

# Laparotomy and laparoscopy differentially accelerate experimental flank tumour growth

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**Background** Surgery depresses host tumoricidal activity and may increase tumour growth. This study compared the effects of laparoscopy with laparotomy on extraperitoneal tumour growth and immune function in a murine model.

**Methods** C57BL/6 female mice aged 8–10 weeks had tumours induced in the right flank ( $n = 45$ ) and were randomized to undergo halothane anaesthesia only, laparoscopy or laparotomy. Flank tumour volume was assessed over 10 days. A second group of animals ( $n = 540$ ) were randomized to undergo the same procedures and killed at 24, 48 and 96 h. Splenocytes were harvested for natural killer (NK) cell and lymphokine activated killer (LAK) cell cytotoxicity studies.

**Results** There was a significant increase in flank tumour growth in the first 48 h after laparotomy and laparoscopy compared with controls ( $P < 0.01$ ). By 96 h the difference was only significant in the laparotomy group ( $P < 0.01$ ). Both NK and LAK cell cytotoxicities were suppressed significantly ( $P \leq 0.03$ ) from 24 h up to 96 h following laparotomy compared with control and laparoscopy groups. There was also a significant suppression in the laparoscopy group compared with controls in the first 48 h after operation ( $P \leq 0.02$ ).

**Conclusion** Extraperitoneal tumour growth was significantly accelerated after laparotomy and correlated with significantly suppressed NK and LAK cytotoxicity for at least 4 days after operation. Laparoscopy had a shorter, less profound effect on tumour growth and immune function.

Observations that local or distant metastatic growth is increased after surgery are well known. This led to investigation of the relationship between operative stress and tumour growth, and it is now recognized that surgery results in decreased host resistance<sup>1</sup>. The precise mechanism underlying the relationship between the effect of surgical and anaesthetic stress on primary tumour growth, the recurrence of tumours and the proliferation of distant metastases remains unknown.

Berti Riboli and colleagues<sup>2</sup> demonstrated that all 100 patients who had a laparotomy experienced postoperative immunosuppression. This was detected from the first day, peaked by the third day after operation and was more severe and prolonged in patients with cancer. Patients with advanced malignancy already suffer from depressed immune function<sup>3</sup>. Macrophage chemotaxis is severely impaired<sup>3</sup>, as is natural killer (NK) cell cytotoxicity<sup>4</sup>. This immune dysfunction may be attributed to malnutrition, altered host cellular metabolism<sup>5</sup> and also to the presence of tumour-derived circulating immunosuppressive factors<sup>3</sup>. Surgical trauma may have a cumulative effect on an already depressed immune system resulting in ideal conditions for tumour growth<sup>1</sup>.

Laparoscopy may cause significantly less immunosuppression than conventional laparotomy. This study observed the effect of peritoneal insufflation and laparotomy on host antitumour defence mechanisms in a non-excisional extraperitoneal tumour model.

## Materials and methods

Viral and antigen-free C57BL/6 female mice (Charles River, Margate, UK), 8–10 weeks old, were studied ( $n = 585$ ). They were housed in a licensed biomedical facility, allowed to

acclimatize for 1 week before the experiments were carried out and fed standard mouse chow and water *ad libitum*. All procedures were carried out under animal licence guidelines of the Ministry of Health, Ireland.

B16 melanoma (NCI Tumour Repository, Frederick, Maryland, USA) syngeneic for C57BL/6 mice was used for these experiments. All experiments were carried out within ten passages of these cells. The cells were prepared for inoculation in calcium- and magnesium-free Hank's Balanced Salt Solution. The B16 melanoma, target-sensitive Yac-1 (for NK cells) and P815 (for lymphokine activated killer (LAK) cells) cell lines (ATCC, Shrewsbury, UK) were used to assess NK and LAK cell cytotoxicity by chromium-51 release assays (CRA).

## Study groups

All experiments except the subcutaneous flank injections were carried out using halothane general anaesthesia. Mice ( $n = 45$ ) received a subcutaneous injection of  $10^6$  B16 tumour cells in the right flank. They were randomized into three groups after flank tumours were induced at 8 days. A control group underwent 20 min of halothane general anaesthesia only. A laparoscopy group had two 18-G and 20-G cannulas (Venflon; Datex-Ohmeda, Dublin, Ireland) placed in four port sites after which a sterile carbon dioxide pneumoperitoneum was established for 20 min (Solos insufflator, St Louis, Missouri, USA) by passing the gas through a 0.2-mm Acrodisc PF filter (Gelman Sciences, Ann Arbor, Michigan, USA) at 4–6 mmHg. A laparotomy group underwent a midline xiphoid to pubis incision exposing the peritoneal contents for 15 min before closure (5 min) with a continuous 3/0 nylon suture.

