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The effect of immune enhancement and suppression on the development of laparoscopic port site metastases

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Abstract

Background: Recent clinical case reports and experimental studies have suggested that laparoscopic cancer surgery is associated with an increased risk of tumor spread to abdominal wall wounds. While the etiology of this problem was initially believed to be related to mechanical contamination of wounds, it is now recognized that there are other contributory factors, including disturbed immune function within the peritoneal cavity. To investigate this question further, we evaluated the effect of immune modulation within an established laparoscopic cancer model.

Methods: Eighteen immune-competent syngeneic rats underwent modulation of their immune system, followed 18 h later by laparoscopy with the introduction of a suspension of adenocarcinoma cells into the peritoneal cavity. Rats were randomly allocated to receive either systemic cyclosporin (immune suppresser), intraperitoneal endotoxin (immune enhancer), or no agent (controls). Seven days later, all rats were killed and their peritoneal cavity was inspected for tumor implantation and port site metastases.

Results: Cyclosporin did not influence the study outcome, but tumor growth ($p = 0.008$) and port site metastases ($p < 0.0001$) were less common following the administration of intraperitoneal endotoxin.

Conclusion: The results of this study suggest that the immune system plays a role in the genesis of port site metastases. A preventive role for endotoxin in patients undergoing laparoscopic cancer surgery, however, remains speculative.

Key words: Laparoscopy — Wound — Cancer — Immune system — Metastasis — Port site metastasis — Cyclosporin — Endotoxin

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Recent clinical and experimental evidence now suggests that the incidence of metastasis to surgical wounds following cancer resection is increased by laparoscopic approaches [12, 13, 21, 27]. Indeed, its occurrence following surgery for early-stage cancers indicates that the prognosis may be adversely influenced [17, 28]. But these findings conflict with those from other experimental studies, which have shown a beneficial effect for laparoscopy for cancer due to less systemic immune suppression and reduced primary tumor growth [1–3, 5].

The underlying cause of port site metastases following laparoscopic surgery is likely to be multifactorial. There is now clear evidence that the laparoscopic manipulation of cancers can spread tumor cells to port sites due to direct spread from contaminated instruments [7]. In addition, it is possible that cells are transported by an indirect mechanism due to aerosolization in the insufflation gas [4, 10, 14, 22]. Furthermore, metabolic and immunological factors, acting at the level of the peritoneal membrane, due to an effect of CO_2 insufflation, may also be important [23, 25, 26]. The use of CO_2 insufflation has been shown to be associated with impaired peritoneal macrophage function as well as an acidotic intraperitoneal environment [25, 26]. These factors could also facilitate tumor implantation and growth.

To further assess the role of the immune system in the genesis of port site metastases, we modulated immune function in a laparoscopic cancer surgery model. Endotoxin, which increases levels of tumor necrosis factor alpha (TNF-α) was used to simulate immune enhancement [19]; and Cyclosporin A, which produces a decrease in TNF-α levels, was used to suppress immunity [20].

Materials and methods

The study was performed in a laparoscopic cancer model that used an immune-competent syngeneic rat strain [13, 14]. A suspension of tumor cells, derived from a mammary adenocarcinoma cell line native to the rat strain used in the study, was introduced into the peritoneal cavity of rats undergoing laparoscopy, and tumor growth and implantation patterns were

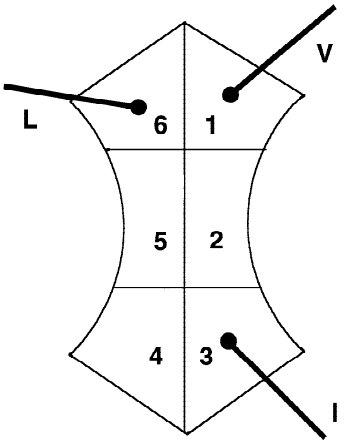

Fig. 1. Division of the abdominal cavity into six sectors and location of trocar entry sites (I = cannula for introduction of tumor, V = venting cannula, L = laparoscope cannula).

assessed 1 week later. This model has been described in detail previously [14].

