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**THE PATHOGENESIS OF MURINE INTRA-ABDOMINAL ABSCESSSES:
ULTRASTRUCTURAL AND QUANTITATIVE STUDIES**

A Thesis presented in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy in Microbiology at
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TABLE OF CONTENTS

	<u>Page</u>
Abstract	(vii)
Declaration	(x)
Acknowledgements	(xi)
List of Tables Appearing in the Text	(xii)
List of Figures Appearing in the Text	(xiv)
Abbreviations	(xviii)
CHAPTER 1: INTRODUCTION	1
1.1 Human Intra-abdominal Abscesses	3
1.2 Virulence of <u>B. fragilis</u> in Animal Models of Intra-abdominal Sepsis	4
1.2.1 Animal Models of Intra-abdominal Sepsis	4
1.2.2 Encapsulation of <u>B. fragilis</u>	5
1.2.3 Bacterial Synergy	8
1.2.4 Other Virulence factors of <u>B. fragilis</u>	13
1.3 Host Defences in the Peritoneal Cavity	16
1.3.1 Lymphatic Clearance	16
1.3.2 Humoral Defence Mechanisms	17
1.3.3 Phagocytosis	19
1.3.4 Cell-mediated Immunity	21
1.3.5 Abscesses	22
1.4 Phagocytosis	23
1.4.1 Methodology	23
1.4.2 Opsonization	26
1.4.3 Attachment and Ingestion	28
1.4.4 Intracellular Events	30

Page

1.5	Phagocytosis of <u>Bacteroides</u> Species	35
1.5.1	Chemotaxis	35
1.5.2	Opsonization and Phagocytic Killing	36
1.5.3	Killing by Abscess Neutrophils	37
1.5.4	Inhibition of Phagocytic Killing of Facultative Anaerobes	38
1.5.5	Effect of Bacterial Capsules	40

	<u>Page</u>
CHAPTER 2: MATERIALS AND METHODS	41
2.1 Animals	42
2.2 Media	42
2.3 Bacteria	43
2.4 Capsules	44
2.5 Bran	44
2.6 Abscess-Inducing Mixtures	45
2.7 Evaluation of Abscesses	45
2.8 Sera	46
2.9 Indirect Fluorescent Antibody Test	47
2.10 Opsonization <u>in vitro</u>	48
2.11 <u>In vitro</u> Phagocytic Killing Assay	48
2.12 <u>In vitro</u> Intracellular Killing Assay	50
2.13 Enumeration of Bacteria and Leukocytes from the Murine Peritoneal Cavity	50
2.14 Transmission Electron Microscopy	51
2.14.1 Standard Procedure	51
2.14.2 Peroxidase Cytochemistry	53
2.14.3 Periodate-Thiocarbohydrazide-Silver Protein Staining	54
2.14.4 Reprocessing of Paraffin Wax - Embedded Abscesses	55
2.15 Statistics	55

	<u>Page</u>
CHAPTER 3: A MODEL OF INTRA-ABDOMINAL ABSCESS DEVELOPMENT IN C3H MICE	56
3.1 Introduction	57
3.2 Results	59
3.2.1 Encapsulation of Bacteria	59
3.2.2 Abscess Development	59
3.2.3 Abscess Histology	67
3.2.4 Ultrastructure of Murine Peritoneal Neutrophils	72
3.2.5 Abscess Ultrastructure	80
3.2.6 Initiation of Abscess Development	84
3.3 Discussion	112
3.3.1 Abscess Development: <u>B. fragilis</u> , <u>E. coli</u> , and Bran	112
3.3.2 Abscess Development: Other Abscess- Inducing Mixtures	117
3.3.3 Initiation of Abscess Development	119

