNFSF10, CASP4, CASP9, CASP7, CASP1, LIMK1, REL, HCK, SMAD1, KRIT1, LMBNB1, ASAP1, GOSR2, PPARG, NFKB2, PTEN, FKBPA1, CSNK1A1, YWHAB, MAPK14, PAG1, MAPK4, ERC1, GRB2, PTPN6, EIF4E, PRKCD, CTSD, TNF, XIAP, MAP3K10 | <0.0001 | MAP3K7, APP, MAP3K8, MAPK1, MAPK3, NFKBIB, NFKBIA, ASAH1, LYN, AIF1M, SLC3A2, NRG5, GSK3B, BCL10, RELB, ARRBD2, EGF, CDKN1A, CASP4, CASP9, CASP7, CASP8, ASAP1, CASP2, CYCS, NSMAF, LMBNB1, GOSR2, PPARG, PTEN, BID, YWHAZ, FKBPA1, BCL3, CSNK1A1, YWHAB, BCL2, VIM, DDIT3, IFNGR1, PRKCD, PTEN, YWHAZ, ZFYVE16, MAPK10, MAP3K7, MAP3K8, MAPK1, MAPK3, NFKBIB, NFKBIA, ASAH1, LYN, AIF1M, SLC3A2, NRG5, GSK3B, BCL10, RELB, ARRBD2, EGF, CDKN1A, CASP4, CASP9, CASP7, CASP8, ASAP1, CASP2, CYCS, NSMAF, LMBNB1, GOSR2, PPARG, PTEN, BID, YWHAZ, FKBPA1, BCL3, CSNK1A1, YWHAB, BCL2, VIM, DDIT3, IFNGR1, PRKCD, PTEN, YWHAZ, ZFYVE16 | <0.0001 |
Table 2. Cont.

| Pathway                                                                 | Melioidosis (total = 651) | Tuberculosis (total = 847) |
|------------------------------------------------------------------------|---------------------------|----------------------------|
| **Plasma membrane estrogen receptor signalling**                       |                           |                            |
| Pathway Genes List                                                     | p-value                   | Pathway Genes List          | p-value |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| Plasmamembrane estrogen receptor signalling                             |                           |                            |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| TNF-α/NF-κB signalling                                                 |                           |                            |
| Pathway Genes List                                                     | p-value                   | Pathway Genes List          | p-value |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| TGF-β receptor signalling; regulation of cytoplasmic and nuclear SMAD2/3 signalling |                           |                            |
| Pathway Genes List                                                     | p-value                   | Pathway Genes List          | p-value |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| IL-1-mediated signalling                                               |                           |                            |
| Pathway Genes List                                                     | p-value                   | Pathway Genes List          | p-value |
| p-value                                                                | Genes List                | Genes List                  | Genes List |
| Chemokine signalling                                                   |                           |                            |
| Pathway Genes List                                                     | p-value                   | Pathway Genes List          | p-value |
| p-value                                                                | Genes List                | Genes List                  | Genes List |

**Note:** The table continues with more entries for each pathway as specified in the document.
calculated from the moderated t-statistic (B-statistic) using Bayesian methods [14]. Illumina probe IDs were mapped to HUGO gene symbols [15] by illuminaHumanv3.db [16]. Networks were clustered by pathway by the Reactome [17] functional interaction network [18] plug-in for Cytoscape 2.8.1 [19], restricting the analysis to modules larger than 10 proteins. The p-values reported are for the hypergeometric test. The top 1000 probes were used to construct networks for presentation in figures. We searched specifically for interferon-regulated gene signatures on Interferome also [20]. Heat maps were drawn with gplots 2.8.0 [21] using colour blind-safe colour ramps generated by RColorBrewer 1.0–2 [22,23]. We divided controls and patients by unsupervised k-means [24] and verified stability of the clusters under 5 random starts.

Table 2. Cont.