Eighteen male Dark Agouti (DA) rats were randomized to undergo laparoscopic surgery, with the introduction of cancer cells into the peritoneal cavity, within one of the following groups (six rats per group):

Group I: control. No immune enhancement or suppression was used in this group.
Group II: Cyclosporin. Rats in this group were injected subcutaneously with 50 mg/kg of Cyclosporin (a suppresser of the immune system) 18 h before surgery.
Group III: endotoxin. Rats in this group were injected intraperitoneally with endotoxin (*E. coli* 0111β, a nonspecific stimulator of the immune system) 18 h before surgery.

Feed was withheld from all rats from the time of injection until surgery. Food was also withheld from rats in the control group for a similar time period. All rats were anesthetized during the operative procedures with a combination of halothane and nitrous oxide, supplemented by oxygen and administered through a close-fitting mask. All surgical procedures were performed under sterile operating conditions.

Pneumoperitoneum was initiated using a conventional Verres needle placed through a right hypochondrial stab wound (Fig. 1), over which a disposable mini-laparoscopy cannula (Imagyn Medical, Laguna Niguel, CA, USA) was passed, to provide access for a 2-mm mini-laparoscope (Imagyn Medical) with an attached conventional laparoscopy camera. Two additional "ports" were inserted—an 18-gauge cannula in the left hypochondrium, which was left open throughout the procedure to vent the insufflation gas, and a 16-gauge cannula in the left lower quadrant.

Once all the ports were placed and insufflation was commenced, a 200-μl volume of a mammary adenocarcinoma cell suspension (containing 2.5×10^5 tumor cells) was introduced under laparoscopic vision through the 16-gauge cannula. It was then sealed to prevent gas leakage. The tumor suspension was prepared using a previously described standardized technique [13, 14]. Gas was insufflated at a rate of 0.4 L/min and a pressure of 2 mmHg for a further 40 min. A constant gas flow was maintained by venting the gas through the 18-gauge cannula. The ports were then removed, and the wounds were closed with sutures.

Seven days later, all the rats were killed. Their abdomens were opened and inspected for the presence of tumor. The abdomen was divided into six sectors (Fig. 1). Peritoneal tumor deposits were assessed in each sector and scored for tumor density using the following peritoneal cancer index proposed by Eggermont et al. [6]:

0 no intraperitoneal tumor
I fewer than three minute tumor foci
II moderate tumor
III abundant or confluent tumor

Representative samples were obtained for histological confirmation of the macroscopic assessment. The port sites were also examined for evidence of tumor implantation. The chi-square test was used for the analysis of the data sets expressed as 2 × 3 contingency tables. A one-way analysis of variance (ANOVA) was used for the comparison of rat weights.

The protocol for this study was approved by the Animal Ethics Committees of the Institute of Medical and Veterinary Science and the University of Adelaide, Adelaide, South Australia.

Results

The mean weights of the rats in each group were similar preoperatively, and all rats gained similar amounts of weight during the postoperative period ($p = 0.83$) (Table 1). Tables 2 and 3 summarize the pattern of tumor implantation found in each study group. One rat in the group receiving intraperitoneal endotoxin did not develop any intraabdominal tumor, whereas all rats in the other study groups developed tumor somewhere in the peritoneal cavity.

Tumor growth occurred in an equal number of sectors in the control and cyclosporin groups, but it was less common following endotoxin administration ($p = 0.008$ by chi-square) (Table 2). The tumor growth was also less dense following endotoxin. Port site metastases were significantly less common following the administration of intraperitoneal endotoxin ($p < 0.0001$ by chi-square) (Table 4). There was no overall predilection for tumor growth at the site where the tumor cell suspension had been introduced (sector 3), and tumor was evenly distributed to all sectors (Table 3).

