

Attenuation of the Tumor-promoting Effect of Surgery by Spinal Blockade in Rats

Shahar Bar-Yosef, M.D.,* Rivka Melamed, M.Sc.,† Gayle G. Page, D.N.Sc.,‡ Guy Shakhar, M.A.,† Keren Shakhar, M.A.,† Shamgar Ben-Eliyahu, Ph.D.§

Background: The perioperative period is characterized by a state of immunosuppression, which was shown in animal studies to underlie the promotion of tumor metastasis by surgery. As this immunosuppression is partly ascribed to the neuroendocrine stress response, the authors hypothesized that spinal blockade, known to attenuate this response, may reduce the tumor-promoting effect of surgery.

Methods: Fischer-344 rats were subjected to a laparotomy during general halothane anesthesia alone or combined with either systemic morphine (10 mg/kg) or spinal block using bupivacaine (50 µg) with morphine (10 µg). Control groups were either anesthetized or undisturbed. Blood was drawn 5 h after surgery to assess number and activity of natural killer cells, or rats were inoculated intravenously with MADB106 adenocarcinoma cells, which metastasize only to the lungs. Metastatic development was assessed by quantifying lung retention of tumor cells 24 h after inoculation or by counting pulmonary metastases 3 weeks later.

Results: Laparotomy conducted during general anesthesia alone increased lung tumor retention up to 17-fold. The addition of spinal block reduced this effect by 70%. The number of metastases increased from 16.7 ± 10.5 (mean \pm SD) in the control group to 37.2 ± 24.4 after surgery and was reduced to 10.5 ± 4.7 during spinal block. Systemic morphine also reduced the effects of surgery, but to a lesser degree. Natural killer cell activity was suppressed to a similar extent by surgery and by anesthesia alone.

Conclusions: The addition of spinal blockade to general halothane anesthesia markedly attenuates the promotion of metastasis by surgery.

SURGICAL resection of tumors is an important aspect of cancer treatment. Unfortunately, it is becoming apparent that the perioperative period is characterized by pronounced immunosuppression,^{1,2} including a reduction in the activity of natural killer (NK) cells^{3,4} and macro-

phages,² known to spontaneously recognize and kill a variety of tumor cells. This fact may increase metastatic development at this susceptible period, hence the description of the surgical option for cancer as a "double-edged sword."⁵

Many factors have been shown to contribute to perioperative immunosuppression, among them anesthetic agents,^{2,6} blood transfusion,⁷ hypothermia,⁸ and psychological stress.⁹ An additional important factor is the physiologic and neuroendocrine responses accompanying surgery.^{1,10,11} Importantly, regional anesthesia has been repeatedly shown to attenuate this stress response,^{11,12} a fact that may underlie the reduced immunosuppression elicited by surgical procedures conducted with regional anesthesia (either spinal or epidural block) compared with general anesthesia.^{11,12} Attenuating immunosuppression may reduce the metastasis-promoting effects of surgery.

Thus, in the current study we sought to assess whether the use of regional anesthesia, specifically spinal block, attenuates the promotion of metastasis by surgery. To this end, we used an animal model that we have previously used to study the influence of surgical stress on metastatic development.^{4,13} After laparotomy with halothane anesthesia, Fischer-344 rats were intravenously inoculated with syngeneic MADB106 adenocarcinoma cells. These tumor cells are retained in the lungs and consequently develop metastases only in this organ. Surgery increased both the retention of tumor cells in the lungs and the number of metastases detected 3 weeks later.⁴

Because our previous studies indicated that the systemic use of low doses of morphine could attenuate the promotion of metastasis by laparotomy,^{14,15} in the current study we also compared the effects of regional block with those of systemic morphine administration. As a preliminary attempt to elucidate cellular mechanisms underlying the potential protective influence of regional anesthesia, we also assessed the number and activity of circulating NK cells, as lung colonization by MADB106 cells is highly sensitive to this activity.^{13,16}

Materials and Methods

Animals

Fischer-344 male rats (Harlan Laboratories, Jerusalem, Israel) were housed four in a cage with free access to food and water in a 12:12-h lighting regimen. Animals were acclimatized to the vivarium for at least 4 weeks before any experiment and were handled three times during the week before each experiment. All experi-

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

* Resident, Department of Anesthesiology, Rabin Medical Center-Beilinson Campus, Petach Tikva, Sackler School of Medicine, † Doctoral Research Fellow, § Head, Psychobiology Research Unit, Department of Psychology, Tel Aviv University. ‡ Associate Professor and Independence Foundation Chair, School of Nursing, Johns Hopkins University.

