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Exploring the bidirectional interface between stress and innate immunity: a focus on glucocorticoid and TLR4-MyD88 signalling

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by

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Thesis abstract

Multiple bidirectional interactions between stress neuroendocrine and innate immunity have been identified in previous research, but the extent of these interactions remain unresolved. This thesis thus aimed to explore the bidirectional interface between stress and innate immunity, by focusing on glucocorticoid and TLR4-MyD88 signalling. The research was undertaken in a series of 3 studies.

Study 1 investigated the impact of baseline TLR4-MyD88 signalling on the neuroendocrine and behavioural responses to acute stress. Through examining stress responses in mice lacking *Tlr4* or *Myd88,* this study found an intrinsic influence of TLR4-MyD88 signalling on both neuroendocrine and behavioural responses. Aadaptations to the feedforward and feedback pathways of glucocorticoid signalling were also identified.

Studies 2 and 3 explored the effects of glucocorticoid signalling on innate immune function in immunocompetent cells. In these 2 studies, BV2 microglia-like cells, RAW264.7 macrophage-like cells and adult primary microglia were utilised. Study 2 demonstrated a biphasic innate immune response to glucocorticoids, where low concentrations of corticosterone pre-exposure primed, while a high stress-like concentration of corticosterone suppressed TLR4-NF-κB-IL-1β responses. Using pharmacological antagonists, it was further revealed that the priming effect on IL-1β was mediated by mineralocorticoid receptor (MR) signalling, while immunosuppressive actions were mediated by glucocorticoid receptor (GR) signalling.

Study 3 further assessed glucocorticoid actions on non-cytokine innate immune responses via measurements of cell motility, cell death and danger-associated molecular pattern (DAMP)-related protein release. Here, a low concentration of corticosterone increased ATP-induced BV2 cell motility. Signalling via GR, a stress-like concentration of Corticosterone and Dexamethasone caused an increase in cytotoxicity and HMGB1 DAMP protein release.

Collectively, the findings in this thesis provide support for multiple intrinsic connections between the stress neuroendocrine and TLR4 signalling pathway, both *in vivo* and *in vitro.* Further mechanistic insights such as GR and MR signalling were revealed, and the implications of this work to health and disease were also discussed.

Declaration

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"If your confusion leads you in the right direction, the results can be uncommonly rewarding."

-Haruki Murakami, Hardboiled wonderland, and the end of the world

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List of Abbreviations

ACTH	Adrenocorticortropic Hormone
AIM1	Absent in Melanoma 1 Protein
AP-1	Activator Protein 1
ATP	Adenosine Triphosphate
BCL-2	B-cell lymphoma 2
CBG	Corticosteroid Binding Globulin
CD11B	Cluster of Differentiation 11B
CD14	Cluster of Differentiation 14
CORT	Corticosterone
CRF	Corticotropin releasing factor
DAMP	Danger Associated Molecular Pattern
DEX	Dexamethasone
FASLG	Fas Ligand
GC	Glucocorticoid
GR	Glucococrticoid receptor
HMGB1	High Mobility Group Box 1
HPA	Hypothalamus Pituitary Adrenal
IBA-1	Ionised Calcium-binding Adapter 1
IFN-β	Interferon β
IkB	Nuclear Factor of Kappa Light Polypeptide
IKK complex	IkB kinase
IL-18	Interleukin 18
IL-1β	Interleukin 1β
IL-1R1	Interleukin 1 Receptor 1
IL-1RA	Interleukin 1 Receptor Antagonist
IL-6	Interleukin 6
IRAK1	Interleukin 1 Receptor-Associated Kinase 1
IRAK4	Interleukin 1 Receptor-Associated Kinase 4
IRF3	Interferon Regulatory Factor 3
LDH	Lactate Dehydrogenase
LPS	Lipopolysaccharide
MAMP	Microbe-Associated Molecular Pattern
MAP kinases	Mitogen-Activated Protein Kinase
MD2	Myeloid Differentiation Factor 2
MR	Mineralocorticoid Receptor
MyD88	Myeloid Differentiation Primary Response 88
Myd88$^{-/-}$	MyD88 knockout
NF-kB	Nuclear Factor Kappa B
NLRP1	NACHT, LRR and PYD domains-containing protein 1
NLRP3	NACHT, LRR and PYD domains-containing protein 3

P2X7	P2X purinoceptor 7
PAMP	Pathogen-Associated Molecular Pattern
PVN	Paraventricular Nucleus of the Hypothalamus
RAAS	Renin-Angiotensin-Aldosterone System
TAB1	TGF-β activated kinase 1/MAP3K7 binding protein 1
TAB2	TGF-β activated kinase 1/MAP3K7 binding protein 2
TAK1	Transforming growth factor β-activated Kinase 1
TBK1	TANK Binding Kinase 1
TLR	Toll-like Receptor
Tlr4$^{-/-}$	Toll-like Receptor 4 knockout
TNF- α	Tumor Necrosis Factor α
TRAF6	TNF Receptor Associated Factor 6
TRIF	TIR-domain-containing adaptor-inducing interferon-B
XAMP	Xenobiotics-Associated Molecular Pattern