

Heat shock proteins: Interactions with bone and immune cells

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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:

Date:

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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.

CONTENTS

Acknowledgements	i
Abstract	ii
List of contents	iii
List of figures	x
List of tables	xv
Abbreviations	xvii

CHAPTER 1 - INTRODUCTION

1.1	Project background	1
1.2	Heat shock proteins	1
1.2.1	HSP 60	4
1.2.2	HSP70	5
1.3	Extracellular Hsps	7
1.4	Cells of the immune system	8
1.4.1	Lymphocytes	8
1.4.2	Monocytes and Macrophages	10
1.5	Hsps as promoters of disease	11
1.6	Cytokine roles of Hsp60 and Hsp70	12
1.7	Anti-inflammatory role of Hsps	14
1.8	Release of Hsps from cells.	16
1.8.1	Classical/non-classical protein secretion	17
1.9	Regulation of bone remodelling - Bone cells	18
1.9.1	Osteoblasts	18

1.9.2	Osteoclasts	20
1.9.3	Osteocytes and Bone lining cells	21
1.10	Regulation of bone remodelling - RANKL/OPG/RANK system	21
1.10.1	OPG	21
1.10.2	RANKL	22
1.10.3	OPG/RANKL/RANK interaction	24
1.10.4	Markers of bone turnover	25
1.11	Relationship between bone and the immune system	25
1.12	Bone and Hsps	27
1.13	Project aims	28
CHAPTER 2 - MATERIALS AND GENERAL METHODS		
2.1	Equipment	29
2.2	Reagents	30
2.3	General Methods	39
2.3.1	MG63 (osteosarcoma) cell line culture	39
2.3.2	Giant Cell Tumour (GCT) cell line culture	39
2.3.3	U937 (human monocytic leukaemia) cell line culture	39
2.3.4	Jurkat (human T-cell leukaemia) cell line culture	40
2.3.5	Isolation and culture of osteoclast precursors from bone marrow	40
2.3.5.1	Removal of murine femora and tibiae	40
2.3.5.2	Isolation of osteoclast precursors	40
2.3.5.3	Culture of osteoclast precursors	41
2.3.6	Isolation and culture of Peripheral Blood Mononuclear Cells (PBMCs)	41
2.3.7	Cell counting	42
2.3.8	MTS Assay	42

2.3.9	mRNA extraction from cells	42
2.3.9.1	Binding of mRNA to Oligo dT cellulose	42
2.3.9.2	Washing Steps	43
2.3.9.3	Elution of the mRNA	43
2.3.10	cDNA synthesis using the First-Strand cDNA Synthesis Kit	43
2.3.11	cDNA synthesis using Ready To Go You Prime First-Strand Beads	44
2.3.12	Reverse Transcription Polymerase Chain Reaction (RT-PCR)	44
2.3.12.1	Primer Sequences	44
2.3.12.2	RT-PCR Reaction Mix	47
2.3.12.3	PCR cycles	48
2.3.13	Agarose Electrophoresis	49
2.3.14	SDS-PAGE gels	50
2.3.14.1	Denaturing gel preparation	50
2.3.14.2	Stacking gel preparation	50
2.3.14.3	Running SDS-PAGE gels	50
2.3.15	Western Blotting	51
2.3.15.1	Protein transfer	51
2.3.15.2	Immunostaining of Western Blots	51
2.3.16	Quantification of OPG using ELISA	52
2.3.17	Quantification of Hsp60 using ELISA	53
2.3.18	Quantification of Hsp70 using ELISA	53
2.3.19	LDH Assay	54
2.3.20	Trap activity assay	54
2.3.21	Trap Staining	54