Received from the Department of Anesthesiology, Rabin Medical Center-Beilinson Campus, Petach Tikva, Sackler School of Medicine, and Psychobiology Research Unit, Department of Psychology, Tel Aviv University, Tel Aviv, Israel; and the School of Nursing, Johns Hopkins University, Baltimore, Maryland. Submitted for publication May 10, 2000. Accepted for publication December 15, 2000. Supported by grant No. CA73056-02 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland (to Dr. Ben-Eliyahu), and grant No. 0962 from the Chief Scientist, Israeli Ministry of Health, Jerusalem, Israel (to Dr. Ben-Eliyahu).

Address reprint requests to Dr. Ben-Eliyahu: Department of Psychology, Tel Aviv University, Tel Aviv 69978, Israel. Address electronic mail to: shamgar@post.tau.ac.il. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

Table 1. Experimental Design: Groups in Each Experiment and Number of Animals in Each Group

Experiment	Age (weeks)	Weight (g ± SD)	Control Group	Anesthesia-only Groups			Surgery Groups		
				H	H + Mo	H + Sp	H	H + Mo	H + Sp
1: Lung tumor retention	10–12	245 ± 25	8	10	7	9	10	11	11
2: Pulmonary metastases	15–16	273 ± 16	11	8	—	8	7	6	7
3: <i>Ex vivo</i> NK assays	9–10	158 ± 21	5	6	—	—	7	6	7

H = halothane only; H + Mo = halothane with systemic morphine; H + Sp = halothane with spinal block.

ments were conducted during the first half of the light phase. In any given experiment, all animals were of the same age. The Institutional Animal Care and Use Committee of Tel Aviv University approved all experiments.

Experimental Design and Procedures

The experimental groups included a control group, three subgroups of anesthesia without surgery, and three subgroups of surgery. The latter six subgroups were exposed to one of the following anesthetic regimens: halothane only, halothane with systemic morphine, or halothane with spinal block. Rats from the control group remained undisturbed in their home cages. Rats from the anesthesia groups were kept anesthetized for 1 h, at the same time as rats from the surgery groups were undergoing laparotomy, as described below. Morphine was injected or spinal block was performed immediately after the induction of general anesthesia, and rats belonging to the surgery subgroups underwent laparotomy immediately thereafter.

The specific design of the three experiments conducted, including the number of animals in each group, is depicted in table 1. Each experiment was conducted separately, and rats were not used for more than one experiment. In the first and second experiments, MADB106 cells were injected 4–5 h after induction of anesthesia. In the first experiment, lungs were removed 24 h after tumor inoculation for the assessment of lung tumor retention, while in the second experiment, lungs were removed 3 weeks after tumor inoculation for counting pulmonary metastases. In the third experiment, blood was withdrawn 4–5 h after the induction of anesthesia, and analyses of the number and activity of NK cells were conducted *in vitro*. Because of the large number of animals needed in experiment 1, two replicates were conducted on separate days, using approximately half the rats from each experimental group.

To avoid potential order effects, in each experiment, the time and order of anesthesia–operation, blood withdrawal, and tumor injection were counterbalanced across all subgroups (*i.e.*, rats from the different experimental groups were alternately treated throughout the experiment). Blood withdrawal or tumor injection was completed within 2 h in all animals.

General Anesthesia, Morphine, and Spinal Block

Anesthesia was induced with halothane in room air and maintained at 2–3% halothane in room air *via* a vaporizer. Rats breathed spontaneously throughout the anesthesia, and the halothane concentration was adjusted according to the animal's respiratory pattern and response to incision.