| Pathway                          | Genes List | p-value | Pathway                          | Genes List | p-value |
|----------------------------------|------------|---------|----------------------------------|------------|---------|
| p75 (NTR) signalling             | BCL2L11, NFKBIA, NOD2, SORT1, RELB, CDC42, CASP9, CDK5, APH1B, REL, NCSTN, STAT5A, MYD88, TRPC3, RIT1, NFKB2, ADAM17, YWHAB, STAT3, MAPK14, ER1C1, GRB2, DYNLT1, PRKCD, TNF, XIAP, RGS19, CRK | 0.0001 | APP, MAPK1, MAPK3, NFKBIA, SORT1, NRAS, GSK3B, FRS3, BCL10, RELB, ARRB2, BEX1, CASP9, CYCS, MYD88, RIT1, YWHAZ, BCL3, YWHAB, BIRC2, YWHAH, GRB2, UBE2D3, RAP1A, PRKCD, SOS1, FBXW11, TNFAP3, TRAF6, CDC42, APH1B, NEDD4L, GB2, NFGRA1, PSEN1, ADAM17, MAPK10, STAT3, MAPK14, RIPK2 | <0.0001 |
| Phagosome                        | NCF4, MSR1, HLA-B, HLA-G, VAMP3, STX7, ATP6V1H, NOX3, CYBA, CYBB, CD36, TUBA4A, CTS1, ATP6V0E1, FCGRA1, THBS1, ATP6V1E1, FGRG2A, CD14, TLR2, TLR4, ITG82, ITG83, ITGAM, FCAR, TAP2, TAP1 | <0.0001 | HLA-DRA, HLA-B, HLA-F, VAMP3, RAB7A, HLA-DMB, CYBB, CD36, STX12, ATP6V0E1, RAB5C, FCGRA3A, FCGRA3, RAB5A, FCGR1, TUBB8, FGRG2A, CD14, ITGB3, FCAR, TUBA1A, TUBA1B, TAP2, TAP1, NCF2, NCF1, HLA-DPA1, HLA-DPB1, MSR1, EEA1, STX7, TLRG1, CTL1, DYNCL1, ATP6V1G1, ATP6V1E1, TLR2, TLR4, TLR6, ACTB | <0.0001 |
| Apoptosis                        | H1F0, BCL2L11, FADD, TNF5F10, CASP9, CASP7, LMBN1, YWHAB, PSMD12, TJP2, PSMA6, PSMA4, PSMA3, PSMB7, PSMB3, PSMB2, BMX, PSMB8, PSMB9, PSMC1, PSMD6, PSMD9, PRKCD, TNF, XIAP, CASP10 | <0.0001 | PIK3CB, NFKBIA, AIFM1, IL1B, CASP9<0.0001, CASP7, CASP8, CYCS, MYD88, BID, BIRC2, IRA4K, IRA3, IRAK2, FAS, TNF5F10, PRKAR1A, CSF2RB, TNF5F10C, PPP3R1, CASP10 | <0.0001 |
| Toll-like receptor signalling    | PIK3CG, NFKBIA, CXCL10, IL1B, FADD, TRAF3, MYD88, TOLLIP, LY96, STAT1, MAPK13, MAPK14, CD14, TBK1, TLR1, TLR2, TLR4, TLR5, TLR6, TRB8, IRAK4, IFNAR1, IFRN2, TNF, IRF7 | <0.0001 | MAP3K7, MAP3K8, PIK3CB, MAPK1, MAPK3, NFKBIA, CXCL10, IL1B, CASP8, MYD88, CD40, LY96, CD14, IRA4K, CD86, IRF7, TRAF5, MAPK10, STAT1, MAPK14, TBK1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, IFRN2, IFRN1 | <0.0001 |
| IL-12 mediated signalling events | NFKBIA, NOD2, IL1B, IL27, RELB, IL18RAP, REL, JAK2, STAT5A, NFKB2, GADD45G, GADD45B, IL18R1, STAT1, STAT3, MAPK14, SOCS1, ERC1, TNF, IL2RG, IRF1 | 0.0001 | HLA-DRA, NFKBIA, IL1B, BCL10, IL27<0.0001, RELB, ARRB2, BAX7A, IL12RB1, IL18RAP, IL6ST, PIAS2, BCL3, BIRC2, UBE2D3, CD86, IRF7, TRAF5, MAPK10, STAT1, MAPK14, TBK1, TLR2, TLR4, TLR5, TRB8, IRAK4, IFNAR1, IFRN2 | <0.0001 |
| IL-2 mediated signalling         | UGCG, NFKBIA, NOD2, PTK2B, RELB, REL, SMPD1, JAK3, STAT5A, STAT3B, NFKB2, STAT1, STAT3, MAPK14, SOCS3, SOCS2, ERC1, GRB2, TNF, IL2RG | 0.0001 | SGM51, UGCG, MAPK1, MAPK3, NFKBIA, ELF1, NRAS, BCL10, RELB, ARRB2, STAT3B, BCL3, BIRC2, GRB2, UBE2D3, TERT, CCNA2, PTK2B, SOS1, FBXW11, TNFAP3, TRAF6, JAK2, CREBBP, GADD45G, GADD45B, IL18R1, STAT1, STAT3, STAT2, MAPK14, SOCS1, RIPK2 | <0.0001 |
| NOD-like receptor signalling     | NFKBIA, NOD2, NLR4C, IL1B, TRIP6, CASP5, CASP1, MEV, ERBB2IP, MAPK13, MAPK14, TNF, XIAP, PYCARD | 0.0001 | MAP3K7, MAPK1, MAPK3, NFKBIA, NFKBIA, NLR4C, IL1B, CASP5, CASP8, CASP1, BIRC2, CARD6, PYCARD, TNFAP3, TRAF6, MEV, MAPK10, ERBB2IP, MAPK14, RIPK2, HSP90AA1 | <0.0001 |
### Table 3. Genes downregulated in melioidosis and tuberculosis, arranged by pathway.