Discussion

Surgical intervention is known to impair systemic immune function, with the degree of effect proportional to the extent of operative trauma [11, 15]. Therefore, it has been claimed that laparoscopic techniques—because they result in less wound-related trauma and cause less disturbance of systemic immune function—might be advantageous for patients undergoing cancer surgery [3, 5]. This hypothesis is supported by clinical studies that have demonstrated that postoperative immune function is better preserved following laparoscopy than laparotomy [3, 5]. Allendorf et al. [1, 2] have also demonstrated in a murine model that tumor cells inoculated into the dorsal skin of laboratory mice grow more easily and aggressively following laparotomy than laparoscopy with CO_2 insufflation, suggesting a systemic benefit for laparoscopic treatment of malignancy, presumably due to less systemic suppression of cell-mediated immune function.

However, despite these potential benefits for laparoscopy, the use of laparoscopic techniques for the staging and manipulation of intraabdominal malignancies has been followed by many case reports that describe tumor metastasis to laparoscopic port sites [12]. Although the true incidence of this phenomenon is unclear, it appears to pose a significant risk for the application of laparoscopic techniques to the diagnosis and excision of cancers. In addition, many experimental studies in small animal models now suggest that carbon dioxide pneumoperitoneum facilitates intraperitoneal tumor growth and wound implantation [8, 16, 24].

It has been argued that port site metastasis is due to

Table 1. Rat weight before and after surgery

	Study group		
	Control	Cyclosporin	Endotoxin
Weight at surgery (g)	224 (203–301)	210 (200–255)	205 (162–235)
Weight at autopsy (g)	250 (222–310)	227 (218–271)	218 (176–256)
% increase in weight	9.5%	7.2%	8.7%

All figures are median and range

Table 2. Number of sectors involved with each peritoneal tumor index grade (36 sectors per study group)

Tumour index grade	Study group		
	Control	Cyclosporin	Endotoxin
0	4	4	23
I	6	7	7
II	12	10	3
III	14	15	3
Total number of sectors with tumor	32 (89%)	32 (89%)	13 (36%)

Table 3. Number of sectors with macroscopic tumor growth of any grade

Sector	Study group			Total
	Control	Cyclosporin	Endotoxin	
1	5	5	1	11
2	6	6	1	13
3	6	6	2	14
4	5	5	3	13
5	5	5	3	13
6	5	5	3	13

Table 4. Number of port sites developing tumor metastases (18 port sites per study group)

Study group		
Control	Cyclosporin	Endotoxin
16 (89%)	15 (83%)	2 (11%)

mechanical contamination of port wounds due to direct contact with contaminated instruments, or to indirect contact due to aerosolization of tumor cells [4, 10, 14, 22]. However, this explanation fails to account for recent experimental studies that have demonstrated that port site metastases can be prevented by using helium gas as the insufflation agent [8, 16]. One possible explanation for this finding is that the development of port site metastases might, at least in part, be influenced by locally acting factors that influence immune function at the level of the peritoneal membrane. This hypothesis is supported by work reported by Volz et al. [23], who showed that carbon dioxide pneumoperitoneum results in significant changes to mechanical, ventilatory, cellular, hormonal, and immunologic parameters, and that these parameters are influenced by intraabdominal pressure, the insufflation gas used, and the duration of insufflation. The specific adverse effects identified include acidosis involving the peritoneal surface and the underlying connective tissue, disturbances in electrical surface charges, and the release of various mediators, such as endotoxin.

In addition, insufflation with CO_2 has been shown to compromise intraperitoneal macrophage activity and depress immunological responses in CD-1 mice, as compared to laparoscopy with air insufflation [25]. Jacobi et al. [9] have demonstrated that laparoscopy with carbon dioxide causes a significant reduction in plasma TNF-α production and an increase in interleukin-10, confirming that CO_2 pneumoperitoneum exerts an influence not just on the local peritoneal environment but also systemically. When attempting to understand the phenomenon of port site metastases, one must consider that while it is possible that at least some systemic immune defense mechanisms are better preserved following laparoscopy than with laparotomy [5], this condition may not reflect the local response to tumor cells at the level of the peritoneal membrane.