For systemic injection, morphine sulfate was prepared in a concentration of 5 mg/ml in phosphate-buffered saline and injected intraperitoneally in a dose of 10 mg/kg using a 25-gauge, 5/8-inch needle. Injection was performed after halothane induction, at least 5 min before abdominal incision.

Spinal block was performed according to a method described by Mestre *et al.*¹⁷ After halothane induction, a 25-gauge, 1.5-inch needle (connected to 50- μ l Hamilton glass syringe) was used to percutaneously locate the spinal canal at L5–L6 or L4–L5 intervertebral spaces. Tail or hind-leg movement was used to confirm penetration into the spinal canal, and 10 μ l bupivacaine 0.5% containing 10 μ g morphine sulfate was injected. We allowed at least 5 min for analgesia to commence before starting laparotomy. This drug regimen has been previously used and was found to be highly synergistic.¹⁸

Validation of Analgetic Techniques

To validate the analgesic effect of systemic morphine and spinal blockade, we conducted a pilot study using 9–10-week-old rats. Morphine was injected or spinal block performed with light halothane anesthesia (lasting 5–10 min). Pain threshold was assessed by measuring the latency to tail withdrawal after immersion in 50°C water. A 20-s cutoff time was set to avoid tissue damage. Measurements were conducted in triplicate and performed once before interventions and at 5, 15, 30, 60, 120, 180, 240, 300, and 360 min after awakening from anesthesia. Analgesic regimens that we tested included systemic injection of morphine (5 or 10 mg/kg intraperitoneally) and four variants of spinal block (50 μ g bupivacaine alone, 25 μ g bupivacaine with 5 μ g morphine, 50 μ g bupivacaine with 10 μ g morphine, and 75 μ g bupivacaine with 15 μ g morphine; n = 1, 2, 1, 1, 2, and 3 per group, respectively). The concentrations for all intrathecal injections were 5 μ g/ml bupivacaine and 1 μ g/ml morphine. In addition, two rats served as control (halo-

thane anesthesia only), and two received intrathecal injection of 10 μ l normal saline.

Laparotomy

After hair trimming and scrubbing with 70% alcohol, a 4-cm midline abdominal incision was performed, and the small and large intestines were externalized and rubbed at four places with a gauze pad. The intestines were then covered with phosphate-buffered saline-soaked gauze, and the abdomen was left open. Finally, the abdomen was closed in one layer with 3-0 nylon sutures. Total procedure time from induction of anesthesia to the last stitch was set to 1 h. Rats awoke 5 min later.

The MADB106 Tumor Line

MADB106 is a selected variant cell line obtained from a pulmonary metastasis of a mammary adenocarcinoma (MADB100) chemically induced in the inbred F344 Fischer rat.¹⁶ MADB106 cells were maintained as a monolayer cell culture in complete medium at 100% humidity, 5% CO₂ at 37°C, and detached from the flask using 0.25% trypsin.

Radiolabeling of Tumor Cells and Assessment of Lung Tumor Retention

For assessment of lung tumor retention, DNA radiolabeling of tumor cells was accomplished by adding 0.4 mCi/ml of ¹²⁵I-iododeoxyuridine (Amersham Biotech, Piscataway, NJ) to the growing cell culture 1 day before collecting the cells for injection.

For tumor cell injection, rats were lightly anesthetized with halothane, and 4×10^5 /kg ¹²⁵I-iododeoxyuridine-labeled MADB106 tumor cells in approximately 0.5 ml of phosphate-buffered saline (supplemented with 0.1% bovine serum albumin) was injected into their tail vein. This procedure lasted 2-3 min, and the rats woke quickly and were returned to their cages. Twenty-four hours later, rats were killed with halothane, and their lungs were removed and placed in a γ -counter for assessment of radioactive content. Lung tumor retention was calculated as the ratio of radioactivity measured in the lungs to radioactivity in the injected tumor cells suspension. Our previous studies have indicated that the level of lung radioactivity reflects the number of viable tumor cells in the lungs.¹³

Induction and Counting of Tumor Metastases

Rats were lightly anesthetized with halothane, and 10^5 MADB106 tumor cells were injected into their tail vein in 0.5 ml of phosphate-buffered saline supplemented with 0.1% bovine serum albumin. Rats were killed with halothane 3 weeks after tumor inoculation, and their lungs were removed and placed for 24 h in Bouin solution (72% saturated picric acid solution, 23% formaldehyde [37% solution], and 5% glacial acetic acid). After lungs were washed in ethanol, visible surface metastases were counted.