	<u>Page</u>
CHAPTER 4: THE INTERACTION OF ABSCESS-INDUCING BACTERIA WITH MURINE PERITONEAL LEUKOCYTES <u>IN VITRO</u>	123
4.1 Introduction	124
4.2 Results	126
4.2.1 Phagocytic Killing of Stationary Phase Bacteria Compared to Logarithmic Phase Bacteria	126
4.2.2 Cytological Assessment of the Phagocytosis of <u>B. fragilis</u> , <u>B. vulgatus</u> and <u>E. coli</u>	126
4.2.3 Phagocytic Killing of <u>B. fragilis</u> , <u>B. vulgatus</u> and <u>E. coli</u>	130
4.2.4 The Effect of Oxygen on the Phagocytic Killing of <u>B. fragilis</u> and <u>E. coli</u>	134
4.2.5 The Effect of Bacterial Concentration on the Activity of Peritoneal Leukocytes	136
4.2.5a Phagocytic Killing of <u>B. fragilis</u> and <u>E. coli</u> in the Presence of Ongoing Phagocytosis	136
4.2.5b Ultrastructural Observations on the Phagocytic Killing of <u>B. fragilis</u> and <u>B. vulgatus</u>	141
4.2.5c Ultrastructural Observations on the Phagocytic Killing of <u>E. coli</u>	159
4.2.5d Intracellular Killing of <u>B. fragilis</u> , <u>B. vulgatus</u> and <u>E. coli</u> in the Absence of Ongoing Phagocytosis	162
4.2.6 The Effect of Bran on the Phagocytic Killing of <u>B. fragilis</u> and <u>E. coli</u>	171
4.3 Discussion	176
4.3.1 Phagocytic Killing of Logarithmic and Stationary Phase Bacteria	176
4.3.2 Phagocytic and Intracellular Killing of <u>B. fragilis</u> , <u>B. vulgatus</u> and <u>E. coli</u>	177
4.3.3 Ultrastructural Observations on the Phagocytosed Bacteria	187
4.3.4 Conclusion	193

Page

CHAPTER 5: CONCLUDING DISCUSSION	195
APPENDIX	206
REFERENCES	212

ABSTRACT

A murine model of intra-abdominal (IA) abscess formation was used to study the interaction of murine strains of bacteria with peritoneal neutrophils. The intraperitoneal (IP) inoculation of non-immune mice with mixtures containing either 5×10^8 Bacteroides fragilis or Bacteroides vulgatus combined with 1×10^6 Escherichia coli and 1 mg of bran as a potentiating agent induced abscess formation after three days. Ten weeks after the IP inoculation of mice with B. fragilis, E. coli and bran IA abscesses containing viable bacteria at concentrations similar to those in the inoculum persisted in 71% of the mice.

During the first 24 hrs of infection B. fragilis and B. vulgatus were readily phagocytosed by neutrophils and some macrophages in the murine peritoneal cavity. However, after the first 4.5 hrs of infection, there were significantly more viable intracellular B. fragilis than B. vulgatus. Furthermore, after up to 24 hrs of phagocytosis in vivo, B. fragilis was more resistant than B. vulgatus to killing when the leukocytes were incubated with normal serum (NS) in vitro. This suggests that B. fragilis persists in IA abscesses because of its resistance to the bactericidal mechanisms of neutrophils after phagocytosis. To test this hypothesis, the extent of fusion of peroxidase-labelled primary granules with neutrophil phagosomes containing B. fragilis or B. vulgatus was examined by electron microscopy. After the in vivo phagocytosis of either B. fragilis or B. vulgatus, primary granules had fused with some bacteria-containing phagosomes of neutrophils. Intact primary granules were also visible in the neutrophils' cytoplasm. However, more damaged intracellular B. vulgatus than B. fragilis were observed. This was consistent with the significant reduction in the number of viable B. vulgatus in IA abscesses three weeks after the IP inoculation of mice with B. vulgatus, E. coli and bran.

The E. coli strain was encapsulated and was relatively resistant to in vitro phagocytosis in either NS or NS and immune serum (IS). This may be important in the persistence of the

infection. E. coli, either alive or killed, had no detectable effect on the phagocytic killing of B. fragilis in vitro. Although capsules were also detected on the B. fragilis and B. vulgatus strains by electron microscopy, both were readily phagocytosed in the presence of NS (a source of complement). In vitro phagocytic killing of B. vulgatus, at a ratio of one bacterium per ten peritoneal leukocytes, occurred in NS alone, whereas the maximal phagocytic killing of B. fragilis and E. coli required NS and IS. Phagocytic killing of B. fragilis and E. coli was significantly reduced in anaerobic conditions.

