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:

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
1.9.3 Osteocytes and Bone lining cells
1.10 Regulation of bone remodelling - RANKL/OPG/RANK system
1.10.1 OPG
1.10.2 RANKL
1.10.3 OPG/RANKL/RANK interaction
1.10.4 Markers of bone turnover
1.11 Relationship between bone and the immune system
1.12 Bone and Hsps
1.13 Project aims

CHAPTER 2 - MATERIALS AND GENERAL METHODS

2.1 Equipment
2.2 Reagents
2.3 General Methods
2.3.1 MG63 (osteosarcoma) cell line culture
2.3.2 Giant Cell Tumour (GCT) cell line culture
2.3.3 U937 (human monocytic leukaemia) cell line culture
2.3.4 Jurkat (human T-cell leukaemia) cell line culture
2.3.5 Isolation and culture of osteoclast precursors from bone marrow
2.3.5.1 Removal of murine femora and tibiae
2.3.5.2 Isolation of osteoclast precursors
2.3.5.3 Culture of osteoclast precursors
2.3.6 Isolation and culture of Peripheral Blood Mononuclear Cells (PBMCs)
2.3.7 Cell counting
2.3.8 MTS Assay
2.3.9 mRNA extraction from cells
2.3.9.1 Binding of mRNA to Oligo dT cellulose
2.3.9.2 Washing Steps
2.3.9.3 Elution of the mRNA
2.3.10 cDNA synthesis using the First-Strand cDNA Synthesis Kit
2.3.11 cDNA synthesis using Ready To Go You Prime First-Strand Beads
2.3.12 Reverse Transcription Polymerase Chain Reaction (RT-PCR)
2.3.12.1 Primer Sequences
2.3.12.2 RT-PCR Reaction Mix
2.3.12.3 PCR cycles
2.3.13 Agarose Electrophoresis
2.3.14 SDS-PAGE gels
2.3.14.1 Denaturing gel preparation
2.3.14.2 Stacking gel preparation
2.3.14.3 Running SDS-PAGE gels
2.3.15 Western Blotting
2.3.15.1 Protein transfer
2.3.15.2 Immunostaining of Western Blots
2.3.16 Quantification of OPG using ELISA
2.3.17 Quantification of Hsp60 using ELISA
2.3.18 Quantification of Hsp70 using ELISA
2.3.19 LDH Assay
2.3.20 Trap activity assay
2.3.21 Trap Staining
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)_{2}D_{3} 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)_{2}D_{3} 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)_{2}D_{3} 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)_{2}D_{3} 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)_{2}D_{3} 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)_{2}D_{3} conditioned GCT and non-conditioned media 66

3.3.5.1 Effect of Hsps and LPS treated media (non-conditioned) on Trap activity 66
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.5.2</td>
<td>Effect of conditioned media from Hsp and LPS treated GCT cells on Trap activity</td>
<td>67</td>
</tr>
<tr>
<td>3.3.5.3</td>
<td>Effect of Hsp, LPS and 1,25(OH)<em>{2}D</em>{3} treated media (non-conditioned) on Trap activity in the presence of M-CSF</td>
<td>67</td>
</tr>
<tr>
<td>3.3.5.4</td>
<td>Effect of conditioned media from GCT cells treated with Hsp, LPS and 1,25(OH)<em>{2}D</em>{3} in the presence of M-CSF</td>
<td>67</td>
</tr>
<tr>
<td>3.4</td>
<td>Discussion</td>
<td>90</td>
</tr>
</tbody>
</table>

**CHAPTER 4 - RELEASE OF HSPS 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

4.4 Discussion

CHAPTER 5 - THE EFFECT OF GLUCOCORTICOIDS AND PHA ON HSP60 AND HSP70 EXPRESSION AND RELEASE IN PBMCS.

5.1 Introduction

5.2 Methods

5.2.1 Detection of Hsp expression in PBMcs

5.2.2 Detection of Hsp expression in Whole Blood

5.2.3 Treatment of PBMcs with PHA

5.2.4 Addition of PHA, dexamethasone and prednisolone to PBMcs

5.2.5 Addition of PHA to negative T-cells

5.2.6 Cell counts

5.3 Results

5.3.1 Optimisation of RT-PCR to detect Hsp gene expression in PBMcs and whole blood

5.3.2 Optimisation of RT-PCR to detect RANKL in PBMcs

5.3.3 The effect of PHA activation on the expression of Hsps, RANKL, IFN-β, and TNF-α in PBMcs

5.3.4 Optimisation of CD25 primers

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

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

5.3.7 Effect of PHA and steroids on the production and release of Hsp60 and Hsp70 in PBMcs and T-cells

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

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

viii
5.3.7.3 The effect of PHA on Hsp70 release and intracellular levels in T-cells.