Flow Cytometry

Fluorescence-activated cell sorter analysis was used to assess the number of NK cells in the blood. NK cells were identified as NKR-P1^{bright} lymphocytes using fluorescein isothiocyanate-conjugated anti-NKR-P1 monoclonal antibody (BD PharMingen, San Diego, CA). To assess the absolute number of NK cells per milliliter blood, a fixed number of polystyrene microbeads (20 μ m; Duke Scientific, Palo Alto, CA) was added to the blood samples before they were prepared for cytometric analysis. For further details, see Shakhar and Ben-Eliyah.¹⁹

Whole-Blood Natural Killer Cytotoxicity Assay

The activity of NK cells was assessed using the whole-blood cytotoxicity assay described in detail elsewhere.¹⁹ Briefly, this procedure assesses antitumor cytotoxicity of NK cells per milliliter blood without prior purification of monocytes. One milliliter of blood was drawn by cardiac puncture, plasma was replaced with culture medium, and aliquots of the washed blood were then placed in the first row of a microtiter plate. To assess NK cytotoxicity at six different effector-to-target ratios, successive twofold dilutions of blood with complete media were performed. As target cells, a fixed amount of ⁵¹Cr-radio-labeled YAC-1 lymphoma cells were added to the blood. Spontaneous and maximal releases of radioactivity from target cells were determined by substituting blood with the culture medium or Triton-X (Sigma Chemicals, St. Louis, MO), respectively. After a 4-h incubation period, samples of supernatant were recovered from each well for the assessment of radioactivity. Specific killing was calculated as [(sample release - spontaneous release) / (maximal release - spontaneous release)] \times 100. A correction for changing hematocrit and supernatant volume over different effector-to-target ratios was included. Earlier studies have indicated that cytotoxicity measured using this procedure is attributable to NK cells rather than other cell types or soluble factors.

Statistical Analysis

A two-way analysis of variance (ANOVA; treatment and replicate as the two factors) was used to analyze lung tumor retention. In experiment 2, large differences in variance between subgroups violated ANOVA assumptions, and separate *t* tests with Bonferroni compensation for multiple tests were used instead.

Natural killer cell activity was analyzed using repeated-measures ANOVA for the different effector-to-target ratios. Lytic units were calculated using the formula $100/ET_{7.5}$, where $ET_{7.5}$ is the effector-to-target ratio needed to lyse 7.5% of target cells. The regression exponential fit method was used to infer $ET_{7.5}$ from the data.²⁰ One-way ANOVA was used to analyze the number of NK cells and lytic units. To eliminate the effect of a change in NK cell number on NK cell activity, the num-

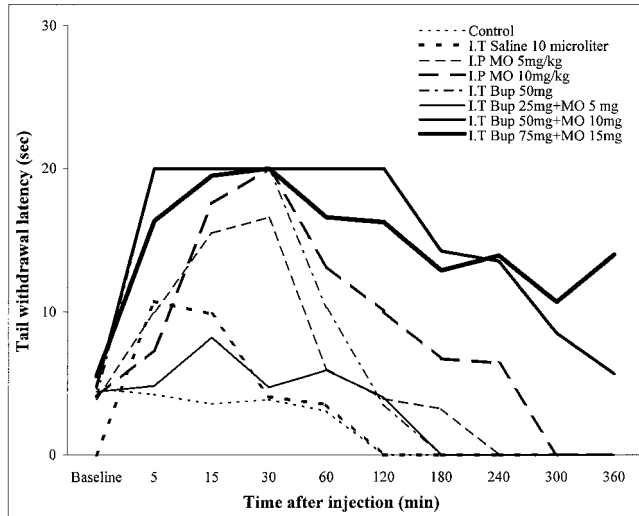


Fig. 1. Duration of analgesia as measured by tail withdrawal latency (in seconds) after injection of either morphine (MO) intraperitoneally (IP) or bupivacaine (Bup) ± morphine intrathecally (IT). Control rats did not receive any injection. Data are presented as mean values.

ber of lytic units divided by NK cell number was calculated as a measure of single NK cell cytotoxic activity.