| GeneSet                        | Melioidosis (total = 1007) | p-value | Tuberculosis (total = 1138) | p-value |
|-------------------------------|-----------------------------|---------|-----------------------------|---------|
| Glypican network              | MEF2C, MAP3K4, PPP1CA, ZAP70, ATM, CARD11, PPP3CC, IL8, MEF2D, RALA, ATF2, CCM2, CDKN1B, HDAC1, CD4, NFATC3, MAF, CABIN1, WWP1, CAMK4, CTBP1, AES, RBP7, MAP3K4, BCL2, CD22, FYN, YWHAQ, FOXO1, FOXO4, GATA3, BLK, EIF4B, SPTBN1, CD79A, PLEKH2, PLEKH1, CLT8, RANBP3, PRKCH, LEF1, UBE2I, PRKCD, PRKRF, CDT81, CAMK2G, CDT83, MAP3K3, PKR2, CTGF, GNG2, TGF1, TGF2, RCSE, RPS6, TGFB3, MAPT, MYC, ARHGEF7, GNB1, DKG1, TBC1D4, CTDP2, EF2, LAT, TSC1, SGK1, LCK, NR3C1, HSPA8, CREB1, CDC25B, SNIP1, SIN3A, CD40LG, SMAD4, SMAD3, CBLB, CYLD, STRAP, MAP4K1, SRF, AKIN, RUNX2, RUNX3, AXIN1, ITK, PTPN6, MAL, PLCG1, AKT1, GSC, PTPRC | <0.0001 | HRAS, PPP2R5D, MAP3K7, MAP3K4, ZAP70, ATM, EEF2K, CARD11, PPP3CB, PPP3CC, IL4, MEF2D, DUSP8, IL10, RALA, ARFGAP1, MAPKAPK5, HDAC2, CDKN1B, HDAC1, RKBK, PPP2R2A, CD4, NFATC3, MAF, CABIN1, WWP1, TGFBRA1, CD72, ETS1, AES, CTBP1, RBBP4, RBBP7, MAP3K4, BCL2, CD22, CD19, FYN, FOXO1, FOXO3, GATA3, ATX1, CD79B, CD79A, SHC1, PRKC, LEF1, PRKCI, UBE2I, PRKAC, CDT81, CAMK2G, MAP3K1, YES1, IKB, TGFBR2, RPS6, TGFB3, MYC, ARHGEF7, TLE1, GNB1, TBC1D4, CTDP2, EF2, TAF2, LAT, TSC1, TSC2, SGK1, CD4, LCK, INPP5D, HSPA8, CREB1, SIN3B, SIN3A, CD40LG, MACF1, SMAD7, SMAD3, DCP1A, CYLD, FZD3, MAPK13, CALM1, AKIN, RUNX2, RUNX3, AXIN1, IN, MAL, PLCG1, AKT1, PAK2, CSK | <0.0001 |
| Protein synthesis, RNA translation, ribosome | RPLP2, RPLP0, RPLP1, PABPC1, EIF4A2, EE1G, EE1F, RPL27A, RPL35A, RPL10A, DHD1, RPL37A, RPL13A, CTT3, CTT7, RPL16A, EIF5A, EEF1B2, EIF3D, EIF3B, EIF3G, EIF3H, EIF3E, EIF3F, EIF3K, EIF1A, EIF4B, EIF4H, DHPS, GSP2, RPL18, RPL17, RPL19, RPL14, RPL13, RPL15, RPL10, RPL12, EIF253, RPS18, RPS19, RPS16, RPS14, RPS15, RPS12, RPS13, RPS10, RPS25, RPS27, RPS28, RPS29, RPS20, RPS23, RPS6, RPS5, RPS35, RPL36, RPL38, RPL30, RPL32, RPL27, RPL29, RPL22, RPL23, EIF2, EF2, UBAS2, RPL7, RPL6, RPL8, RPL3, RPL5, RPL4, RPL7A, RPL3A, RPS25, RPS3, RPS4X, RPL5A, RPL27A, EIF2B1, PIK3G, PIK5P | <0.0001 | CCT2, CCT3, CTT6A, CCT7, EE1F1B2, EE1F1D, EE1F2, EIF2B3, EIF3A, EIF3B, EIF3C, EIF3E, EIF3F, EIF3G, EIF3H, EIF3I, EIF3K, IF4A2, FBXW7, PABPC1, RPL10, RPL10A, RPL11, RPL13, RPL13A, RPL14, RPL15, RPL18, RPL19, RPL22, RPL23, RPL23A, RPL26, RPL27, RPL29, RPL3, RPL31, RPL32, RPL35, RPL36, RPL36A, RPL37A, RPL4, RPL5, RPL6, RPL7, RPL7A, RPL8, RPL9, RPL10, RPL11, RPL51, RPS13, RPS15, RPS15A, RPS16, RPS18, RPS19, RPS20, RPS23, RPS24, RPS25, RPS27A, RPS28, RPS29, RPS33, RPS4X, RPS35, RPS5, RPS5P, RPS5, RPS5P, UBA52 | <0.0001 |
| TGF-β receptor signalling, regulation of SMAD2/3 | MEF2C, MAP3K4, PPP1CA, ATM, IL8, MEF2D, RALA, ATF2, CCM2, HDAC1, NFATC3, MAF, CABIN1, WWP1, CAMK4, CTBP1, RBBP7, BCL2, FYN, YWHAQ, FOXO1, FOXO4, GATA3, BLK, SPTBN1, PRKCH, UBE2I, PRKCD, PIK3K, MAP3K3, PTG32, CTGF, TGFB2, TGFB3, TGF1, TGF2, CTSF, TGFB3, MAPT, MYC, DOKA, CTLA4, RPS6K5, CDTS2, LCK, NR3C1, HSPA8, CREB1, CDC25B, SNIP1, SIN3A, CD40LG, SMAD4, SMAD3, CBLB, STRAP, SRF, RUNX2, RUNX3, AXIN1, GSC, TGFB3, FNTA, E2F4, ANAPC1, ANAPC5, ET51, SNW1, LEF1, CAMK2G, PIK3R1, PIK3R2, FOSB, RBL2, JUND, CTGF, CDC23, GIPC1, AXIN2 | <0.0001 | MAP3K7, MAP3K4, ATM, EEF2K, IL4, MEF2D, DUSP8, IL10, RALA, MAPKAPK5, HDAC2, HDAC1, PPP2R2A, NFATC3, MAF, CABIN1, WWP1, TGFBRA1, CTBP1, RBBP4, RBBP7, BCL2, FYN, FOXO1, FOXO3, GATA3, BLK, SHC1, PRKCA, PRKCH, UBE2I, PRKCD, MAP3K1, YES1, IKB, TGFBR2, TGFBR3, MYC, CTLA4, RPS6K5, RPS6K1B, CDTS1, CDTS2, TAF2, TSC2, CDK4, LCK, HSPA8, CREB1, SIN3B, SIN3A, CD40LG, SMAD7, SMAD3, DCP1A, MAPK13, CALM1, RUNX2, RUNX3, AXIN1, PAK2 | <0.0001 |
| GeneSet | Genes | List | p-value | Genes | List | p-value |
|---------|-------|------|---------|-------|------|---------|