CHAPTER 3 - HEAT SHOCK PROTEINS AND BONE REMODELLING

3.1	Introduction	56
3.2	Methods	58
3.2.1	Heat shock of MG63 cells	58
3.2.2	Restriction Digest of Hsp primer PCR products	58
3.2.3	Addition of GroEL and 1,25(OH)₂D₃ to MG63 cells	58
3.2.4	Addition of Hsp60, Hsp70 and GroEL to MG63 cells	59
3.2.5	Treatment of GCT with Hsp60, Hsp70, GroEL, LPS and 1,25 (OH)₂D₃	59
3.2.6	Addition of Hsp60, Hsp70, LPS and a range of GroEL concentrations +/- Polymyxin B to GCT cells	60
3.2.7	Treatment of osteoclast precursors with Hsp60, Hsp70, GroEL, LPS and 1,25(OH)₂D₃ conditioned GCT media	61
3.3	Results	63
3.3.1	Heat shock and restriction digest	63
3.3.2	Effect of GroEL and 1,25(OH)₂D₃ on osteocalcin and RANKL expression in MG63 cells	63
3.3.3	Addition of Hsp60, Hsp70 and GroEL to MG63 cells	64
3.3.3.1	Effect on OPG production	64
3.3.3.2	Effect on cell proliferation/metabolic activity	64
3.3.3.3	Effect on gene expression	64
3.3.4	Treatment of GCT with Hsp60, Hsp70, GroEL, LPS and 1,25 (OH)₂D₃	65
3.3.4.1	Effect on OPG production	65
3.3.4.2	Effect on cell proliferation/metabolic activity	65
3.3.4.3	Effect on gene expression	66
3.3.5	Treatment of osteoclast precursors with Hsp60, Hsp70, GroEL, LPS and 1,25(OH)₂D₃ conditioned GCT and non-conditioned media	66
3.3.5.1	Effect of Hsps and LPS treated media (non-conditioned) on Trap activity	66

3.3.5.2	Effect of conditioned media from Hsp and LPS treated GCT cells on Trap activity	67
3.3.5.3	Effect of Hsps, LPS and 1,25(OH) ₂ D ₃ treated media (non-conditioned) on Trap activity in the presence of M-CSF	67
3.3.5.4	Effect of conditioned media from GCT cells treated with Hsps, LPS and 1,25(OH) ₂ D ₃ in the presence of M-CSF	67
3.4	Discussion	90

CHAPTER 4 - RELEASE OF HSPTS FROM IMMUNE CELLS

4.1	Introduction	94
4.2	Methods	96
4.2.1	Measurement of Hsp release from immune cells in normal and heat shock conditions	96
4.2.1.1	Cell preparation	96
4.2.1.2	Heat treatment	96
4.2.1.3	Hsp release time course	96
4.2.2	Treatment of PBMCs with inhibitors of protein release	96
4.2.3	Collection, storage and analysis of cellular and media samples	97
4.2.4	Isolation of T and B cells	98
4.2.5	Hsp release from T and B cells	98
4.3	Results	100
4.3.1	Hsp60 release from U937, Jurkat and PBMCs	100
4.3.2	Hsp70 release from U937, Jurkat and PBMCs	100
4.3.3	Western blot and gene expression	101
4.3.4	LDH and cell viability counts	101
4.3.5	Protein release inhibitors	102
4.3.5.1	Inhibition of Hsp60 release	102
4.3.5.2	Inhibition of Hsp70 release	102