Provided ANOVA indicated significant group differences, *post hoc* Scheffé analysis was used to conduct pairwise comparisons.

All data were normally distributed and are expressed as mean ± SD unless stated otherwise. $P < 0.05$ was considered significant, except when using the Bonferroni correction, where $P < 0.005$ was required.

Results

Validation of Analgetic Techniques

Systemic injection of 10 mg/kg morphine resulted in analgesia that started shortly after injection and lasted for 120–180 min (fig. 1).

Spinal injection of 50 µg bupivacaine with 10 µg

morphine resulted in analgesia that started 5 min after injection and lasted for 300 min; lower doses, or the use of bupivacaine without morphine, had a substantially shorter duration of action, and higher doses were not more effective. Signs of motor block were confined to the hind legs and dissipated 15 min after spinal injection, regardless of the dose used.

Experiment 1: The Effect of Surgery and Anesthesia on MADB106 Lung Tumor Retention

In rats anesthetized without surgery, no differences in lung tumor retention were found between the different anesthesia regimens (halothane only, $0.52\% \pm 0.34$; halothane + morphine, $0.42\% \pm 0.1$; halothane + spinal block, $0.32\% \pm 0.18$) ($F_{2,18} = 1.2, P = 0.326$). Therefore, for further analysis these subgroups were combined to form the anesthesia-only group.

In both replicates of the experiment, surgery increased lung tumor retention compared with either the anesthesia-only or the control groups (although the size of the effect was different in the two replicates: 17.7- and 2.5-fold, respectively). As shown in figure 2, in both replicates the addition of spinal block, and to a lesser extent systemic morphine, attenuated this effect of surgery. To combine the replicates and analyze for specific effects, a two-way ANOVA was conducted with replicate as a factor in the analysis. Surgery with general halothane anesthesia increased lung tumor retention compared with the control group ($P = 0.0003$, Scheffé *post hoc* test), as well as compared with the anesthesia-only group ($P < 0.0001$, Scheffé *post hoc* test). The effect of surgery was reduced by 50–62% with the addition of systemic morphine, although this reduction did not reach statistical significance ($P = 0.28$), and lung tumor retention remained significantly elevated compared with the control group ($P = 0.012$). On the other hand, the addition of spinal block significantly attenuated the effect of surgery by 66–74% ($P = 0.0171$ compared with surgery with halothane only), rendering the difference

Fig. 2. The effect of surgery on lung tumor retention of MADB106 tumor cells. Compared with control (C) and anesthesia-only (A) groups, surgery with halothane (H) significantly increased lung tumor retention. The addition of spinal block (H + Sp) significantly attenuated this metastasis-promoting effect of surgery, whereas systemic morphine (H + Mo) did not cause such attenuation, and lung tumor retention remained significantly higher than control levels. Data are presented as mean ± SD, separately for each of the two replicates conducted.