Flank tumours were measured with a Vernier calipers in two dimensions (shortest (*a*) and longest (*b*) diameters) every second day for 10 days after operation. Flank tumour volume (*V*) was calculated using the formula:  $V = \pi/6(a^2 + b^2)^{6-8}$ . This is a reliable and reproducible measure of tumour volume and correlates closely with other parameters for assessing tumour volume such as tumour weight/carcass weight ratios<sup>7</sup>. The change in FTV at each time point was determined by subtracting the FTV of the previous time point from that of the current time point and is referred to as the difference in FTV at each time point. Initial flank tumour experiments were carried out when tumours were

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larger, with the surgical interventions performed at day 14 after tumour inoculation (data not shown); findings were similar to previous experiments performed in this laboratory<sup>9</sup>. The initial pattern of tumour growth in the laparotomy and laparoscopy groups at day 2 was similar to the present findings. However, at day 4 the increase in flank tumour volume was significant in the laparotomy group compared with controls only. This was attributed to the fact that manipulation was performed 2 weeks after injection of the tumour cells when the tumour burden was relatively large and the tumour cells were in a proliferative growth phase, and consequently the trauma of laparotomy would probably have less of an effect on tumour growth than if intervention was performed earlier. For this reason, surgical interventions were performed at 8 days.

#### Determination of immune function in vitro

C57BL/6 female mice ( $n = 540$ ) were randomized into the three groups and the procedures performed as described. They were killed at 24, 48 and 96 h after operation and the spleens were harvested for *in vitro* cytotoxicity assays. Three spleens were pooled for each sample within each group, yielding 20 samples per group at each time point. Ten samples were used for NK and LAK cytotoxicity assays against syngeneic B16 melanoma and the other ten samples for assays against target-sensitive cell lines at each time point. Harvesting of splenocytes, preparation of NK and generation of LAK cells, and 4-h CRA are described elsewhere<sup>4,10</sup> and were modified for use in this study as appropriate. Standard *in vitro* CRA<sup>4,10</sup> were performed using fresh NK and LAK cells against syngeneic B16 melanoma and target-sensitive cells (Yac-1 for NK cells and P815 for LAK cells) at an effector to target ratio of 100 : 1. Spontaneous release was consistently 6 per cent or less of the total incorporated label. Percentage cytotoxicity for each effector cell was calculated as: (experimental counts per minute – spontaneous counts per minute/total incorporated label counts per minute)  $\times$  100.

#### Statistical analysis

Statistical analysis was performed using Statworks and Data Desk application programs on a Macintosh IIfx personal computer (Apple; Cupertino, California, USA; Statworks; Cricket Software, Philadelphia, Pennsylvania, USA; DataDesk 4.1; Data Description, Ithaca, New York, USA). Data are expressed as mean (s.e.m.) and were compared using analysis of variance with least significant difference *post hoc* correction.

## Results

#### Effect of surgery on flank tumour growth

The mean size of the flank tumours at 8 days was 0.50(0.18) cm<sup>3</sup> ( $n = 45$ ). The difference in flank tumour volume was significant in both surgery groups compared with the control group at 48 h. The difference in flank tumour growth in the control group was 0.08(0.02) cm<sup>3</sup> compared with 0.25(0.02) cm<sup>3</sup> in the laparoscopy group and 0.34(0.05) cm<sup>3</sup> in the laparotomy group ( $P < 0.01$ ). This significant increase in growth was maintained in the laparotomy group (0.43(0.05) cm<sup>3</sup>) only over the next 48 h compared with both the control (0.18(0.02) cm<sup>3</sup>) and laparoscopy (0.20(0.02) cm<sup>3</sup>) groups ( $P < 0.01$ ). The change in FTV at day 2 after laparotomy was significantly greater compared with both laparoscopy and control; this was also true at day 4. There was no further significant increase in flank tumour growth in either group between days 4 and 8 (4 days). Between days 8 and 10 after operation the difference in flank tumour volume in the laparotomy group (0.45(0.04) cm<sup>3</sup>) was again significant compared with both the control (0.27(0.03) cm<sup>3</sup>) and laparoscopy (0.29(0.04) cm<sup>3</sup>) groups ( $P < 0.01$ ).