4.3.6	T and B cell Hsp release	102
4.4	Discussion	128
CHAPTER 5 - THE EFFECT OF GLUCOCORTICOIDS AND PHA ON HSP60 AND HSP70 EXPRESSION AND RELEASE IN PBMCs.		
5.1	Introduction	131
5.2	Methods	132
5.2.1	Detection of Hsp expression in PBMCs	132
5.2.2	Detection of Hsp expression in Whole Blood	132
5.2.3	Treatment of PBMCs with PHA	132
5.2.4	Addition of PHA, dexamethasone and prednisolone to PBMCs	133
5.2.5	Addition of PHA to negative T-cells	133
5.2.6	Cell counts	134
5.3	Results	135
5.3.1	Optimisation of RT-PCR to detect Hsp gene expression in PBMCs and whole blood	135
5.3.2	Optimisation of RT-PCR to detect RANKL in PBMCs	135
5.3.3	The effect of PHA activation on the expression of Hsps, RANKL, IFN- β , and TNF- α in PBMCs	135
5.3.4	Optimisation of CD25 primers	136
5.3.5	The effect of a 24 h treatment with PHA or a combination of PHA and dexamethasone or prednisolone on Hsp gene expression in PBMCs	136
5.3.6	The effect of a 24 h treatment with PHA or a combination of PHA and dexamethasone or prednisolone on CD25, RANKL, IFN- β and TNF- α gene expression in PBMCs	137
5.3.7	Effect of PHA and steroids on the production and release of Hsp60 and Hsp70 in PBMCs and T-cells	137
5.3.7.1	The effect of PHA activation and prednisolone on release and intracellular levels of Hsp60 in PBMCs at 8 and 24 h time points	137
5.3.7.2	The effect of PHA activation and prednisolone on release and intracellular levels of Hsp70 in PBMCs at 8 and 24 h time points	138

5.3.7.3	The effect of PHA on Hsp70 release and intracellular levels in T-cells.	138
5.4	Discussion	158
CHAPTER 6 - THE EFFECT OF HSP AND LPS TREATMENT ON HSP60 AND HSP70 RELEASE FROM IMMUNE AND OSTEOBLAST- LIKE CELLS		
6.1	Introduction	161
6.2	Methods	162
6.2.1	Addition of Hsp60, Hsp70, GroEL and LPS to Jurkat cells and PBMCs	162
6.2.2	Addition of GroEL to T and B cells	162
6.2.3	Addition of Hsp60, Hsp70, GroEL and LPS to GCT cells	163
6.3	Results	164
6.3.1	Effect of incubation with Hsp70, GroEL and LPS on the release of Hsp60 from PBMCs and Jurkat cells	164
6.3.2	Effect of incubation with Hsp60, GroEL and LPS on the release of Hsp70 in PBMCs and Jurkat cells	164
6.3.3	LDH and MTS results following treatment of PBMCs and Jurkat cells with Hsps and LPS	165
6.3.4	Gene expression for Hsp60, Hsp70 and CD25 in PBMCs following 24 h treatment with Hsp60, Hsp70, GroEL and LPS	166
6.3.5	Effect of GroEL on Hsp70 release from isolated negative T and B cells	166
6.3.6	Effect of Hsp70, GroEL and LPS on Hsp60 release from GCT cells	166
6.3.7	Effect of Hsp60, GroEL and LPS on Hsp70 release from GCT cells	167
6.4	Discussion	187
CHAPTER 7 – DISCUSSION		
7.1	Release of Hsps from Immune cells	190
7.2	Hypothesis 1 – Hsps cause increased bone resorption by acting on the RANKL/OPG system.	192
7.3	Hypothesis 2 – Hsps act as a danger signal to the innate immune system	198
7.4	Conclusions	204

FIGURES

Figure 1.1	The role of heat shock factor in heat shock protein production.	4
Figure 1.2	The role of HSP60 and HSP10 in protein folding	6
Figure 1.3	Lineage of immune cells.	9
Figure 1.4	Relationship between osteoblast and osteoclast lineages.	19
Figure 1.5	The lineage and location of bone cells.	20
Figure 1.6	A summary of the RANKL/OPG system	24
Figure 3.1	Restriction digest of RT-PCR products for Hsp60, Hsp90 α , and Hsp90 β . PCR products were amplified from control and heat shocked MG63 cells.	68
Figure 3.2	Restriction digest of RT-PCR products for Hsp27 and Hsp70.	69
Figure 3.3	The effect of a 24 h treatment with GroEL and/or 1,25(OH) $_2$ D $_3$ on RANKL expression in MG63 cells.	71
Figure 3.4	The effect of a 24 h treatment with GroEL and/or 1,25(OH) $_2$ D $_3$ on osteocalcin expression in MG63 cells.	72
Figure 3.5	The effect of a 24 h treatment with Hsp60, Hsp70 and GroEL on OPG production by MG63 cells.	73
Figure 3.6	MTS assay results - The effect of a 24 h treatment with Hsp60, Hsp70 and GroEL on MG63 cells.	74
Figure 3.7	MTS assay results - The effect of a 24 h treatment with a range of GroEL concentrations on MG63 cells.	75
Figure 3.8	The effect of a 24 h treatment with Hsp60, Hsp70 and GroEL on Hsp60, Hsp70, OPG and RANKL expression in MG63 cells.	76
Figure 3.9	The effect of a 24 h treatment with Hsp60, Hsp70, GroEL, LPS and 1,25(OH) $_2$ D $_3$ on OPG production by GCT cells.	77
Figure 3.10	The effect of a 24 h treatment with Hsp60, Hsp70, GroEL, LPS and 1,25(OH) $_2$ D $_3$ on intracellular OPG in GCT3 cells.	78
Figure 3.11	MTS assay results - The effect of a 24 h treatment with Hsp60, Hsp70, GroEL +/- Polymyxin B (PB) and LPS on GCT cells.	79

