An integrated metagenomic approach to investigating disease heterogeneity in sepsis due to community acquired pneumonia

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Michaelmas Term, 2019

Abstract

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Acknowledgements

Acknowledgements

Declarations

I declare that, unless otherwise stated, all work presented in this thesis is my own. Several aspects of the study relied upon collaboration where part of the work was conducted with or by others.

Details here.

Submitted Abstracts

Abstract Title
Poster: Intensive Care Society State of the Art Meeting (2014)
KL Burnham, J Radhakrishnan, EE Davenport, E Svoren, P Humburg, AC Gordon, P Hutton, C Garrard, CJ Hinds, JC Knight, and the GAinS investigators

Associated Publications

Paper title
Journal (2016)
EE Davenport, **KL Burnham**, J Radhakrishnan, P Humburg, P Hutton, TC Mills, A Rautanen, AC Gordon, C Garrard, AVS Hill, CJ Hinds, JC Knight

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Abbreviations

ACN	Acetonitrile
ARDS	Acute respiratory distress syndrome
AS	Ankylosing spondylitis
WTCHG	Wellcome Trust Centre for Human Genetics

1

Introduction

This chapter presents the aims of this thesis, and provides an overview of pre-existing knowledge relevant to these goals

The overall objective of this thesis is to

1.1 Genetics and gene expression

The human genome was first sequenced completely by the Human Genome Project Consortium in 2003 (**Collins2003**).

Types of genetic variation

A polymorphism is a genetic locus that exists in multiple forms, or alleles, at appreciable frequencies in a population (e.g. minor allele ≥ 1%).

1.2 Specific aims and objectives

The aims of this thesis are to:

- **Investigate the host transcriptome in sepsis of different sources (Ch. 3)**

  The two most common causes of sepsis in the UK are community acquired

pneumonia (CAP) and faecal peritonitis (FP). The host transcriptome in sepsis due to FP has not yet been described, and the clinical differences observed between CAP and FP suggest that variation may also be observed in the molecular response to disease. I aim to:

1. describe the host transcriptome in sepsis due to FP and CAP for peripheral blood leukocytes
2. directly compare gene expression between CAP and FP patients
3. identify temporal changes in gene expression in CAP and FP

- **Compare the sepsis transcriptomic response to related conditions (Ch. 3)**

  The sepsis response is related to the systemic inflammatory response to insults such as surgery and trauma. Comparison of the infectious and sterile responses might be aid interpretation of the biological processes involved. I will therefore:

  1. define the transcriptomic response in:
     (a) sepsis due to CAP and FP, in contrast to non-septic samples
     (b) traumatic injury, in contrast to healthy volunteers
     (c) cardiac surgery, using pre- and post-operative samples
  2. compare these responses across conditions

Bonsall2015

2

Materials and Methods

This chapter describes

2.1 Genomic Advances in Sepsis

The UK Genomic Advances in Sepsis (GAinS) study (http://ukccggains.com) is a multicentre prospective study initiated in 2005 by the UK Critical Care Genomics group to characterise genetic variants that affect susceptibility to and outcomes from sepsis. A bioresource arising from this study includes biological samples and phenotypic information from over 2000 individuals with sepsis from community acquired pneumonia (CAP) or faecal peritonitis (FP) admitted to intensive care units (ICUs). This thesis focuses only on the subset of individuals with sepsis from CAP. Recruitment was initially carried out in 34 ICUs and remains ongoing in 4 ICUs across the UK.

2.1.1 GAinS patient recruitment and exclusion criteria

Sepsis patients were recruited through the GAinS study from 34 participating ICUs between 2005 and 2018. Patients were recruited if they met the diagnostic criteria for severe sepsis in use at the time of study initiation (Sepsis-2, 1992 ACCP/SCCM consensus definition). CAP was defined as febrile illness associated with cough, sputum production, breathlessness, leukocytosis and radiological features of pneumonia acquired prior to or within 48 hours of hospital admission. Exclusion criteria included immunocompromise, admission for palliative care only, and pregnancy.

2.2 Other cohorts

Two other cohorts were studied: (a) patients with hepatitis C virus infection, and (b) patients undergoing cardiac surgery.

2.2.1 Hepatitis C virus infection patient recruitment and exclusion criteria

The hepatitis C virus infection (HCV) cohort was recruited through the NIHR Oxford Biomedical Research Centre Prospective Cohort Study in Hepatitis C.

