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

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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 $5x10^8$ Bacteroides fragilis or Bacteroides vulgatus combined with $1x10^6$ 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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ABBREVIATIONS

AIM	An abscess-inducing mixture containing: 1 mg bran, $5x10^8$ colony forming units of *Bacteroides fragilis*, $1x10^6$ colony forming units of *Escherichia coli* in 0.05 ml of RPMI-1640 medium
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