Figure 3.12	MTS assay results - The effect of a 24 h treatment with a range of GroEL concentrations +/- Polymyxin B (PB) on GCT cells.	80
Figure 3.13	The effect of a 24h treatment with Hsp60, Hsp70, GroEL and LPS on Hsp60, Hsp70 and RANKL expression in GCT cells.	81
Figure 3.14	Trap assay results showing osteoclast activity following the incubation of osteoclast precursors with Hsp60, Hsp70, GroEL, LPS and 1,25(OH)₂.	82
Figure 3.15	Trap + multinucleated osteoclasts formed from osteoclast precursors incubated in the presence of M-CSF and RANKL for 7 days.	83
Figure 3.16	Osteoclast precursors incubated with LPS only (10µg/ml) for 7 days.	84
Figure 3.17	Osteoclast precursors following a 7 day incubation in cell culture media only (non-treated control).	85
Figure 3.18	Trap assay results showing osteoclast activity following the incubation of osteoclast precursors with conditioned media from treated GCT cells.	86
Figure 3.19	Osteoclast precursors following a 7 day incubation in conditioned media from untreated GCT cells.	87
Figure 3.20	Trap assay results showing osteoclast activity following the incubation of osteoclast precursors with Hsp60, Hsp70, GroEL, LPS and 1,25(OH)₂D₃ in the presence of M-CSF.	88
Figure 3.21	Trap assay results showing osteoclast activity following the incubation of osteoclast precursors with conditioned media from treated GCT cells in the presence of M-CSF.	89
Figure 4.1	Release of Hsp60 into cell culture media from U937 cells in normal and heat shock conditions.	105
Figure 4.2	Intracellular Hsp60 levels in U937 cells cultured in normal and heat shock conditions.	106
Figure 4.3	Release of Hsp60 into cell culture media from Jurkat cells in normal and heat shock conditions.	107
Figure 4.4	Intracellular Hsp60 levels in Jurkat cells cultured in normal and heat shock conditions.	108
Figure 4.5	Release of Hsp60 into cell culture media from PBMCs in normal	109

and heat shock conditions.