| IFN-γ pathway | MEF2C, MAP3K4, PPP1CA, ATM, DOK2, IL8, MEF2D, RAL, AT2F, CCM2, HDAC1, NFATC3, MAF, CABIN1, WWP1, CAMK4, ETS1, CTBP1, RBBP7, BCL2, FYN, YWHAQ, FOX01, FOX04, GATA3, BLK, EIF4B, SPTBN1, PRKCH, UBE2I, PRKQ, CAMQG, MAPK3, PIK3R1, PTGS2, TGF, TGIF1, TGIF2, TCF3, RPS56, TGFB, MAP, MYC, DGKA, CTLA4, RPSK6, CTDSF, EF2, TSC1, LCK, NR3C1, HSPA8, CREB1, CDC25B, JAK1, SNIP1, SIN3A, RAPGF1, CD40LG, FCR2, SMAD4, SMAD3, PTPN11, CBLB, STRAP, SRF, RUNX2, RUNX3, AXIN1, PTEN, AKT1, GSC | <0.0001 | PPI2R5D, MAP3K7, MAP4K4, ATM, EEF2K, DOK2, IL4, MEF2D, DUSP8, IL10, RAL, MAPKAPK5, TFF3, HDAC2, HDAC1, PPI2R2A, NFATC3, MAF, CABIN1, WWP1, TGFBAP1, PIAS1, ETS1, CTBP1, RBBP4, RBBP7, MAP3K1, BCL2, FYN, FOX01, FOX03, GATA3, BLK, EIF3A, SHC1, PRKCA, PRKCH, UBE2I, PRKQ, CAMQG, MAPK3, YES1, IRF4, TGFB, RPS56, TGFB, MAP, MYC, CTLA4, RPSK6, RPSK6B1, CTDSF, CTDSF, EF2, TFA2, TSC1, TSC2, CD4, HMGA1, LCK, INPP5D, HSPA8, CREB1, SIN3A, RAPGF1, CD40LG, FCR2, SMAD4, SMAD3, PTPN11, DCP1A, GF3A, STAT6, MAPK13, CALM1, RUNX2, RUNX3, AXIN1, AKT1, PAK2, LTA | <0.0001 |
| TRAIL (TNF superfamily, member 10) signalling | ZAP70, ATM, PARP1, CARD11, PPP3CC, IL8, LMA, MEF2D, CDK18, CASP8, CASP2, CYCS, CD4, NFATC3, MAF, CABIN1, CAMK4, ETS1, MAPK3, PIK3R1, PTGS2, BCL2, CD22, FYN, YWHAQ, FOX01, FOX04, GATA3, BLK, EIF4B, CD79B, CD79A, PLEXH2, PLEXH1, CLT4, PRKCH, PRKQ, CFL2, CYTH1, CAMQG, CYTH3, PIK3R1, PTGS2, RPS6, MYC, ARHGEF7, DGKA, NUMA1, TBC1D4, CTLA4, PDE3B, EF2, TRADD, LAT, TSC1, SKG1, LCK, CD40L, CBLB, CYLD, SATB1, MADD, MAP4K1, ITK, PTEN6, MALT1, PLCG1, DAP3, AKT1, PTPRC, SPTAN1 | <0.0001 | HRAS, PPI2R5D, MAP3K7, ZAP70, ATM, PARP1, AIF1, CARD11, PPP3CB, PPP3CC, IL4, MEF2D, DUSP8, IL10, RAL, MAPKAPK5, TFF3, HDAC2, HDAC1, PPI2R2A, NFATC3, MAF, CABIN1, WWP1, TGFBAP1, PIAS1, ETS1, CTBP1, RBBP4, RBBP7, MAP3K1, BCL2, FYN, FOX01, FOX03, GATA3, BLK, EIF3A, SHC1, PRKCA, PRKCH, UBE2I, PRKQ, CAMQG, MAPK3, YES1, IRF4, TGFB, RPS56, TGFB, MAP, MYC, CTLA4, RPSK6, RPSK6B1, CTDSF, CTDSF, EF2, TFA2, TSC1, TSC2, CD4, HMGA1, LCK, INPP5D, HSPA8, CREB1, SIN3A, RAPGF1, CD40LG, FCR2, SMAD4, SMAD3, PTPN11, DCP1A, GF3A, STAT6, MAPK13, CALM1, RUNX2, RUNX3, AXIN1, AKT1, PAK2, LTA | <0.0001 |
| Class I PI3K signalling events | ZAP70, ATM, CARD11, PPP3CC, IL8, MEF2D, CDK18, CD4, NFATC3, MAF, CABIN1, CAMK4, ETS1, MAPK3, PIK3R1, BCL2, CD22, FYN, YWHAQ, FOX01, FOX04, GATA3, BLK, EIF4B, CD79B, CD79A, PLEXH2, PLEXH1, CLT4, PRKCH, PRKQ, CYTH1, CAMQG, CYTH3, PIK3R1, PTGS2, RPS6, ARHGEF7, DGKA, NUMA1, TBC1D4, CTLA4, PDE3B, EF2, TRADD, LAT, TSC1, SKG1, LCK, CD40L, CBLB, CYLD, SATB1, MADD, MAP4K1, ITK, PTEN6, MALT1, PLCG1, DAP3, AKT1, PTPRC, SPTAN1 | <0.0001 | HRAS, PPI2R5D, MAP3K7, ZAP70, ATM, PARP1, AIF1, CARD11, PPP3CB, PPP3CC, IL4, MEF2D, DUSP8, IL10, RAL, MAPKAPK5, TFF3, HDAC2, HDAC1, PPI2R2A, NFATC3, MAF, CABIN1, WWP1, TGFBAP1, PIAS1, ETS1, CTBP1, RBBP4, RBBP7, MAP3K1, BCL2, FYN, FOX01, FOX03, GATA3, BLK, EIF3A, SHC1, PRKCA, PRKCH, UBE2I, PRKQ, CAMQG, MAPK3, YES1, IRF4, TGFB, RPS56, TGFB, MAP, MYC, CTLA4, RPSK6, RPSK6B1, CTDSF, CTDSF, EF2, TFA2, TSC1, TSC2, CD4, HMGA1, LCK, INPP5D, HSPA8, CREB1, SIN3A, RAPGF1, CD40LG, FCR2, SMAD4, SMAD3, PTPN11, DCP1A, GF3A, STAT6, MAPK13, CALM1, RUNX2, RUNX3, AXIN1, AKT1, PAK2, LTA | <0.0001 |
| TNF- α/NF-κB | MEF2C, ATM, PARP1, IL8, LMA, MEF2D, RAL, AT2F, CCM2, CASP8, CASP2, CYCS, NFATC3, MAF, CABIN1, CAMK4, GNB2L1, MAPK3X4, PRF1, BCL2, FYN, YWHAQ, GATA3, BLK, PRKCH, PRKQ, CFL2, MAP3K, PIK3R1, PTGS2, TCF3, MYC, DGKA, NUMA1, CTLA4, RPSK6, TRADD, LCK, CREB1, CDC25B, CD40L, CBLB, CYLD, SATB1, MADD, MAP4K5, MAP4K2, SRF, MALT1, AKT1, SPTAN1 | <0.0001 | SMARCA4, SMARCB1, IKBKE, HDAC2, <0.0001 | HDAC1, IKBKE, FAF1, MAP3K4, IRF3, AKAP8, MAP3K2, RPS15, BTRC, RPSK6B1, LRRPC, TRAF1, TRAF2, RPL6, RPL4, TRAF5, TRADD, FB, PTEN11, POL1R1C, POL2R2H, HS90AB1, AKT1, MCM7, PSMD1, MCM5, PNA6, PEBP1, PNA3 | <0.0001 |
Table 3. Cont.