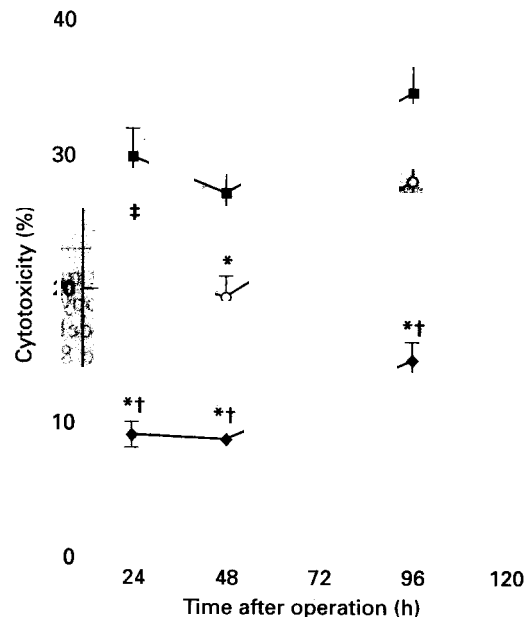


Fig. 1 Natural killer cytotoxicity against Yac-1 after laparotomy (◆), laparoscopy (○) and in controls (■) 24–96 h after operation. \* $P < 0.01$  versus control; † $P < 0.01$  versus laparoscopy; ‡ $P = 0.02$  versus control; (ANOVA with LSD *post hoc* correction)

#### Effect of surgery on immune function

NK cell cytotoxicity against the target-sensitive Yac-1 tumour cell line was significantly decreased in the laparotomy and laparoscopy groups compared with controls at 24 h after operation (Fig. 1). Furthermore, there was also a significant reduction in cytotoxicity between laparotomy and laparoscopy groups ( $P < 0.01$ ). This suppression continued to be significant in both groups compared with controls over the next 24 h ( $P < 0.01$ ) and between the two surgical groups ( $P < 0.01$ ). By day 4 the suppression remained significant only in the laparotomy group ( $P < 0.01$ ). The same pattern was observed for NK cell cytotoxicity against syngeneic B16 melanoma. At 24 h percentage cytotoxicities were: control 7.9(1.0), laparoscopy 5.1(0.6) and laparotomy 2.2(0.4) ( $P < 0.01$  and  $P = 0.01$  laparotomy versus control and laparoscopy respectively;  $P = 0.01$  laparoscopy versus control). Suppression continued at 48 h with control 7.5(0.8) per cent, laparoscopy 5.6(0.4) per cent and laparoscopy 2.4(0.3) per cent ( $P < 0.01$  laparotomy versus both groups;  $P = 0.01$  laparoscopy versus control). At 4 days after operation, NK cell dysfunction persisted significantly in the laparotomy group (5.8(0.3) per cent) only compared with laparoscopy (10.1(0.8) per cent) and controls (12.0(0.8) per cent) ( $P < 0.01$  laparotomy versus both groups).

LAK cytotoxicity against the target-sensitive P815 cell line was also significantly reduced in the two operative groups compared with controls up to 48 h after surgery (Fig. 2). There was also a significant decrease in cytotoxicity between the laparotomy and laparoscopy groups ( $P \leq 0.01$ ). This suppression persisted only in the laparotomy group at day 4 ( $P < 0.01$  versus control;  $P = 0.02$  versus laparoscopy). A similar pattern emerged again in the cytotoxicity against B16 melanoma. Suppression was evident at 24 h in mice that had a laparotomy (7.7(0.4) per cent) and those undergoing laparoscopy (11.2(0.5) per cent) ( $P < 0.01$  both groups versus control and laparotomy versus laparoscopy). This

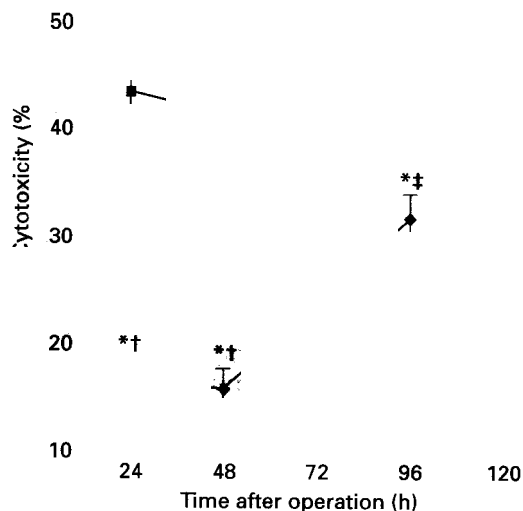


Fig. 2 Lymphokine activated killer cytotoxicity against P815 after laparotomy (◆), laparoscopy (○) and in controls (■) 24–96 h after operation. \* $P < 0.01$  versus control; † $P = 0.01$  versus laparoscopy; ‡ $P = 0.02$  versus laparoscopy; (ANOVA with LSD *post hoc* correction)

persisted at 2 days with percentage cytotoxicities of 19.1(1.6) in controls, 10.2(1.1) in the laparoscopy group and 6.1(1.0) in the laparotomy group ( $P < 0.01$  laparotomy and laparoscopy versus control;  $P = 0.03$  laparotomy versus laparoscopy). By 96 h significant suppression occurred only in the laparotomy group (8.6(1.2) per cent) compared with control (26.0(2.5) per cent) and laparoscopy (20.0(2.4) per cent) groups ( $P < 0.01$ ).