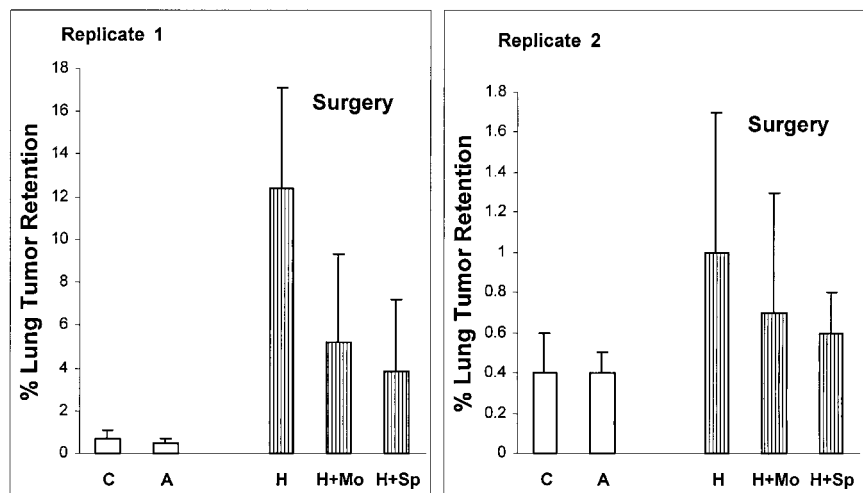


Table 2. Effects of Anesthesia and Surgery on Blood Number of NK Cells, Polymorphonuclear Cells (PMN), and Mononuclear Cells (Monocytes and Lymphocytes)

	Control	Anesthesia	Surgery		
			H	H + Mo	H + Sp
NK cells	164 (\pm 40)	168 (\pm 47)	174 (\pm 52)	131 (\pm 26)	113 (\pm 27)*
PMN cells	1,283 (\pm 175)	951 (\pm 236)	2,759 (\pm 1,282)†	1,781 (\pm 408)	1,233 (\pm 305)*
Mononuclear cells	6,220 (\pm 836)	4,223 (\pm 735)†	2,661 (\pm 853)†	2,107 (\pm 742)	1,830 (\pm 1,060)†

Values are mean (\pm SD), per 1 μ l blood. H = halothane only; H + Mo = halothane with systemic morphine; H + Sp = halothane with spinal block.

* $P < 0.05$ compared with surgery with halothane only. † $P < 0.05$ compared with corresponding control group.

between surgery with spinal block and the control or anesthesia-only groups not significant. No significant differences were found between the groups that underwent surgery with halothane plus morphine and surgery with halothane plus spinal block.

Experiment 2: The Effect of Surgery and Anesthesia on Pulmonary Metastases

As in lung tumor retention, no significant difference existed between the two subgroups of anesthesia without surgery (halothane alone [15.1 ± 13.9 metastases per animal] or halothane with spinal block [18.3 ± 6.2 metastases]; $t_{12} = -0.5$, $P = 0.59$), and the two subgroups were combined for further analysis. Surgery conducted with general halothane anesthesia increased the number of metastases 2.2-fold, from 16.7 ± 10.5 in the control group (16.9 ± 10.4 in the anesthesia-only group) to 37.2 ± 24.4 ($P = 0.0047$ and $P = 0.0066$, respectively). The addition of spinal block to general halothane anesthesia significantly reduced the number of metastases from 37.2 ± 24.4 to 10.5 ± 4.7 ($P = 0.0043$), whereas the addition of systemic morphine had no significant effect (modestly reducing the number of metas-

tases to 31 ± 13.9 , $P = 0.46$) (fig. 3). However, the difference between the systemic morphine subgroup and the spinal block subgroup was not significant.

Experiment 3: The Effect of Surgery and Anesthesia on Number and Activity of Natural Killer Cells

Flow cytometry indicated that surgery with halothane anesthesia did not change the number of circulating NK cells at 4 h after the end of surgery. However, the addition of spinal block to the general anesthesia caused a significant decrease in the number of NK cells in rats undergoing surgery (table 2). On the other hand, surgery caused an increase in the number of polymorphonuclear cells, which was abolished by the addition of spinal block (table 2).

The cytotoxic activity of NK cells was suppressed by general anesthesia alone (although not significantly), and further by surgery, reaching statistically significant levels ($P = 0.02$) compared with control (fig. 4). There were no significant differences between the anesthesia-alone group and the surgery groups. The addition of spinal block to general anesthesia in rats undergoing surgery decreased NK activity even more, but this additional decrease was not statistically significant and is at least partly attributable to a significant decrease in the blood number of NK cells in this group (table 2).