| GeneSet                          | Genes | List | p-value | Genes | List | p-value |
|----------------------------------|-------|------|---------|-------|------|---------|
| Formation and maturation of mRNA transcript | 47    |      | <0.0001 | 49    |      | <0.0001 |
| Spliceosome                      | 37    |      | <0.0001 | 39    |      | <0.0001 |
| T-cell receptor signalling       | 34    |      | <0.0001 | 38    |      | <0.0001 |
| IL-2-mediated signalling events  | 18    |      | 0.022   | 30    |      | <0.0001 |

Results

The melioidosis cohort consisted of 30 patients and 30 controls. Baseline characteristics are in Table 1. In the melioidosis cohort, 6,755 probes were differentially expressed (that is, either up or downregulated) representing 4632 unique genes. Annotation was available for 1,658 of these genes, of which 651 were upregulated and 1,007 were downregulated. The tuberculosis cohort consisted of 30 patients and 30 controls. In the tuberculosis cohort, 6911 probes were differentially expressed (that is, either up or downregulated) representing 4632 unique genes. Annotation was available for 1,658 of these genes, of which 651 were upregulated and 1,007 were downregulated. The tuberculosis cohort consisted of 20 patients and 24 controls. In the tuberculosis cohort, 6911 probes were differentially expressed (that is, either up or downregulated) representing 4632 unique genes. Annotation was available for 1,658 of these genes, of which 651 were upregulated and 1,007 were downregulated. The tuberculosis cohort consisted of 20 patients and 24 controls. In the tuberculosis cohort, 6911 probes were differentially expressed (that is, either up or downregulated) representing 4632 unique genes. Annotation was available for 1,658 of these genes, of which 651 were upregulated and 1,007 were downregulated.

Pathway Analysis

Interferon-mediated responses were the dominant pathway seen in both melioidosis and in tuberculosis (p<0.0001 for both, Tables 2 & 3). Class 1 and class 2 interferons were prominent in both (Table 4). Of the immune-related pathways, TRAIL (TNF family member 10), tumour necrosis factor α (TNFα), transforming growth factor β (TGFB), interferukin (IL)-1, IL-2, IL-12, chemokine and Toll-like receptor (TLR) pathways were all differentially regulated (Tables 2 & 3). There was no gene signature that distinguished melioidosis from tuberculosis, and for each of the pathways differentially expressed in melioidosis, we were able to find a counterpart in tuberculosis (Tables 2 & 3). Class 1 and class 2 interferons were prominent in both (Table 4). Of the immune-related pathways, TRAIL (TNF family member 10), tumour necrosis factor α (TNFα), transforming growth factor β (TGFB), interferukin (IL)-1, IL-2, IL-12, chemokine and Toll-like receptor (TLR) pathways were all differentially regulated (Tables 2 & 3). There was no gene signature that distinguished melioidosis from tuberculosis, and for each of the pathways differentially expressed in melioidosis, we were able to find a counterpart in tuberculosis (Tables 2 & 3).

Modular Analysis

In a modular analysis of the upregulated genes (Figure 2A), interferon and cytokine signalling clustered together in the centre of the network, causing the complement (cluster 1), NOD-like receptor (cluster 2) and TLR (cluster 3) pathways to gain
prominence. In the downregulated genes (Figure 2B), the most prominent clusters were the ribosomal proteins (cluster 1) and zinc finger proteins (cluster 2).

PAMP-specific Responses

*B. pseudomallei* expresses lipopolysaccharide on its outer membrane, while *M. tuberculosis* does not and has a lipid-rich cell wall. Lipopolysaccharide is recognized by TLR4 and CD14, and both are upregulated in melioidosis (*P* = 0.0016 and 1.5 × 10^-6, respectively); however, TLR4 and CD14 are also upregulated in tuberculosis (*P* = 1.5 × 10^-6 and 9.4 × 10^-4, respectively). *B. pseudomallei* is a flagellated, motile bacterium, while *M. tuberculosis* is immotile with no flagellum. Flagellin is a ligand for TLR5 [25] and NLRC4 [26]. Both TLR5 and NLRC4 were upregulated in melioidosis (*P* = 5.4 × 10^-13 and 4.2 × 10^-10, respectively), but both were upregulated in tuberculosis also (*P* = 8.1 × 10^-10 and 2.4 × 10^-11).

**Discussion**

There were 4632 genes differentially expressed in melioidosis and 5045 genes in tuberculosis, thus approximately 20% of the human genome is differentially regulated in each disease. The most prominent pathway in melioidosis was interferon (IFN-γ) and the same was true of tuberculosis. There were no pathways differentially regulated in melioidosis that were not also differentially regulated in tuberculosis, and there was no signature which reliably distinguished melioidosis and tuberculosis.

Berry *et al.* identified an 86-gene signature as being specific for tuberculosis after eliminating differentially regulated genes common to *Staphylococcus pyogenes* and *Staphylococcus aureus* infections, and to two auto-inflammatory diseases (systemic lupus erythematosus and Still’s disease). This signature was also present in melioidosis, which is surprising given that all melioidosis patients recruited had acute rather than chronic melioidosis, which is clinically distinct from tuberculosis.

