Heat shock proteins: Interactions with bone and immune cells

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

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DECLARATION

The work in this thesis is original and has not been submitted previously in support of any qualification or course.

Signed:

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ABSTRACT

Heat shock proteins (Hsps) are increasingly being seen as having roles other than those of intracellular molecular chaperones, particularly with regard to their potential to act as cytokines, and to stimulate the innate immune system. Hsps have also been found to promote bone resorption and osteoclast formation *in vitro*, although the mechanism has not been previously identified. The overall aims of this thesis were to determine whether Hsps could stimulate bone resorption by affecting the RANKL/OPG pathway, and to address the hypothesis that Hsps can act as a danger signal to the innate immune system.

In order for Hsps to affect either the RANKL/OPG system of bone resorption or act as danger signals they would need to be actively released from cells, ideally in a controlled manner following exposure to the source of stress. Hsp60 and Hsp70 were found to be released from a range of immune cells including the cell lines Jurkat and U937, and also PBMCs, T-cells and B-cells. This release was not due to cell damage. The release of Hsp60 and Hsp70 were downregulated by inhibitors of protein secretion, in particular Hsp70 release was reduced by compounds that inhibited lysosomal pathways and Hsp60 release by classical secretion inhibitors.

Hsp60, Hsp70, GroEL and LPS all affected the RANKL/OPG system of bone regulation; OPG production and release was down-regulated in the MG63 and GCT osteoblast-like cell lines following treatment with Hsp60, Hsp70 and LPS, and RANKL expression was upregulated following treatment with Hsp60, Hsp70, GroEL and LPS. This effect on the RANKL/OPG system was found to translate into an effect on osteoclast formation when conditioned media from treated osteoblasts was added to osteoclast precursors in the presence of M-CSF.

A range of different factors that affected Hsp release were identified; PHA activation of PBMCs was found to upregulate Hsp60 release from PBMCs. GroEL and LPS caused an upregulation in Hsp70 release from PBMCs and GCT osteoblast like cells, and Hsp70 was found to stimulate Hsp60 release from PBMCs and GCT cells. These responses of Hsp release were used to form a theory of a cascade-like danger signal that may occur when cells are exposed to bacterial infection and which would result in activation of antigen presenting cells *via* previously identified receptors for Hsps such as CD14/TLR4 or by unidentified pathways. The elevated release of Hsps in response to GroEL and LPS was also identified as a mechanism that could stimulate bone loss during infection or autoimmunity by affecting the RANKL/OPG system.

In conclusion, Hsp60 and Hsp70 can be released from immune cells under normal conditions, and from both immune and osteoblast-like cells following stimulation with LPS and other Hsps. The observed release responses provide a mechanism through which Hsps can act as danger signals to the innate immune system, and also as promoters of bone resorption via the RANKL/OPG system.

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ABBREVIATIONS

$1,25(OH)_2D_3$	1,25 dihydroxyvitamin D_3
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
CD	Cluster designation
cDNA	Complimentary DNA
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic Reticulum
GCT	Giant cell tumour
HSE	Heat shock element
HSF	Heat shock factor
HSP	Heat shock protein
IFN	Interferon
IL	Interleukin
M-CSF	Macrophage colony stimulating factor
MG63	Osteosarcoma cell line
mRNA	Messenger RNA
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
OPG	Osteoprotegerin
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGE_2	Prostaglandin E2
PHA	Phytohaemagglutinin
PTH	Parathyroid hormone
RA	Rheumatoid arthritis
RANK	Receptor activator of NF-kappaB

RANKL	Receptor activator of NF-kappaB ligand
RT-PCR	Reverse transcription polymerase chain reaction
SDS-PAGE	Sodium dodecyl sulfate - Polyacrylamide gel electrophoresis
SEM	Standard error of the mean
TCR	T-cell antigen receptor
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TRAIL	Tumour necrosis factor (TNF)-related apoptosis inducing ligand
U937	Human monocytic leukaemia cell line