## Discussion

Since the work by Buinauskas *et al.*<sup>11</sup> and Lewis and colleagues<sup>12</sup>, the trauma of major surgery, as well as various forms of anaesthesia<sup>13–18</sup>, have been known to induce significant immunosuppression. However, minimal access surgery can now be accomplished with lower morbidity rates and a faster recovery, largely as a result of reduced surgical trauma<sup>19</sup>. There are concerns, however, about the safety of laparoscopic surgery for malignancy<sup>20</sup>. There were fears about port-site recurrences<sup>21–24</sup>. However, results of short-term clinical studies have suggested that laparoscopic resection of colorectal tumours is not associated with an adverse outcome compared with traditional open resection<sup>25–27</sup>. Experimentally, minimally invasive surgery preserves T cell mitogen responses and delayed hypersensitivity<sup>28,29</sup>, and tumours develop more rapidly and behave more aggressively after laparotomy than laparoscopy<sup>30</sup>. Redmond *et al.*<sup>31</sup> have shown a preservation of monocyte function in patients undergoing laparoscopic versus open cholecystectomy. None of these studies has examined the cellular defects underlying the effects on tumour growth and correlated these with extraperitoneal tumour growth.

The hypothesis was that laparoscopy would preserve immune function and consequently affect tumour growth less than laparotomy in a tumour-bearing host. NK cells were the focus of the studies of immune function as they have been shown to be involved in the recognition and destruction of early developing tumours, activation in response to tumour cell proliferation and limitation of blood and lymph-borne metastatic cells. NK cells are also

cytotoxic to many fresh solid tumour cells<sup>32</sup>. LAK cells generated by the stimulation of NK cells by interleukin 2 and interferon  $\gamma$  possess highly potent tumoricidal properties compared with NK cells and NK-resistant tumour cells have been demonstrated to be susceptible to LAK killing *in vitro*<sup>32</sup>. Early work demonstrated the effect of experimental surgical stress on NK cytotoxicity<sup>33–35</sup> and, more recently, NK suppression has been demonstrated after surgery for breast and oesophageal carcinoma<sup>36,37</sup>. Griffith and co-workers<sup>38</sup> have shown a significant decrease in T cell proliferation after open cholecystectomy compared with laparoscopic cholecystectomy but, interestingly, found a small but insignificant decrease in NK cell cytotoxicity in both groups of patients.

Both NK and LAK cell cytotoxicity were significantly suppressed following laparotomy and laparoscopy compared with that in controls up to 48 h after surgery. This early depression of an important antitumour defence mechanism may explain the observed changes in tumour growth. This suppression of immune function persisted significantly in mice that had a laparotomy compared with control animals and with those that had a laparoscopy up to 4 days after operation. This suggests that the acceleration in tumour growth in these animals is due, at least in part, to failure of this first-line defence mechanism.

In the first 48 h, the increase in flank tumour volume was significant in both the laparoscopy and laparotomy groups compared with controls but not between the two surgical groups. This is probably explained by the fact that laparoscopic surgery has been shown to elicit similar responses to laparotomy<sup>28</sup> immediately after operation. The persisting significant NK and LAK dysfunction occurring in mice that had a laparotomy compared with animals in the other two groups between 48 and 96 h after operation provides an explanation for the observed significant and sustained increase in tumour growth.

Several mechanisms have been postulated to be responsible for early postoperative immunosuppression, in particular the production of prostaglandin  $E_2$  by macrophages<sup>39</sup> and the adrenocorticot stress response<sup>28,31</sup>, as well as the emergence of 'suppressor' cells that interfere with NK-mediated tumour cell lysis<sup>40</sup>. The size of the surgical wound may be another factor as it is known that the wound is a site of intense immunological activity<sup>41</sup>. However, wound factors could not be responsible for the changes in NK and LAK function and tumour growth in the early postoperative phase as immunosuppressive factors have been found in the wound fluid of rats only 7 days after injury<sup>42</sup>. Although this study only examined the events occurring in the early postoperative period, it could be speculated that the emergence of suppressive factors in the laparotomy wound during the later time points would explain the significant difference in tumour growth 7 days after operation in mice that had a laparotomy.

The immunological changes observed after surgery are complex and multifactorial. This study has shown profound suppression of immune function with correspondingly accelerated tumour growth, even after laparoscopy. The effect after laparoscopy is much less profound and prolonged than after laparotomy, and could have implications for the timing of adjuvant therapy.

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