**Interferon-mediated Responses**

The IFN-γ pathway was reported as the most prominent pathway identified in gene expression studies of a mouse model of melioidosis [27], and blocking IFN-γ dramatically increases host susceptibility to melioidosis [3]. In human studies, plasma IFN-γ concentrations were high in melioidosis [3], and IFN-γ-mediated responses were also the most prominent feature in a gene expression study of melioidosis in another human cohort [26]. The finding here that this feature is shared with tuberculosis is unsurprising, because IFN-γ responses are crucial for the host response against intracellular pathogens such as *B. pseudomallei* and *M. tuberculosis*. IFN-γ treatment has a role in the management of multidrug-resistant tuberculosis, and adjunctive therapy with IFN-γ is beneficial in a mouse model of melioidosis [29], although its role in clinical melioidosis remains undefined [30].

In their original report on this tuberculosis cohort, Berry *et al.* noted that type 2 IFN-γ responses were prominent, but noted that type 1 IFN-αβ responses were present also [7]. We found that type 1 interferon-αβ responses were just as prominent in melioidosis, but the clinical relevance of this remains to be defined.

Type 1 interferons can be produced by almost any cell type (leukocytes, fibroblasts and endothelial cells) and are induced by a range of bacterial pathogens, whereupon they proceed to modulate the host response in a manner that is as yet incompletely understood [31]. The signalling pathways initiated by type 1 interferons are best described in terms of their activation of signal transducer and activator of transcription (STAT) family members (STAT1 to STAT6) [32], the best studied of which are STAT1 and STAT3. STAT1 activation is dependent on both type 1 and type 2 interferons and results in a pro-inflammatory response, with recruitment of inflammatory cells and the enhancement of antigen presentation [31]. On the other hand, STAT3 activation is a key mediator of IL-10 signalling, and results in inhibition of inflammatory responses and directly inhibits STAT1 activation [31]. The role of STAT4 is less well described, but STAT4 activation may play a role in T helper 1 lymphocyte differentiation, which is an essential part of the host response to intracellular pathogens. Type 1 interferons are also necessary for the production of inducible nitric oxide synthase [33], which is in turn necessary for the clearance of intracellular bacteria. Interestingly, type 1 interferons are able to inhibit IL-1β production and inflammasome assembly by two separate mechanisms: the first is via inhibition of NLRP1 and NLRP3 inflammasomes in a STAT1-dependent manner; the second, is a reduction in pro-IL-1 levels via a STAT3-dependent pathway [34]. It has previously been shown that host response to *B. pseudomallei* is inflammasome-dependent [35].

The role of type 1 interferons in tuberculosis is unclear, since mice deficient in the production of type 1 interferons are better able to control *M. tuberculosis* infections [36], but type 1 interferons also play a non-redundant protective role in the absence of type 2 interferon signaling [37]. The role of type 1 interferons in the pathogenesis of melioidosis remains to be studied.

Table 4. Interferon signatures for melioidosis and tuberculosis.

| Melioidosis | Tuberculosis |
|-------------|-------------|
| Type 1      | Both        | Type 2 | Type 1 | Both | Type 2 |
| AIM2        | TLR5        | TNFSF10 | GBP5   | CASP1 | SEPT4  |
| CTS1        | GYG1        | DRAM   | ANKRD22| DUSP3 | CD274  |
| SH3GLB1     | DUSP3       | NEUR1  | FCGR1A | GBP1  | NLRC5  |
| TNC2        | CASP1       | CASP5  | AIM2   | GBP2  | PSMB10 |
| NEU1        | IFITM1      | SEPT4  | CEACAM1| GBP4  | P2RY14 |
| FCGR1A      | UPPI1       | CD274  | WDPY1  | IL15  | RNF213 |
| H2AFJ       | SERPING1    | SYN2   | EPST1  | ATF3  |        |
| IFITM3      | VAMP5       | H4     | LY96   | PSEME |
| LACTB       | SOCS3       | HIST2H4| GADD45B| PSEME |
| DYSF        | SAT1        | RAB24  | LACTB  | UBE2L6|
| VNN1        | SLC30A1     | SPT140 | GSTO1  | GCH1  |
| PG51        | JAG1        | SERTAD3| SH3GLB1| TAP1  |
| CXCL16      | LIMK2       | SH3GLB1| TAP1   | TRIM22|
| DYNLT1      | TXN         | MYD88  | STAT1  | TAP2  |
| MYD88       | TAP1        | VAMP5  | PSMB8  |        |
| JAK2        | IL15        |        | WARS   |        |
|             |             |        | SECTM1 |        |
|             |             |        | GYG1   |        |
|             |             |        | IFITM1 |        |
|             |             |        | IRF1   |        |
|             |             |        | SAT1   |        |
|             |             |        | RTP4   |        |
|             |             |        | CLUC1  |        |
|             |             |        | CASP4  |        |
|             |             |        | PLAIR   |        |
|             |             |        | DYNLT1 |        |
|             |             |        | SLC30A1|        |
|             |             |        | ACTA2  |        |