Figure 4.6	Intracellular Hsp60 levels in PBMCs cultured in normal and heat shock conditions.	110
Figure 4.7	Release of Hsp70 into cell culture media from U937 cells in normal and heat shock conditions.	111
Figure 4.8	Intracellular Hsp70 levels in U937 cells cultured in normal and heat shock conditions.	112
Figure 4.9	Release of Hsp70 into cell culture media from Jurkat cells in normal and heat shock conditions.	113
Figure 4.10	Intracellular Hsp70 levels in Jurkat cells cultured in normal and heat shock conditions.	114
Figure 4.11	Release of Hsp70 into cell culture media from PBMCs in normal and heat shock conditions.	115
Figure 4.12	Intracellular Hsp70 levels in PBMCs cultured in normal and heat shock conditions.	116
Figure 4.13	Release of Hsp70 into cell culture media from PBMCs after culture at 37°C for 2, 4, 6 and 24 h (minus t0 control).	117
Figure 4.14	Intracellular Hsp70 in PBMCs after culture at 37°C for 2, 4, 6 and 24 h.	118
Figure 4.15	Western blot results for Hsp60 and Hsp70 in media and cell lysates from heat shocked U937 cells.	119
Figure 4.16	Western blot results for Hsp60 and Hsp70 in media and cell lysates from heat shocked Jurkat cells.	120
Figure 4.17	Hsp60 and Hsp70 expression in U937 cells in response to a 2 h heat shock.	121
Figure 4.18	Hsp60 and Hsp70 expression in Jurkat cells in response to a 2 h heat shock.	122
Figure 4.19	Release of Hsp70 into cell culture media from T and B-cells after culture at 37°C for 5 h at 37°C (minus t0 control).	123
Figure 4.20	Effect of protein release inhibitors on Hsp60 release from PBMCs during 5 h incubation at 37°C.	124
Figure 4.21	Effect of protein release inhibitors on intracellular Hsp60 in PBMCs during 5 h incubation at 37°C.	125

Figure 4.22	Effect of protein release inhibitors on Hsp70 release from PBMCs during 5 h incubation at 37°C.	126
Figure 4.23	Effect of protein release inhibitors on intracellular Hsp70 in PBMCs during 5 h incubation at 37°C.	127
Figure 5.1	Detection of Hsp expression using RT-PCR in PBMC samples.	139
Figure 5.2	Detection of Hsp expression in whole blood samples using RT-PCR.	140
Figure 5.3	Testing of RANKL primers using cDNA from control and PHA activated PBMCs	141
Figure 5.4	The effect of PHA activation followed by a 24 h incubation on heat shock protein expression in PBMCs.	142
Figure 5.5	The effect of PHA activation followed by a 24 h incubation on RANKL and IFN-β expression in PBMCs.	143
Figure 5.6	The effect of PHA treatment of PBMCs on the gene expression of Hsp60, Hsp70, TNF-α, IFN-β, and RANKL at 3 h and 24h time points.	144
Figure 5.7	Optimisation of CD25 primers: The effect of PCR cycle number on band clarity in control and PHA activated PBMC samples.	145
Figure 5.8	The effect of a 24h treatment with PHA, or combination of PHA and Dexamethasone or Prednisolone on the expression of Hsp27, Hsp60 and Hsp70 in PBMCs.	146
Figure 5.9	The effect of a 24h treatment with PHA, or combination of PHA and Dexamethasone or Prednisolone on the expression of CD25, TNF-α, IFN-β, and RANKL in PBMCs.	147
Figure 5.10	The effect of PHA and Prednisolone on Hsp60 release into cell culture media from PBMCs cultured in optimal conditions for 8 h.	148
Figure 5.11	The effect of PHA and Prednisolone on Hsp60 release into cell culture media from PBMCs cultured in optimal conditions for 24 h.	149
Figure 5.12	The effect of PHA and Prednisolone on intracellular Hsp60 levels in PBMCs cultured in optimal conditions for 8 h.	150
Figure 5.13	The effect of PHA and Prednisolone on intracellular Hsp60 levels in PBMCs cultured in optimal conditions for 24 h.	151
Figure 5.14	The effect of PHA and Prednisolone on Hsp70 release into cell culture media from PBMCs cultured in optimal conditions for 8 h.	152
Figure 5.15	The effect of PHA and Prednisolone on Hsp70 release into cell culture media from PBMCs cultured in optimal conditions for 24 h.	153