2.2.2 Cardiac surgery patient recruitment and exclusion criteria

Patients undergoing elective cardiac surgery requiring cardiopulmonary bypass (coronary artery bypass grafting, valve replacement, or valve repair) were recruited by Dr Eduardo Svoren and Professor Charles Hinds (Bart's and the London NHS Trust). This study aimed to investigate the host inflammatory response induced by elective cardiac surgery involving cardiopulmonary bypass.

Patients were excluded if they were immunocompromised, undergoing an emergency operation, had malignancy, or were unable to provide informed consent.

2.3 Metagenomics

2.3.1 Nucleic acid extraction

Total nucleic acid extraction was performed using the NucliSENS easyMag platform (Biomerieux). Typically, 500ul of extracted plasma was eluted in 25 μ l of buffer. Postextraction quality control was performed using the Agilent 2100 Bioanalyzer platform and/or the Qubit dsDNA HS Assay (Thermo Fisher Scientific).

2.3.2 Library preparation methods

Four library prepration methods were evaluated.

1. RNA: We used the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs) with several modifications to the manufacturers guidelines including: fragmentation for 4 minutes at 94°C, omission of Actinomycin D at first-strand reverse transcription, library amplification for 15 PCR cycles using custom indexed primers and post-PCR clean-up with 0.85x volume Ampure XP (Beckman Coulter).

2: DNA: The Nextera DNA Library Preparation Kit (Illumina) was used according to the manufacturer's guidelines.

3: Combined with Fragmentation (CF): This involved the RNA protocol (1) followed by the DNA protocol (2).

4. This involved the RNA protocol (1), with omission of fragmentation, end repair, and adaptor ligation steps, followed by the DNA protocol (2).

2.3.3 Spike ins

RNA: We used the Ambion ERCC RNA Spike-In Mix 1 (Thermo Fisher Scientific) consisting of 92 synthetic transcripts between 250-2000 nucleotides in length, at a range of pre-specified concentrations**Rna2005**

DNA: Multiple restriction enzyme digest of three synthetic plasmids was performed according to manufacturer instructions (New England BioLabs) (Table 2.1).

Plasmid	Original size (bp)	Restriction enzymes	Fragment sizes (bp)
pHBV	6820	AccI, AlwNI, HindIII, NdeI	800, 1178, 1652, 3190
p1990	4808	AccI, AlwNI, HindIII	401, 588, 1796, 2023
p2022	3356	AccI, AlwNI, NdeI	379, 1099, 1878

Table 2.1: DNA spike-in controls. Plasmids, restriction enzymes, and resulting fragment sizes.

The three plasmids were pooled in equal mass ratios and spiked-in at 3% sample DNA concentration by mass.

2.3.4 Probe-based enrichment

In collaboration with a paediatric meningitis study, a custom probe panel covering bacterial and viral pathogens relevant to meningitis and pneumonia was designed using the Agilent SureDesign service. This included probes complementary to three ERCCs (ERCC14, ERCC25, ERCC116) and the pHBV plasmid fragment. The probe set included 52,101 120nt RNA oligonucleotide probes (5.87×10^6 bp).

1μg of each indexed pooled library was enriched using the Agilent SureSelect^{XT}

Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library protocol with one major modification to the recommended protocol. This involved capture on a post-PCR indexed pool with use oligonucleotide blockers complementary to adapter sequences.

2.3.5 Data processing

2.4 Digital droplet PCR

Digital droplet PCR (ddPCR) was performed for targets from several microorganisms: (a) influenza, (b) *S. pneumoniae*, (c) Epstein-Barr virus, and (d) cytomegalovirus. The assay was performed on samples following nucleic acid extraction as above. For the RNA-based pathogen (influenza) following nucleic acid extraction, we performed first strand cDNA synthesis (SuperScript III First-Strand Synthesis System, Invitrogen).

Sample processing was performed in triplicate (1.5μl per replicate) following the recommended workflow (QX200 ddPCR system, Bio-Rad). Custom-designed PrimeTime (IDT) primer/probe sets targeting the influenza A matrix (M) and *S. pneumoniae* capsular polysaccharide biosynthesis (*cpsA*) genes were designed based on published sequence data.