5.4 Discussion

CHAPTER 6 - THE EFFECT OF HSP AND LPS TREATMENT ON HSP60 AND HSP70 RELEASE FROM IMMUNE AND OSTEOSTROMAL-LIKE CELLS

6.1 Introduction

6.2 Methods

6.2.1 Addition of Hsp60, Hsp70, GroEL and LPS to Jurkat cells and PBMCs

6.2.2 Addition of GroEL to T and B cells

6.2.3 Addition of Hsp60, Hsp70, GroEL and LPS to GCT cells

6.3 Results

6.3.1 Effect of incubation with Hsp70, GroEL and LPS on the release of Hsp60 from PBMCs and Jurkat cells

6.3.2 Effect of incubation with Hsp60, GroEL and LPS on the release of Hsp70 in PBMCs and Jurkat cells

6.3.3 LDH and MTS results following treatment of PBMCs and Jurkat cells with Hsps and LPS

6.3.4 Gene expression for Hsp60, Hsp70 and CD25 in PBMCs following 24 h treatment with Hsp60, Hsp70, GroEL and LPS

6.3.5 Effect of GroEL on Hsp70 release from isolated negative T and B cells

6.3.6 Effect of Hsp70, GroEL and LPS on Hsp60 release from GCT cells

6.3.7 Effect of Hsp60, GroEL and LPS on Hsp70 release from GCT cells

6.4 Discussion

CHAPTER 7 – DISCUSSION

7.1 Release of Hsps from Immune cells

7.2 Hypothesis 1 – Hsps cause increased bone resorption by acting on the RANKL/OPG system.

7.3 Hypothesis 2 – Hsps act as a danger signal to the innate immune system

7.4 Conclusions
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, Hsp90α, and Hsp90β. 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)2D3 on RANKL expression in MG63 cells. 71
Figure 3.4 The effect of a 24 h treatment with GroEL and/or 1,25(OH)2D3 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)2D3 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)2D3 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.

Figure 3.13  The effect of a 24h treatment with Hsp60, Hsp70, GroEL and LPS on Hsp60, Hsp70 and RANKL expression in GCT cells.

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.

Figure 3.15  Trap + multinucleated osteoclasts formed from osteoclast precursors incubated in the presence of M-CSF and RANKL for 7 days.

Figure 3.16  Osteoclast precursors incubated with LPS only (10μg/ml) for 7 days.

Figure 3.17  Osteoclast precursors following a 7 day incubation in cell culture media only (non-treated control).

Figure 3.18  Trap assay results showing osteoclast activity following the incubation of osteoclast precursors with conditioned media from treated GCT cells.

Figure 3.19  Osteoclast precursors following a 7 day incubation in conditioned media from untreated GCT cells.

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.

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.

Figure 4.1  Release of Hsp60 into cell culture media from U937 cells in normal and heat shock conditions.

Figure 4.2  Intracellular Hsp60 levels in U937 cells cultured in normal and heat shock conditions.

Figure 4.3  Release of Hsp60 into cell culture media from Jurkat cells in normal and heat shock conditions.

Figure 4.4  Intracellular Hsp60 levels in Jurkat cells cultured in normal and heat shock conditions.

Figure 4.5  Release of Hsp60 into cell culture media from PBMCs in normal
and heat shock conditions.

Figure 4.6 Intracellular Hsp60 levels in PBMCs cultured in normal and heat shock conditions.

Figure 4.7 Release of Hsp70 into cell culture media from U937 cells in normal and heat shock conditions.

Figure 4.8 Intracellular Hsp70 levels in U937 cells cultured in normal and heat shock conditions.

Figure 4.9 Release of Hsp70 into cell culture media from Jurkat cells in normal and heat shock conditions.

Figure 4.10 Intracellular Hsp70 levels in Jurkat cells cultured in normal and heat shock conditions.

Figure 4.11 Release of Hsp70 into cell culture media from PBMCs in normal and heat shock conditions.

Figure 4.12 Intracellular Hsp70 levels in PBMCs cultured in normal and heat shock conditions.

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).

Figure 4.14 Intracellular Hsp70 in PBMCs after culture at 37°C for 2, 4, 6 and 24 h.

Figure 4.15 Western blot results for Hsp60 and Hsp70 in media and cell lysates from heat shocked U937 cells.

Figure 4.16 Western blot results for Hsp60 and Hsp70 in media and cell lysates from heat shocked Jurkat cells.

Figure 4.17 Hsp60 and Hsp70 expression in U937 cells in response to a 2 h heat shock.

Figure 4.18 Hsp60 and Hsp70 expression in Jurkat cells in response to a 2 h heat shock.

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).

Figure 4.20 Effect of protein release inhibitors on Hsp60 release from PBMCs during 5 h incubation at 37°C.

Figure 4.21 Effect of protein release inhibitors on intracellular Hsp60 in PBMCs during 5 h incubation at 37°C.
Figure 4.22 Effect of protein release inhibitors on Hsp70 release from PBMCs during 5 h incubation at 37ºC.

Figure 4.23 Effect of protein release inhibitors on intracellular Hsp70 in PBMCs during 5 h incubation at 37ºC.

Figure 5.1 Detection of Hsp expression using RT-PCR in PBMC samples.

Figure 5.2 Detection of Hsp expression in whole blood samples using RT-PCR.

Figure 5.3 Testing of RANKL primers using cDNA from control and PHA activated PBMCs

Figure 5.4 The effect of PHA activation followed by a 24 h incubation on heat shock protein expression in PBMCs.