When cytotoxic activity was calculated as the number of lytic units at 7.5% specific killing, the same results emerged (data not shown). Calculating the number of

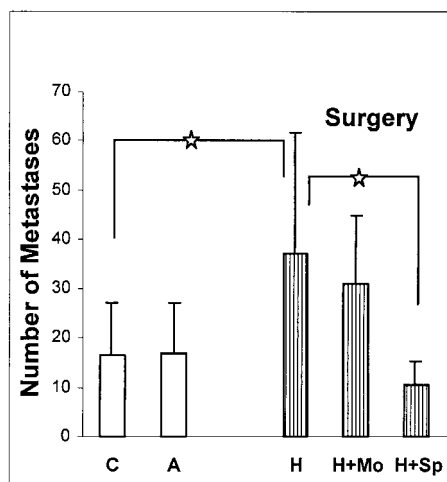


Fig. 3. The effect of surgery on the number of pulmonary metastases. Surgery performed with halothane anesthesia (H) increased the number of pulmonary metastases compared with the control (C) and anesthesia-only (A) groups. Adding spinal block (H + Sp), but not systemic morphine (H + Mo), abolished this increase. *Statistically significant differences. Data are presented as mean \pm SD.

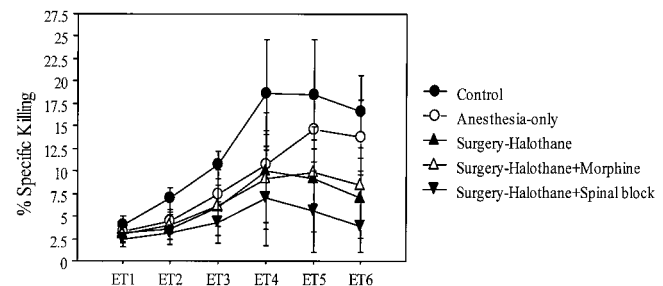


Fig. 4. Effects of anesthesia and surgery on natural killer (NK) cytotoxic activity (expressed as percent specific killing at six effector-to-target [ET] ratios), assessed 5 h after the beginning of surgery. Surgery suppressed NK activity compared with the control group, with no significant difference between the various anesthetic regimens. Data are presented as mean percent specific killing \pm SD.

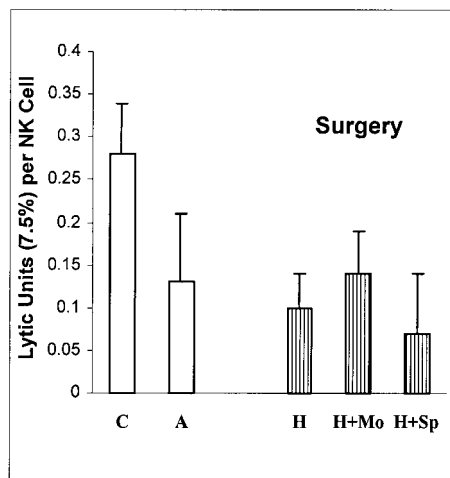


Fig. 5. The effect of anesthesia and surgery on single natural killer (NK) cell activity levels, expressed as number of lytic units (LU_{7.5%}) per NK cell. NK activity was reduced by anesthesia (A) and surgery (H) to the same extent compared with the control group (C). Adding systemic morphine (H + Mo) or spinal block (H + Sp) did not attenuate this effect. Data are presented as mean ± SD.

lytic units per NK cell reduced the differences between the various surgical subgroups (fig. 5) while maintaining the significant difference between the control group and the surgery groups combined ($P = 0.011$). Anesthesia alone reduced the number of lytic units per NK cell to a very similar level as surgery, but this difference did not reach statistical significance ($P = 0.095$).

Discussion

This study in a rat model of breast cancer metastasis shows that surgical stress promotes metastatic development and that the use of regional anesthesia markedly attenuates this effect. This conclusion is based both on measuring lung tumor retention 24 h after tumor inoculation (fig. 2) and on counting pulmonary metastases 3 weeks later (fig. 3). It is important to note that lung tumor retention of the MADB106 cells is an early indicator of the number of metastases that would have developed had the rat not been killed, and that both indices are highly sensitive to NK activity.^{13,16,19}