2.5 Epstein Barr Virus Serology

Enzyme linked immunosorbent assay was used to test for the presence of IgG and IgM antibodies against the EBV viral capsid antigen (VCA) using proprietary kits (Abcam). The manufacturer's instructions were followed. Plasma samples (10ul) were diluted to the recommended 1:100 concentration and run in duplicate. Absorbance was measured at 450 nm using the X plate reader. Samples were

considered to be positive if the absorbance value was greater than 10% over the cut-off control absorbance value.

2.6 Transcriptomics

2.6.1 Sample collection

2.6.2 RNA extraction

2.6.3 Microarray analysis

2.6.4 Data processing and analysis

Four microarray datasets were combined for analysis. The datasets included GAinS patients (both CAP and FP) as well as the negative control Cardiac Surgery patients. Quality control was performed for each dataset individually and outliers removed before the individual datsets were combined. Prior to QC, 19 samples from the Radhakrishnan cohort (2 mislabelled, 5 replicates, 12 missing consent, 1 CAP misdiagnosis) and 84 samples from the Davenport cohort (48 failed hybridsation, 34 missing consent, 2 CAP misdiagnosis, 1 withdrawn consent) were excluded.

The QC for each dataset involved filtering out probes with a detection value of ¡0.95 in ¿95% of samples followed by normalisation using the Variance Stabilisation and Normalisation (vsn) R package **Huber2002**

After the four datasets were combined, probe filtering was repeated using the same parameters described above followed by normalisation using vsn. Using principal component analysis, clear batch effects were seen and the ComBat function from the R package sva was used to directly estimate and remove the

Dataset	Samples post-QC	CAP	FP	Cardiac
Radhakrishnan 2010	236	124 (39/45/40)	94 (37/34/23)	18 (6/6/6)
Davenport 2011	339	262 (130/86/46)	0	77 (39/38)
Burnham 2014	159	106 (42/42/22)	53 (25/15/13)	0
Burnham 2016	143	72 (24/24/24)	71 (23/24/24)	0
Total	877	564	218	95

Table 2.2: Summary of GAinS gene expression data
The number of samples is documented in the table with the number of patients at each time point in brackets. The three CAP and FP GAinS time points are day 1, day 3 and day 5 of ICU admission. The three Cardiac time points are prior to induction of anesthesia, immediately post-operative, 24 hours post-operative.

known batch effects.

3

Results1

This chapter explores

3.1 Introduction

3.1.1 Difficulties in defining and diagnosing sepsis

Sepsis is currently defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection".

3.1.2 Aims

1. Aim 1
2. Aim 2
3. Aim 3

3.2 Results: sepsis

3.2.1 Description of sepsis cohorts

In this chapter, I analyse sepsis microarray gene expression data processed in several different batches over a six year period (Fig. 3.1).

3.3 Discussion

3.4 Conclusions

The analysis presented in this chapter combines multiple transcriptomic datasets to compare the systemic inflammatory response across different sources. This demonstrates that a significant proportion of the sepsis transcriptomic response is shared between CAP and FP patients, although there is some specificity according to infection site. These findings may have implications for the management of sepsis, suggesting patient stratification and targeting treatment on the basis of cause of sepsis could be beneficial. In addition, future research and drug development may benefit from the use of more homogeneous cohorts. However, aspects of the transcriptomic response are shared across sepsis due to CAP and FP and sterile SIRS, indicating that findings in one SIRS subtype might be relevant to another and that similar treatment strategies could be considered.