Figure 5.16	The effect of PHA and Prednisolone on intracellular Hsp70 levels in PBMCs cultured in optimal conditions for 8 h.	154
Figure 5.17	The effect of PHA and Prednisolone on intracellular Hsp70 levels in PBMCs cultured in optimal conditions for 24 h.	155
Figure 5.18	Hsp70 release into media from isolated negative T-cells following a 24 h incubation after initial PHA treatment (4µg/ml, 2 h).	156
Figure 5.19	Intracellular Hsp70 levels in isolated negative T-cells following a 24 h incubation after initial PHA treatment (4µg/ml, 2 h).	157
Figure 6.1	The effect of a 24 h treatment with Hsp70 and LPS on Hsp60 release from PBMCs.	170
Figure 6.2	The effect of a 24 h treatment with Hsp70 and LPS on intracellular Hsp60 in PBMCs.	171
Figure 6.3	The effect of a 24 h treatment with Hsp70, GroEL and LPS on Hsp60 release from Jurkat cells.	172
Figure 6.4	The effect of a 24 h treatment with Hsp70, GroEL and LPS on intracellular Hsp60 in Jurkat cells.	173
Figure 6.5	The effect of a 24 h treatment with Hsp60, GroEL and LPS on Hsp70 release from PBMCs.	174
Figure 6.6	The effect of a 24 h treatment with Hsp60, GroEL and LPS on intracellular Hsp70 in PBMCs.	175
Figure 6.7	The effect of a 24 h treatment with Hsp60, GroEL and LPS on Hsp70 release from Jurkat cells.	176
Figure 6.8	The effect of a 24 h treatment with Hsp60, GroEL and LPS on intracellular Hsp70 in Jurkat cells.	177
Figure 6.9	MTS results for PBMCs treated with Hsp60, Hsp70, GroEL and LPS for 24 h.	178
Figure 6.10	MTS results for Jurkat cells treated with Hsp60, Hsp70, GroEL and LPS for 24 h.	179
Figure 6.11	Hsp60 Hsp70 and CD25 gene expression following 24 h incubation with Hsp60, Hsp70, GroEL and LPS.	180
Figure 6.12	Hsp70 release from T cells following a 24 h treatment with GroEL.	181
Figure 6.13	Hsp70 release from B cells following a 24 h treatment with GroEL.	182

Figure 6.14	The effect of a 24 h treatment with Hsp70, GroEL and LPS on Hsp60 release from GCT cells.	183
Figure 6.15	The effect of a 24 h treatment with Hsp70, GroEL and LPS on intracellular Hsp60 levels in GCT cells.	184
Figure 6.16	The effect of a 24 h treatment with Hsp60, GroEL and LPS on Hsp70 release from GCT cells.	185
Figure 6.17	The effect of a 24 h treatment with Hsp60, GroEL and LPS on intracellular Hsp70 levels in GCT cells.	186
Figure 7.1	Summary of the hypothesis that Hsps lead to increased bone resorption.	196
Figure 7.2	Diagram showing an Hsp signalling cascade following cell exposure to bacterial infection.	201

TABLES

Table 1.1	Summary of heat shock proteins and their locations in mammalian cells.	3
Table 1.2	Summary of traditional functions of Hsps in mammalian cells.	3
Table 1.3	Characteristics of T and B cells	9
Table 1.4	Non-classical protein secretion indicators.	17
Table 1.5	Molecules that affect RANKL and OPG levels.	23
Table 1.6	Markers of bone formation and resorption.	25
Table 3.1	Treatments added to osteoclast precursors.	62
Table 3.2	DNA fragment sizes after restriction digest of heat shock protein RT-PCR products.	70
Table 4.1	Summary of inhibitor functions.	97
Table 4.2	Release of Hsp60 and Hsp70 from cultured U937, Jurkat, and PBMCs.	104

Table 6.1	The effect of 24 h incubation with Hsp60, Hsp70, GroEL and LPS on Hsp60 and Hsp70 release from PBMCs.	168
Table 6.2	The effect of 24 h incubation with Hsp60, Hsp70, GroEL and LPS on Hsp60 and Hsp70 release from Jurkat cells	169
Table 7.1	Summary of factors identified in experimental work from this thesis that provide a link between Hsps, LPS and increased bone resorption.	197

ABBREVIATIONS

1,25(OH)₂D₃	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
PGE₂	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