In the presence or absence of on-going phagocytosis, at a ratio of 100 bacteria per peritoneal leukocyte, a proportion of intracellular B. fragilis resisted the bactericidal mechanisms of neutrophils for at least 2 hrs in the in vitro assays. Intracellular B. fragilis were more resistant to ultrastructural damage than were B. vulgatus in the presence of on-going phagocytosis. Ingested B. fragilis were located within the phagosomes of neutrophils, and there was evidence of primary granule fusion with 15% and 13% of these phagosomes in NS and NS plus IS respectively. More phagocytic killing occurred in IS because, although the addition of IS to NS did not alter the percentage of phagocytes with intracellular bacteria, it did result in the phagocytosis of a greater number of bacteria per neutrophil. This resulted in more phagosomes per neutrophil in NS and IS, although the number of bacteria per phagosome and the proportion of peroxidase-positive phagosomes were similar to those in NS alone. Consequently, overall more bacteria were exposed to granule contents in NS and IS and more were killed by the peritoneal neutrophils than in NS alone. However, the small proportions of peroxidase-positive phagosomes in either NS or NS and IS, plus the survival of a greater proportion of B. fragilis when exposed to neutrophils at high vs low ratios of bacteria to peritoneal leukocytes, suggests that the fusion of an insufficient number of primary granules may influence the ability of neutrophils to kill bacteria readily phagocytosed at high ratios of bacteria to leukocytes.

A role for extracellular NS in the process of ~~phagosome~~-granule fusion within neutrophils was demonstrated. After the phagocytosis of pre-opsonized B. fragilis in the presence of NS, which supported intracellular killing of the majority of the bacteria, few peroxidase-positive or peroxidase-negative granules were seen in the cytoplasm of neutrophils, indicating that ~~phagosome~~-granule fusion had occurred. In contrast, in either NS heated to inactivate complement or the absence of NS, which did not support intracellular killing of B. fragilis, many intact granules were visible in the cytoplasm of neutrophils.

Bran was an essential component of the abscess-inducing mixture. In vitro, the phagocytic killing of B. fragilis and E. coli was reduced in the presence of bran. This effect of bran was observed with pre-opsonized bacteria in NS and suggests that bran affects the serum components, probably complement, necessary for the stimulation of intracellular killing.

After 120 mins of in vitro phagocytosis, the coalescence of phagosomes containing B. fragilis was evident in some neutrophils. The disintegration of the membranes of some necrotic neutrophils released bacteria from the phagosomes. Intracellular killing assays indicated that 20-40% of B. fragilis were viable at this time. Furthermore, bacteria were located in extracellular and intracellular sites within the abscesses. Thus, it is suggested that the establishment of a cycle of phagocytosis, limited intracellular killing due to insufficient fusion of primary granules with phagosomes in the presence of large numbers of bacteria, a situation compounded by the low levels of extracellular NS components, followed by release of the bacteria and limited bacterial replication, enabled the survival of bacteria in IA abscesses in mice.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university, and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person, except where due reference is made in the text.

Lesley Hampton

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LIST OF TABLES APPEARING IN THE TEXT

<u>Table</u>	<u>Page</u>
1.1 Bacterial synergy in animal models of IA sepsis	9
1.2 Effect of anaerobes on the phagocytosis and killing of facultative anaerobes	39
3.1 <u>Abscesses induced by B. fragilis, E. coli</u> and bran	61
3.2 <u>Abscesses induced by Bacteroides species, E. coli</u> and bran	64
3.3 Number of leukocytes in the murine peritoneal cavity after inoculation with abscess-inducing mixtures	90
3.4 Cellular content of the murine peritoneal cavity 1 hr post-inoculation with abscess-inducing mixtures	91
3.5 Summary of ultrastructural observations on the initiation of abscess development	97
3.6 Killing <u>in vitro</u> of bacteria phagocytosed <u>in vivo</u>	110
4.1 Phagocytic killing of stationary phase bacteria compared to logarithmic phase bacteria in aerobic conditions	127
4.2 Leukocyte-associated bacteria (LAB) at 60 mins	128
4.3 Neutrophil-associated <u>E. coli</u> after preopsonization in 50% NS and 50% IS	131
4.4 The Effect of oxygen on the phagocytic killing of <u>B. fragilis</u> and <u>E. coli</u>	135
4.5 The Effect of <u>B. fragilis</u> concentration on the activity of peritoneal leukocytes	140
4.6 The Effect of extracellular serum on degranulation	153
4.7 Fusion of neutrophil primary granules with phagosomes containing <u>B. fragilis</u>	156
4.8 Number of viable intracellular bacteria after 3 mins of phagocytosis	165