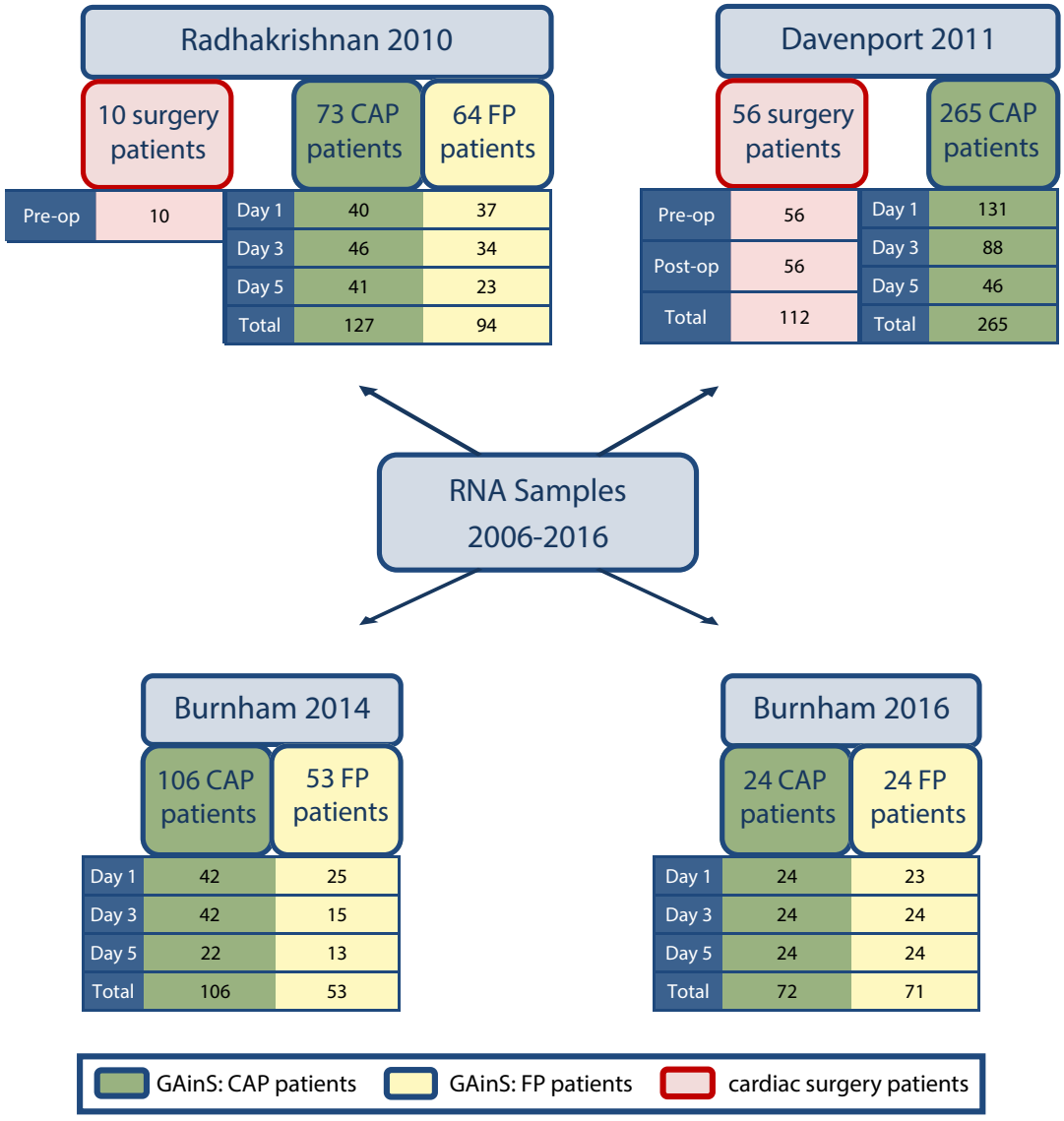

Figure 3.1: Overview of the GAinS gene expression datasets analysed in this thesis. The number of samples for which gene expression data are available following quality control is given for each of the four cohorts analysed in this thesis. This is subdivided according to the cause of sepsis (CAP (*green*) or FP (*yellow*)), given the focus on source of infection in this chapter. Cohorts are named according to the person who generated the data and the year this was done. Samples from elective cardiac surgery patients (*red*), taken before and after their operation by Eduardo Svoren, are also included in the Radhakrishnan 2010 and Davenport 2011 cohorts.

4

General Discussion

This chapter outlines the broader conclusions and future directions suggested by the work described in this thesis

General intro

4.1 A section

4.2 Limitations and future work

4.3 Conclusion

Conclusions.