Figure 5.5 The effect of PHA activation followed by a 24 h incubation on RANKL and IFN-β expression in PBMCs.

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.

Figure 5.7 Optimisation of CD25 primers: The effect of PCR cycle number on band clarity in control and PHA activated PBMC samples.

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.

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.

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.

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.

Figure 5.12 The effect of PHA and Prednisolone on intracellular Hsp60 levels in PBMCs cultured in optimal conditions for 8 h.

Figure 5.13 The effect of PHA and Prednisolone on intracellular Hsp60 levels in PBMCs cultured in optimal conditions for 24 h.

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.

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.
Figure 5.16  The effect of PHA and Prednisolone on intracellular Hsp70 levels in PBMCs cultured in optimal conditions for 8 h.

Figure 5.17  The effect of PHA and Prednisolone on intracellular Hsp70 levels in PBMCs cultured in optimal conditions for 24 h.

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).

Figure 5.19  Intracellular Hsp70 levels in isolated negative T-cells following a 24 h incubation after initial PHA treatment (4μg/ml, 2 h).

Figure 6.1  The effect of a 24 h treatment with Hsp70 and LPS on Hsp60 release from PBMCs.

Figure 6.2  The effect of a 24 h treatment with Hsp70 and LPS on intracellular Hsp60 in PBMCs.

Figure 6.3  The effect of a 24 h treatment with Hsp70, GroEL and LPS on Hsp60 release from Jurkat cells.

Figure 6.4  The effect of a 24 h treatment with Hsp70, GroEL and LPS on intracellular Hsp60 in Jurkat cells.

Figure 6.5  The effect of a 24 h treatment with Hsp60, GroEL and LPS on Hsp70 release from PBMCs.

Figure 6.6  The effect of a 24 h treatment with Hsp60, GroEL and LPS on intracellular Hsp70 in PBMCs.

Figure 6.7  The effect of a 24 h treatment with Hsp60, GroEL and LPS on Hsp70 release from Jurkat cells.

Figure 6.8  The effect of a 24 h treatment with Hsp60, GroEL and LPS on intracellular Hsp70 in Jurkat cells.

Figure 6.9  MTS results for PBMCs treated with Hsp60, Hsp70, GroEL and LPS for 24 h.

Figure 6.10  MTS results for Jurkat cells treated with Hsp60, Hsp70, GroEL and LPS for 24 h.

Figure 6.11  Hsp60 Hsp70 and CD25 gene expression following 24 h incubation with Hsp60, Hsp70, GroEL and LPS.

Figure 6.12  Hsp70 release from T cells following a 24 h treatment with GroEL.

Figure 6.13  Hsp70 release from B cells following a 24 h treatment with GroEL.
Figure 6.14 The effect of a 24 h treatment with Hsp70, GroEL and LPS on Hsp60 release from GCT cells.

Figure 6.15 The effect of a 24 h treatment with Hsp70, GroEL and LPS on intracellular Hsp60 levels in GCT cells.

Figure 6.16 The effect of a 24 h treatment with Hsp60, GroEL and LPS on Hsp70 release from GCT cells.

Figure 6.17 The effect of a 24 h treatment with Hsp60, GroEL and LPS on intracellular Hsp70 levels in GCT cells.

Figure 7.1 Summary of the hypothesis that Hsps lead to increased bone resorption.

Figure 7.2 Diagram showing an Hsp signalling cascade following cell exposure to bacterial infection.

TABLES

Table 1.1 Summary of heat shock proteins and their locations in mammalian cells.

Table 1.2 Summary of traditional functions of Hsps in mammalian cells.

Table 1.3 Characteristics of T and B cells

Table 1.4 Non-classical protein secretion indicators.

Table 1.5 Molecules that affect RANKL and OPG levels.

Table 1.6 Markers of bone formation and resorption.

Table 3.1 Treatments added to osteoclast precursors.

Table 3.2 DNA fragment sizes after restriction digest of heat shock protein RT-PCR products.

Table 4.1 Summary of inhibitor functions.

Table 4.2 Release of Hsp60 and Hsp70 from cultured U937, Jurkat, and PBMCs.
Table 6.1  The effect of 24 h incubation with Hsp60, Hsp70, GroEL and LPS on Hsp60 and Hsp70 release from PBMCs.

Table 6.2  The effect of 24 h incubation with Hsp60, Hsp70, GroEL and LPS on Hsp60 and Hsp70 release from Jurkat cells

Table 7.1  Summary of factors identified in experimental work from this thesis that provide a link between Hsp6s, LPS and increased bone resorption.
ABBREVIATIONS

1,25(OH)₂D₃  1,25 dihydroxyvitamin D₃
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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANKL</td>
<td>Receptor activator of NF-kappaB ligand</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate - Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>TCR</td>
<td>T-cell antigen receptor</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TRAIL</td>
<td>Tumour necrosis factor (TNF)-related apoptosis inducing ligand</td>
</tr>
<tr>
<td>U937</td>
<td>Human monocytic leukaemia cell line</td>
</tr>
</tbody>
</table>