<u>Table</u>		<u>Page</u>
4.9	Intracellular killing of <u>B. fragilis</u> , <u>B. vulgatus</u> and <u>E. coli</u>	166
4.10	The Effect of killed <u>E. coli</u> on the intracellular killing of pre- opsonized <u>B. fragilis</u> in aerobic conditions	169
4.11	The Effect of <u>E. coli</u> on the intracellular killing of pre- opsonized <u>B. fragilis</u> in anaerobic conditions	170

LIST OF FIGURES APPEARING IN THE TEXT

<u>Figure</u>	<u>Page</u>
3.1 Encapsulation of bacteria	60
3.2 Viable bacteria in abscesses induced by <u>B. fragilis</u> , <u>E. coli</u> and bran	62
3.3 Viable bacteria in abscesses induced by <u>Bacteroides</u> species, <u>E. coli</u> and bran	65
3.4 Histology of a Day 3 abscess induced by AIM	68
3.5 Histology of a Day 13 abscess induced by AIM	69
3.6 Histology of a Day 40 abscess induced by AIM	70
3.7 Distribution of <u>B. fragilis</u> antigens within abscesses induced by AIM	71
3.8 A Mature murine peritoneal neutrophil	73
3.9 A Peroxidase-labelled mature murine peritoneal neutrophil	74
3.10 A Peroxidase-labelled mature murine peritoneal neutrophil without lead citrate staining	75
3.11 Peroxidase-labelled mature murine peritoneal neutrophils with the omission of H ₂ O ₂ (a) or 3-3' diaminobenzidine tetrahydrochloride (b) from Graham's and Karnovsky's medium	76
3.12 A PA-TCH-SP stained mature murine peritoneal neutrophil	77
3.13 A PA-TCH-SP stained mature murine peritoneal neutrophil digested with α -amylase	78
3.14 Control mature murine peritoneal neutrophils which illustrate that PA-TCH-SP positive granules contain vicinal glycol-containing glycoconjugates	79
3.15 Ultrastructure of a Day 3 IA abscess induced by AIM	81
3.16 Ultrastructure of a Day 6 IA abscess induced by AIM	82
3.17 Ultrastructure of a Day 13 IA abscess induced by AIM	83
3.18 Ultrastructure of a Day 21 IA abscess induced by AIM	85

<u>Figure</u>		<u>Page</u>
3.19	Ultrastructure of a Day 40 IA abscess induced by AIM	86
3.20	Ultrastructure of a Day 70 IA abscess induced by AIM	87
3.21	Reprocessing of a histologically-fixed Day 13 IA abscess induced by AIM for electron microscopy	88
3.22	Infection of the murine peritoneal cavity - 1 hr	92
3.23	Infection of the murine peritoneal cavity - 4.5 hrs	93
3.24	Infection of the murine peritoneal cavity - 24 hrs	94
3.25	Viable intracellular bacteria after <u>in vivo</u> phagocytosis by murine peritoneal leukocytes	95
3.26	1 hr after the IP inoculation of 5×10^8 <u>B. fragilis</u> and 1 mg of bran	98
3.27	Intraperitoneal inoculation of 5×10^8 <u>B. fragilis</u> , 1×10^6 <u>E. coli</u> and 1 mg of bran - 1 hr infection	99
3.28	Intraperitoneal inoculation of 5×10^8 <u>B. fragilis</u> , 1×10^6 <u>E. coli</u> and 1 mg of bran - 4.5 hr infection	100
3.29	Intraperitoneal inoculation of 5×10^8 <u>B. fragilis</u> , 1×10^6 <u>E. coli</u> and 1 mg of bran - 24 hr infection	102
3.30	Intraperitoneal inoculation of 5×10^8 <u>B. fragilis</u> and 1×10^6 <u>E. coli</u> - 1 hr infection	103
3.31	Intraperitoneal inoculation of 5×10^8 <u>B. fragilis</u> and 1×10^6 <u>E. coli</u> - 24 hr infection	104
3.32	Intraperitoneal inoculation of 5×10^8 <u>B. vulgatus</u> , 1×10^6 <u>E. coli</u> and 1 mg of bran - 1 hr infection	105
3.33	Intraperitoneal inoculation of 5×10^8 <u>B. vulgatus</u> , 1×10^6 <u>E. coli</u> and 1 mg of bran - 4.5 hr infection	106