A

Results1

Clinical covariate	P Value	FDR	CAP	FP	
Sex	0.523	0.777	0.51	0.44	NS
Activated protein C	0.239	0.495	0.10	0.03	NS
Corticosteroid	0.216	0.490	0.16	0.27	NS
Renal failure	0.929	0.963	0.21	0.19	NS
Renal replacement therapy	1.000	1.000	0.08	0.08	NS
Ventilation	0.051	0.164	0.70	0.53	NS
Inotropes	0.735	0.867	0.48	0.45	NS
Comorbidities: Cardiac	0.797	0.867	0.40	0.48	NS
Comorbidities: Respiratory	0.160	0.403	0.34	0.25	NS
Comorbidities: Renal	0.366	0.496	0.05	0.11	NS
Comorbidities: Neurological	0.796	0.867	0.10	0.13	NS
Comorbidities: GI	0.002	0.020	0.04	0.27	**
Comorbidities: Immune	0.103	0.297	0.11	0.25	NS
Comorbidities: Infection	0.706	0.867	0.05	0.05	NS
Comorbidities: Endocrine	0.178	0.434	0.18	0.11	NS

Table A.1: Comparison of discrete clinical covariates between CAP and FP.
Discrete variables were compared by Chi-square test. The proportion for each is given for CAP and FP.

Table A.2: Summary of genes differentially expressed between CAP and FP.
40 most significantly differentially expressed probes.

Gene Symbol	Log_2 fold change	Mean CAP	Mean FP	FDR
EPSTI1	2.25	10.17	7.91	$2.97x10^{-8}$
XAF1	1.70	9.71	8.00	$2.12x10^{-7}$
IFIH1	1.20	9.03	7.83	$2.12x10^{-7}$
HERC5	1.82	10.33	8.51	$2.12x10^{-7}$
LYSMD2	1.06	10.82	9.76	$2.12x10^{-7}$
OAS2	1.58	10.04	8.46	$2.12x10^{-7}$
TDRD9	-1.30	8.80	10.10	$2.12x10^{-7}$
LY6E	1.56	10.56	8.99	$4.12x10^{-7}$
MSRA	-0.83	9.02	9.85	$4.12x10^{-7}$
IFIT1	2.35	10.02	7.67	$4.12x10^{-7}$
IFIT3	1.96	9.91	7.95	$4.12x10^{-7}$
FLJ31222	-0.62	6.22	6.84	$4.12x10^{-7}$
C20ORF3	-0.65	11.05	11.71	$4.50x10^{-7}$
FBXW2	-0.76	7.86	8.62	$4.50x10^{-7}$
ATP9A	-1.11	9.80	10.91	$5.45x10^{-7}$
TDRD9	-1.25	8.88	10.13	$6.71x10^{-7}$
BTN3A3	0.84	7.40	6.56	$6.71x10^{-7}$
IFIT2	1.56	11.90	10.34	$6.71x10^{-7}$
NQO2	-0.84	9.94	10.78	$7.40x10^{-7}$
PARP14	0.99	9.58	8.59	$8.28x10^{-7}$
FAM101B	-0.75	9.08	9.83	$8.80x10^{-7}$
BTN3A1	1.01	8.74	7.73	$8.85x10^{-7}$
GADD45A	-1.10	10.08	11.18	$8.85x10^{-7}$
PSME2	0.68	10.71	10.03	$8.88x10^{-7}$
REC8	0.59	8.94	8.35	$1.34x10^{-6}$
CKAP4	-0.72	11.72	12.44	$1.59x10^{-6}$
LILRA6	-0.74	11.10	11.84	$1.93x10^{-6}$
PFKFB2	-1.41	8.45	9.86	$1.93x10^{-6}$
IFI44L	2.13	8.65	6.52	$1.93x10^{-6}$
ZDHHC19	-2.09	9.84	11.93	$1.94x10^{-6}$
BIRC3	0.75	9.12	8.37	$2.32x10^{-6}$
CA4	-0.96	10.98	11.94	$2.32x10^{-6}$
EPHB1	0.86	6.84	5.98	$2.32x10^{-6}$
MMP9	-0.91	13.37	14.27	$2.40x10^{-6}$
WIPI1	-0.65	8.67	9.31	$2.40x10^{-6}$
KIF3C	-0.62	6.08	6.70	$2.40x10^{-6}$
ISG15	1.63	9.76	8.13	$2.58x10^{-6}$
PFKFB2	-1.03	7.48	8.51	$2.59x10^{-6}$
IFI44	1.58	9.94	8.36	$3.01x10^{-6}$
EFCBP1	-1.32	5.82	7.15	$3.19x10^{-6}$