Note: The interferon signatures for melioidosis (A) and tuberculosis (B) are listed here (analysis from www.interferome.org). Berry *et al.* noted that both type 1 and type 2 interferon responses were prominent in tuberculosis. We find that type 1 interferon responses appear in melioidosis also. doi:10.1371/journal.pone.0054961.t004
Figure 2. Network representation of genes differentially expressed in melioidosis. ‘Canonical’ pathways (such as those presented in a standard biochemistry textbook) are manually curated collections of protein interactions arranged in a manner that aids human understanding, and as artificial constructs the boundaries between pathways are subjective. Pathways that are conceptually distinct often have proteins in common and overlap, so in modular analysis, multiple pathways may collapse into a single module, causing other pathways and relationships to gain prominence. These two networks (A and B) represent those genes that are differentially expressed in melioidosis. For simplicity of presentation, we have used only a subset of genes in these networks. The top 221 upregulated genes (as ranked by p-value) are presented in A, and the top 155 downregulated genes are in B. The same clusters were found in an analysis of the whole gene set and those results are presented in Tables 2 & 3. Network A. IFN-γ, TNF-α, IL-12 signalling pathways cluster together with the glypican network in the centre of the graph, but the complement/chemokine receptor (cluster 1), inflammasome (cluster 2) and Toll-like receptor pathways come to prominence in this analysis (cluster 3). Network B. IFN-γ, TGF-β and TNF signalling again cluster in the middle of the network. The two most prominent clusters are ribosomal proteins (cluster 1) and zinc finger proteins (cluster 2).

doi:10.1371/journal.pone.0054961.g002
PAMP-specific Responses

TLR4 and CD14 are upregulated in both melioidosis and tuberculosis. The classical ligand for TLR4 [38] and for CD14 [39] is lipopolysaccharide (LPS), which would explain this finding for B. pseudomallei. TLR4 will recognize heparin-binding haemagglutinin [40], and CD14 will bind lipoproteinmannan [41], both of which are expressed by M. tuberculosis.

The pattern recognition receptors TLR5 [25] and NLRC4 [26] both recognize flagellin. No alternative ligand has yet been described for TLR5, so it is more difficult to explain why tuberculosis should apparently induce a flagellin-response. One explanation may be upregulation of pattern recognition receptors is not driven by their ligands. TLR5 expression is induced as part of the type 1 interferon response [42], while NLRC4 is upregulated as part of the TNF-α response [43]. Both pathways are prominent in the host response to melioidosis. In support of this hypothesis, the TLRs are upregulated as a group in both melioidosis (TLR1, TLR2, TLR4, TLR5, TLR6, TLR8 and TLR10) and tuberculosis (TLR2, TLR4, TLR5, TLR6, TLR7, TLR8).

Limitations and Future Research

Tuberculosis is strongly associated with HIV infection, but melioidosis is not. HIV targets primarily CD4-positive T-lymphocytes and lymphocyte depletion is a feature of all sepsis. Lymphocytes were depleted in both the melioidosis and the TB cohorts, so lymphocyte-related pathways and modules are missing from the whole blood gene expression data of both cohorts, making it difficult to make any comment about the relative role of CD4-positive cells in melioidosis compared to tuberculosis. The whole blood signature was dominated by neutrophils which may also have obscured any lymphocyte signature. Future studies that use purified lymphocytes harvested from melioidosis patients may shed light on this issue.

Microarrays generate large amounts of data that are useful for the development of hypotheses. Our analysis has identified a number of other pathways that are differentially regulated in melioidosis, but which are unstudied to date. Notably, the TRAIL number of other pathways that are differentially regulated in the development of hypotheses. Our analysis has identified a pathway pathway is differentially regulated in melioidosis, but its role remains undefined at present. The glypicans (cell surface proteoglycans) contribute to cell proliferation and growth, both essential processes in the host response to infection. To date, investigations into the role of glypicans have been confined primarily to cancer biology, although glypican-deficient mice are more susceptable to respiratory infections [44]. In tuberculosis, the glypican network appears to have greater prominence than even the interferon-mediated responses.

Conclusions

Host responses to melioidosis and TB are dominated by interferon-signalling events, despite the fact that the organisms are unrelated and present completely different cell-surface PAMPs to the host. This is likely because they both stimulate host responses common to intracellular pathogens, and because the expression of pattern recognition receptors is not driven by their ligands, but by cytokine responses (primarily IFN-γ and TNF-α). The β2-gene signature identified by Berry et al. clusters melioidosis patients just as effectively as it clusters tuberculosis. It therefore seems likely that whole blood gene signatures will not be able to diagnose tuberculosis in areas where melioidosis and TB are co-endemic, but may find utility when interpreted in combination with clinical features. Further studies using direct comparisons will be required to confirm this finding.

Acknowledgments

We thank the staff of Sappasitphrasong Hospital, Ubon Ratchathani, who cared for the melioidosis patients in this study, and the staff of the Wellcome Trust Mahidol-Oxford Tropical Medicine Research Unit, who recruited the patients and collected the samples. We also thank Roslin Russell, Keith James, Mike Smith, Robert Stojnic, Mark Dunning, Wei Shli for help with scripting for R and Bioconductor; Joost Wiersinga, Tassali Weehuizen, Ana Luisa Toribio for helpful discussions; Damien Chausa-bel, Christine Graham and Anne O’Garra with help using the raw data from their TB cohort.

Author Contributions

Conceived and designed the experiments: GCKWK MFS GD SJP. Performed the experiments: GCKWK MFS RRM DL. Analyzed the data: GCKWK MFS RD SB. Contributed reagents/materials/analysis tools: MFS RD NPD GD SJP. Wrote the paper: GCKWK SD SJ P.