<u>Figure</u>	<u>Page</u>
3.34 <u>Intraperitoneal inoculation of 5x10⁸ B. vulgatus, 1x10⁶ E. coli and 1 mg of bran - 24 hr infection</u>	107
3.35 <u>Intraperitoneal inoculation of 5x10⁸ B. vulgatus, and 1x10⁶ E. coli - 1 hr infection</u>	108
3.36 <u>Intraperitoneal inoculation of 5x10⁸ B. vulgatus and 1x10⁶ E. coli - 24 hr infection</u>	109
4.1 <u>Phagocytosis of B. fragilis, B. vulgatus and E. coli</u>	129
4.2 <u>Phagocytosis of viable and non-viable E. coli</u>	132
4.3 <u>Phagocytic killing of bacteria in aerobic conditions</u>	133
4.4 <u>Effect of B. fragilis concentration on phagocytic killing in aerobic and anaerobic conditions</u>	137
4.5 <u>Effect of E. coli concentration on phagocytic killing in aerobic and anaerobic conditions</u>	138
4.6 <u>Extracellular B. fragilis after 20 mins in 10% NS</u>	142
4.7 <u>Phagocytosed B. fragilis after 20 mins in 10% NS</u>	143
4.8 <u>Phagocytosed B. fragilis after 20 mins in 10% NS - an eosinophil</u>	144
4.9 <u>Phagocytosed B. fragilis after 120 mins in 10% NS</u>	145
4.10 <u>Phagocytosed B. fragilis after 20 mins in 10% NS and 10% IS</u>	147
4.11 <u>Phagocytosed B. fragilis after 120 mins in 10% NS and 10% IS - clumped peritoneal leukocytes</u>	148
4.12 <u>Phagocytosed B. fragilis after 120 mins in 10% NS and 10% IS</u>	149
4.13 <u>Phagocytosed B. fragilis after 120 mins in 10% NS and 10% IS - neutrophils with large phagosomes</u>	150

<u>Figure</u>		<u>Page</u>
4.14	Phagocytosed <u>B. fragilis</u> after 120 mins in 10% NS and 10% IS - necrotic neutrophils	152
4.15	The Effect of extracellular serum on degranulation after 30 mins of phagocytosis	154
4.16	Phagocytosed <u>B. vulgatus</u> in 10% NS	157
4.17	Phagocytosed <u>B. vulgatus</u> in 10% NS and 10% IS	158
4.18	Phagocytosed <u>E. coli</u> after 20 mins in 10% NS	160
4.19	Phagocytosed <u>E. coli</u> after 60 mins in 10% NS and 10% IS	161
4.20	Phagocytosed <u>E. coli</u> (pre-opsonized in 50% NS and 50% IS) after 60 mins in 10% NS	163
4.21	Intracellular pre-opsonized <u>B. fragilis</u> after 60 mins in 10% NS in the absence of ongoing phagocytosis	168
4.22	The Clumping of peritoneal leukocytes in the presence of bran	172
4.23	The Phagocytosis of bran by peritoneal neutrophils after 20 mins in 10% NS	173
4.24	The Effect of bran on the phagocytic killing of <u>B. fragilis</u> in aerobic conditions	174
4.25	The Effect of bran on the phagocytic killing of <u>E. coli</u> in aerobic conditions	175

ABBREVIATIONS

AIM	An abscess-inducing mixture containing:
	1 mg bran)
	5x10 ⁸ colony forming units of)
	<u>Bacteroides fragilis</u>) in 0.05 ml of
	1x10 ⁶ colony forming units of) RPMI-1640 medium
	<u>Escherichia coli</u>)
BHIB	Brain heart infusion broth
cfu	Colony forming units
EM	Electron microscopy
FCS	Foetal calf serum
HNS	Heat-inactivated normal serum
IA	Intra-abdominal
IP	Intraperitoneal
IS	Heat-inactivated immune serum
LPS	Lipopolysaccharide
NS	Fresh normal serum
PA-TCH-SP	Periodate-thiocarbohydrazide-silver proteinate
PBS	Phosphate-buffered saline
SC	Subcutaneous
SD	Standard deviation
WC	Wilkins Chalgren