Gene Symbol	Log_2 fold Change	Mean Viral	Mean Non-Viral	FDR
IFI27	3.47	9.22	5.77	$7.14x10^{-5}$
XAF1	1.59	9.18	7.60	0.014
SPATS2L	1.20	6.84	5.66	0.014
LOC389386	0.86	5.88	5.02	0.014
JUP	1.65	6.43	4.77	0.018
LGALS3BP	1.11	4.87	3.77	0.020
BTN2A2	0.74	4.94	4.21	0.020
SERPING1	1.92	7.50	5.60	0.026
GALM	0.93	8.37	7.46	0.026
MOV10	0.88	7.60	6.73	0.026
EPSTI1	1.89	11.10	9.23	0.029
TGIF2	0.86	6.81	5.96	0.031
LY6E	1.49	11.07	9.60	0.033
Hs.125087	1.23	6.82	5.60	0.033
OAS1	1.20	9.25	8.06	0.033
Hs.72010	0.82	5.74	4.93	0.033
CD2AP	0.76	4.93	4.18	0.033
IFI44L	2.39	9.77	7.40	0.033
IFI44	1.49	10.54	9.06	0.033
BATF2	1.48	5.91	4.45	0.033
OAS2	1.39	5.72	4.34	0.033
TIMM10	1.35	8.89	7.56	0.033
PARP12	0.91	9.81	8.90	0.033
HERC6	0.83	7.70	6.88	0.033
SCO2	0.79	10.44	9.66	0.033
SP140	0.74	8.30	7.57	0.033
ZCCHC2	0.68	3.76	3.08	0.033
PSME2	0.58	11.07	10.49	0.033
OAS3	1.64	8.76	7.14	0.033
LAP3	0.81	9.21	8.41	0.035
LOC652694	1.28	8.48	7.20	0.040
HES4	0.98	7.68	6.70	0.041
RSAD2	1.74	8.24	6.52	0.043
OTOF	1.37	6.06	4.71	0.043
IFIH1	0.96	9.11	8.16	0.043
LAMP3	0.81	5.24	4.44	0.043
RTP4	1.37	7.30	5.94	0.044
ISG15	1.49	10.45	8.97	0.049
C19orf12	0.63	7.92	7.29	0.049
ECHDC3	-1.15	7.40	8.55	0.049

Table A.3: Summary of genes differentially expressed between viral and non-viral patients.

Table A.4: Summary of genes differentially expressed between CAP and FP (validation).
40 most significantly differentially expressed probes.

Gene Symbol	Log_2 fold change	Mean CAP	Mean FP	FDR
PATL2	1.12	8.15	7.04	$2.93x10^{-11}$
LOC197135	0.96	7.62	6.66	$2.35x10^{-10}$
BTN3A1	1.38	9.12	7.74	$2.35x10^{-10}$
CA4	-1.12	11.04	12.17	$7.92x10^{-10}$
IFIT3	2.28	10.14	7.86	$1.22x10^{-9}$
HERC5	1.78	10.14	8.36	$6.54x10^{-9}$
TAP2	1.00	9.04	8.04	$6.54x10^{-9}$
IFIT1	2.44	9.52	7.08	$1.18x10^{-8}$
HS.573264	0.48	7.38	6.91	$1.27x10^{-8}$
GFOD2	-0.57	7.92	8.49	$1.33x10^{-8}$
IFIT2	1.78	11.88	10.10	$1.72x10^{-8}$
ODF3B	0.93	7.93	7.01	$2.27x10^{-8}$
EPSTI1	2.15	9.74	7.59	$2.53x10^{-8}$
IFIH1	1.21	8.71	7.50	$2.95x10^{-8}$
PARP14	1.11	9.73	8.62	$4.25x10^{-8}$
POLB	0.84	9.41	8.57	$6.18x10^{-8}$
XAF1	1.66	9.66	8.00	$6.95x10^{-8}$
PDE4B	0.99	9.30	8.31	$8.53x10^{-8}$
TCTN1	0.57	6.79	6.23	$8.53x10^{-8}$
PSMB9	0.68	8.11	7.43	$1.84x10^{-7}$
PECR	-1.02	8.53	9.55	$1.84x10^{-7}$
TDRD9	-1.49	8.94	10.43	$2.86x10^{-7}$
ZNF282	-0.54	7.37	7.91	$5.20x10^{-7}$
GBP1	1.53	8.84	7.32	$5.65x10^{-7}$
MMP9	-0.99	13.19	14.18	$5.65x10^{-7}$
LYSMD2	1.04	10.69	9.65	$5.65x10^{-7}$
TDRD9	-1.51	8.74	10.25	$6.65x10^{-7}$
SCO2	1.02	10.33	9.32	$8.58x10^{-7}$
GBP1	1.49	9.65	8.16	$9.76x10^{-7}$
UBE2L6	0.87	11.91	11.04	$1.02x10^{-6}$
PCMT1	-0.48	11.56	12.04	$1.15x10^{-6}$
BTN3A3	0.78	7.56	6.77	$1.23x10^{-6}$
GBP5	1.39	9.81	8.41	$1.24x10^{-6}$
STAT1	1.02	9.87	8.84	$1.35x10^{-6}$
ABCG1	1.25	9.00	7.75	$1.42x10^{-6}$
ABCG1	0.93	7.21	6.28	$1.57x10^{-6}$
IER5	0.65	8.73	8.08	$1.57x10^{-6}$
PSME1	0.48	12.52	12.04	$1.57x10^{-6}$
HS.445414	0.68	8.51	7.83	$1.82x10^{-6}$
LOC282997	0.68	7.41	6.74	$1.82x10^{-6}$