References

1. Limmathurotsakul D, Peacock S (2011) Melioidosis: a clinical overview. Br Med Bull 99: 125–139.
2. Wong KT, Puthucheary SD, Chaowagul W, Amornchai P, Cheng AC, et al. (1995) The histopathology of human melioidosis. Histopathology 26: 51–55.
3. Wiersinga WJ, van der Poll T, White NJ, Day NP, Peacock SJ (2006) Melioidosis: insights into the pathogenesis of Burkholderia pseudomallei. Nat Rev Microbiol 4: 272–282. doi:10.1038/nrmicro1385.
4. Collins HL, Kaufmann SH (2001) The many faces of host responses to tuberculosis. Immunology 103: 1–9.
5. Koh GCKW, Maude RR, Schreiber MF, Limmathurotsakul D, Wiersinga WJ, et al. (2011) Glibberase is anti-inflammatory and associated with reduced mortality in melioidosis. Clin Infect Dis 52: 717–725. doi:10.1093/cid/ciq192.
6. Chierakul W, Puthucheary SD, Vadivelu J, Wang JT, Siddique M, et al. (2010) Comparison of background correction methods for two-colour microarrays. Bioinformatics 26: 1398–1407. doi:10.1093/bioinformatics/btq236.
7. Gentelman RC, Carey V, Bates DM, Bolstad B, D'Agostino M, et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5: R80. doi:10.1186/gb-2004-5-10-r80.
8. Dunning MJ, Smith ML, Ritchie ME, Tavare S (2007) beadarray: R classes and methods for Illumina bead-based data. Bioinformatics 23: 2183–2184. doi:10.1093/bioinformatics/btm131.
20. Samarajiwa SA, Forster S, Auchettl K, Hertzog PJ (2009) INTERFEROME: the database of interferon regulated genes. Nucleic Acids Res 37: D852–7.
21. Warnes GR (2010) gplots: Various R programming tools for plotting data. Available: http://CRAN.R-project.org/package = gplots.
22. Neuwirth E (2007) RColorBrewer: ColorBrewer palettes. Available: http://cran.r-project.org/web/packages/RColorBrewer/index.html.
23. Brewer CA, Hatchard GW, Harrower MA (2005) ColorBrewer in print: a catalog of color schemes for maps. Cartography and geographic information science 30: 5–32.
24. Hartigan JA, Wong MA (1979) A K-means clustering algorithm. Applied Statistics 28: 100–108.
25. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, et al. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410: 1099–1103. doi:10.1038/35074106.
26. Sutterwala FS, Flavell RA (2009) NLRC4/IPAF: a CARD carrying member of the NLR family. Clin Immunol 130: 2–6. doi:10.1016/j.clinimm.2008.08.011.
27. Chin C-Y, Monack DM, Nathan S (2010) Genome wide transcriptome profiling of a murine acute melioidosis model reveals new insights into how Burkholderia pseudomallei overcomes host innate immunity. BMC Genomics 11: 672. doi:10.1186/1471–2164–11–672.
28. Pankla R, Buddhisa S, Berry M, Blankenship D, Bancroft G, et al. (2009) Genomic transcriptional profiling identifies a candidate blood biomarker signature for the diagnosis of septicemic melioidosis. Genome Biology 10: R127. doi:10.1186/gb-2009–10–11-r127.
29. Propst KL, Troyer RM, Kellihan LM, Schweizer HP, Dow SW (2010) Immunotherapy markedly increases the effectiveness of antimicrobial therapy for treatment of Burkholderia pseudomallei infection. Antimicrob Agents Chemother 54: 4520. doi:10.1128/AAC.00805–10.
30. Koh GCCW, Limnathurosakul D (2010) Gamma interferon supplementation for melioidosis. Antimicrob Agents Chemother 54: 1792–1799. doi:10.1128/AAC.01513–09.
31. Gonzalez-Navajas JM, Lee J, David M, Rax E (2012) Immunomodulatory functions of type I interferons. Nat Rev Immunol 12: 123–135. doi:10.1038/ nri3133.
32. Hebenstreit D, Wirmberger G, Hoeije-Hoeck J, Duscha A (2009) Signaling mechanisms, interaction partners, and target genes of STAT6. Cytokine Growth Factor Rev 17: 173–188.
33. Utasimcharoen P, Amantagool N, Achjaroen S, Limposuvan K, Chaisiriya P, et al. (2004) Induction of iNOS expression and antimicrobial activity by interferon (IFN)-beta is distinct from IFN-gamma in Burkholderia pseudomallei-infected mouse macrophages. Clin Exp Immunol 136: 277–283.
34. Guarda G, Braun M, Stachl F, Tardivel A, Mattmann C, et al. (2011) Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 34: 213–223.
35. Breitbach K, Sun GW, Koehler J, Eske K, Wongprompitak P, et al. (2009) Caspase-1 mediates resistance in murine melioidosis. Infect Immun. Available: http://www.ncbi.nlm.nih.gov/pubmed/19179418. Accessed 1 February 2009.
36. Stanley SA, Johnsdrow JE, Manzanillo P, Cox JS (2007) The Type I IFN response to infection with Mycobacterium tuberculosis requires ESX-1-mediated secretion and contributes to pathogenesis. J Immunol 178: 3143–3152.
37. Desvignes L, Wolf AJ, Ernst JD (2012) Dynamic roles of type I and type II IFNs in early infection with Mycobacterium tuberculosis. J Immunol 188: 6205–6215.
38. Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, et al. (1999) Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). J Exp Med 189: 615–625.
39. Kitchens RL (2000) Role of CD14 in cellular recognition of bacterial lipopolysaccharides. Chem Immunol 74: 61–82.
40. Jung ID, Jrong SK, Lee G-M, Noh KT, Heo DR, et al. (2011) Enhanced efficacy of therapeutic cancer vaccines produced by co-treatment with Mycobacterium tuberculosis heparin-binding hemagglutinin, a novel TLR4 agonist. Cancer Res 71: 2853–2870. doi:10.1158/0008–5472.CAN–10–3487.
41. Pugin J, Heumann ID, Tomasz A, Kravchenko VV, Akamatsu Y, et al. (1994) CD14 is a pattern recognition receptor. Immunity 1: 509–516.
42. Khoo JJ, Forster S, Mansell A (2011) Toll-like receptors as interferon-regulated genes and their role in disease. J Interferon Cytokine Res 31: 13–25. doi:10.1089/jir.2010.0095.
43. Gutierrez O, Pipaon C, Fernandez-Luna JL (2004) Ipaf is upregulated by tumor necrosis factor-alpha in human leukemia cells. FEBS Lett 568: 79–82. doi:10.1016/j.febslet.2004.01.095.
44. Cano-Gauci DF, Song HH, Yang H, McKeirle C, Choo B, et al. (1999) Glypican-3-deficient mice exhibit developmental overgrowth and some of the abnormalities typical of Simpson-Golabi-Behmel syndrome. J Cell Biol 146: 255–264.