Our study sought to further investigate the possible influence of immune function on the development of port site metastases. Endotoxin (lipopolysaccharide *E. coli* 0111β) was used to stimulate peritoneal immune function. It is derived from the cell wall of Gram-negative bacteria and has been shown to be a potent stimulator of peritoneal macrophage function, with its intraperitoneal administration resulting in increased release of TNF-α, IL-1, and IL-6 [19]. Macrophages are one of the principal effector cells of the immune system and therefore are part of the native or nonspecific immune system. On the other hand, lymphocytes play little role in the peritoneal cavity's immune system. Endotoxin administration resulted in a significant reduction in the incidence of tumor implantation and port site metastases. The most likely explanation for this finding is that the reduction in tumor implantation was mediated by intraperitoneal immune function enhancement, which was due to endotoxin stimulating peritoneal macrophages. Differences in local immune effector cell function, and not cell-mediated immune function differences, are believed to account for the results of this study. A direct toxic effect of endotoxin on the tumor cells is unlikely to explain the study's findings, since the endotoxin was administered into the peritoneal cavity 18 h before the tumor cells were introduced at laparoscopy.

There is evidence that laparotomy enhances macrophage function, including TNF-α release, with its effect similar to the one that occurs following the intraperitoneal injection of endotoxin. Clinical and laboratory studies have shown that circulating blood macrophages release more TNF-α after open surgery than laparoscopic surgery, and neutrophil function is similarly enhanced [18, 25]. These results have been attributed to the translocation of gut-derived lipopolysaccharide across the bowel wall, due to the stimulatory

effect of laparotomy. It is therefore possible that laparotomy is in fact protective against wound tumor formation due to enhanced macrophage function. This concept provides an alternative explanation for the results of previous studies showing reduced rates of wound metastasis, but greater growth of tumors remote to the abdominal cavity, for laparotomy vs laparoscopy [13]. This hypothesis warrants further investigation.

Cyclosporin, has been shown to produce immune suppression in previous studies using the DA rat [20]. However, compared to rats in the control group, it failed to significantly increase tumor implantation or port site metastases. Although this lack of difference could be explained by proposing that immune suppression does not influence the risk of tumor dissemination, the similar pattern of metastases observed in the Cyclosporin and control groups may also be related to the high rate of port site metastases found in the control group. To demonstrate a significant increase in the rate of metastases, a much larger number of rats would have been needed for this experiment. Alternatively, the lack of difference could be due to the fact that cyclosporin's principal action is against T-lymphocyte function rather than peritoneal macrophages. Hence, if macrophages are important for dealing with free tumor cells, this would account for the lack of effect. Furthermore, a single dose of cyclosporin may have been insufficient to adequately suppress the immune system. Further studies using different doses of Cyclosporin, as well as agents that are better at suppressing macrophage function, could clarify this issue.

Further differences between clinical surgery and the animal model used in this study must be acknowledged when assessing the outcome of the current study, as well as other animal studies. All but one animal grew a tumor somewhere in the abdominal cavity—a situation that does not arise clinically. However, there were obvious implantation pattern differences between the various study groups that support our conclusions. Furthermore, the pneumoperitoneum pressure of 2 mmHg is less than that used clinically. This pressure was selected following earlier pilot studies, which determined the appropriate pressure for safe laparoscopy in our rat model [14]. Higher pressures were less reliably tolerated. Furthermore, the gas leak of 0.4 L/min in the small rat abdomen is likely to approximate 10–20 L/min in the clinical setting. Although this is higher than the average gas leak during clinical surgery, such leaks occasionally occur during advanced laparoscopic procedures. Nevertheless, the pressure and leak parameters were identical in all study groups. While clinical laparoscopic surgery cannot be replicated fully, the use of an animal model is an appropriate strategy for the initial testing of hypotheses that require subsequent clinical confirmation.

The results of our study support the hypothesis that the incidence of port site metastases can be influenced by alterations in the immune environment, particularly at the local level of the peritoneal membrane. Further work is under way to investigate the effect of CO_2 on the immune environment within the peritoneal cavity. Furthermore, even though we have demonstrated that immune enhancement can reduce the likelihood of intraperitoneal tumor metastasis following laparoscopy, a role for immune-modulating agents in clinical practice must remain speculative.

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