B

Results2

Table B.1: In vitro studies of the transcriptomic endotoxin tolerance response.

Study	Samples	Platform	ET Analysis Approach	Accession
Foster2007	Mouse macrophages (n=2): untreated (N), stimulated once (N+L) or twice (T+L) with LPS	Affymetrix Mouse Genome 430 2.0 arrays	Only LPS-induced genes considered. Tolerizeable genes defined as (N+L)/(T+L) >3. Non-tolerizeable genes defined as (N+L)/(T+L) ≥ 1	GSE7348
Mages2007	Mouse bone marrow derived macrophages (n=3): prestimulated (1_X) or not (0_X) for 18h, 2h rest, cells either restimulated (X_1) or not (X_0)	Affymetrix GeneChip MOE430 2.0	All pairwise comparisons between treatments, presented as comparisons with untreated cells	GSE8621
Fresno2009	Human monocytes (n=2): unstimulated, stimulated, and tolerant cells with three different exposure times	Illumina Sentrix HumanRef-8_V2 BeadChip	LPS-induced genes classified as tolerizeable and nontolerizeable in the ET phase, genes moving between these groups over time described	GSE15219
Pena2011	Human PBMCs (n=4): untreated or challenged with LPS (10 ng/ml) in a single dose (LPS) or two doses at a 24h interval (LPS/LPS) and incubated for 4h	Illumina/Genome BC Microarray Platform	ET: differentially expressed between LPS/LPS and untreated cells.	GSE22248

Yang2012	Human PBMCs (n=3) left untreated (N) or LPS stimulated for 16h (T), washed and 2h recovery; then given media (N, T) or LPS 6h (N+L, T+L)	NimbleGen array and GenePix Pro 6.0	Gene expression compared between untreated, stimulated, restimulated. T vs N most changes, but 356 non-tolerizeable genes also identified and focussed on as potential regulators of TLR-induced inflammation.	NA
Allantaz-Frager2013	Healthy PBMCs (n=6): unstimulated (medium), 1 dose LPS (LPS unprimed), 2 doses of LPS (LPS primed) or 2 doses of LPS and interferon gamma (LPS primed + IFNg)	GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix)	43 transcripts DE between LPS unprimed and LPS primed, 70 between medium and LPS unprimed - split into tolerizeable and non-tolerizeable. Selected transcripts associated with development of ET identified, expression restored after immunostimulation with IFN-γ. Validation by qRT-PCR in healthy donors and septic patients	GSE46914
OCarroll2014	Mouse bone marrow derived macrophages (n=3). Untreated (N), acute response to LPS (LPS activation group), LPS tolerance (T) and recovered (RM).	Agilent mouse 8x60K arrays	*k*-means clustering showed unique transcriptional signature for each treatment group, 10 gene expression profiles across groups. 30 up and downregulated genes compared with recovered macrophages. Focus on recovery from endotoxin tolerance, no gene list for tolerance given.	GEO accession number: GSE47783 raw and processed data