As a preliminary study of potential immunologic mechanisms mediating the protective effects of spinal block, we assessed the number and activity of NK cells at the time tumor cells were injected. NK cells are a subset of lymphocytes that spontaneously recognize and kill tumor cells and have been shown to be especially important in controlling circulating tumor cells and micrometastases.²¹ However, in the current study, at the time point studied, NK activity was reduced to a similar degree by general anesthesia alone and by surgery, and was further reduced by spinal block, despite its favorable

effect on metastatic development (fig. 4). This discordance is intriguing considering that the metastatic process of the MADB106 line is extremely sensitive to NK cell activity: selective depletion of NK cells increases MADB106 metastasis more than a 100-fold.^{13,16,19} A similar discordance between the *in vivo* and *in vitro* results has been described in respect to the protective effects of systemic morphine administration using the same tumor model.¹⁴ A number of factors may account for this discordance: (1) although the *in vivo* findings reflect a cumulative effect on immune function over the 24 h after tumor inoculation, the *in vitro* assay only evaluates NK activity at the single time point of inoculation; (2) although in the current study NK activity was assessed in the blood, metastases are formed in the lungs, and thus pulmonary NK activity may be of greater importance; (3) the NK ⁵¹Cr-release assay may fail to reflect various aspects of *in vivo* NK tumoricidal activity (e.g., inducing apoptosis²²); and (4) other effector cells may be involved in controlling pulmonary metastases of the MADB106 (e.g., alveolar macrophages¹⁶).

Considering the potential clinical implications of our findings, it would be instructive to understand the underlying mechanisms. It is our hypothesis that immunosuppression is a major factor mediating the promotion of metastasis by surgery and that the protective effects of regional anesthesia are mediated, at least partly, by attenuation of the neuroendocrine stress response to surgery and its accompanying immunosuppression. Several lines of evidence support this hypothesis. Studies using various animal models have reported promotion of metastasis by surgery.^{4,23-27} In humans, such a promotion is suggested by anecdotal reports of metastatic flare-ups after cytoreductive procedures,²⁸ as well as by lower survival rates after staged procedures for colon cancer as compared with a single operation.²⁹ Although various mechanisms may underlie these effects of surgery, one that has received much attention and support is perioperative immunosuppression. This adverse effect of surgery is well established experimentally and clinically and has been shown to be proportional to the magnitude of the surgical procedure.^{27,30} Perioperative immunosuppression is manifested as both humoral and cellular dysfunction,^{1,2} including a reduction in NK cell activity.^{3,4} Suppression of NK activity may enable dormant micrometastases to grow, especially in the context of reduced level of angiostatin caused by surgery.³¹ Tumor cells released into circulation as a result of tumor manipulation³² may have a greater chance of implantation during such conditions.

The stress response to surgery is believed to be a major factor mediating perioperative immunosuppression, acting through multiple neuroendocrine pathways. These include the secretion of endogenous opioids,³³ hormones of the hypothalamic-pituitary-adrenal axis,¹⁰ and catecholamines.¹⁹ Although catecholamines were re-

ported both to enhance NK activity³⁴ and to suppress it,³⁵ we recently reported that in our model, activation of the sympathetic nervous system, or the administration of a β -adrenergic agonist, suppresses single-cell NK activity in rats, consequently enhancing MADB106 metastasis.¹⁹

Studies in humans have indicated that regional anesthesia can attenuate the perioperative increase in the levels of these hormones.³⁶⁻³⁹ Accordingly, several studies have indicated that surgeries conducted with regional anesthesia preserve various immunologic indices better than those conducted with general anesthesia. These indices include blastogenic response of lymphocytes,⁴⁰ lymphocyte concentration,⁴¹ NK cell activity,^{38,39,42} and Th1 to Th2 cell ratio.⁴³ Although not tested in the current study, we suggest that the evident protective effect of spinal block results from the attenuation of the surgical stress response. In support of this suggestion, the current study indicated that spinal block indeed abrogated the surgery-induced neutrophilia, a phenomenon known to be related to elevated corticosteroid levels.⁴¹

It is also feasible that the neuroendocrine changes, which are associated with surgery and are modified by anesthesia, may affect metastatic development through nonimmunologic mechanisms, including modulation of tumor angiogenesis, cytokine release, or direct influences on malignant cells. In addition, both spinal anesthesia and systemic morphine induce other physiologic changes besides modulation of the stress response. Clearly, rats exposed to different anesthetic regimens may have somewhat different blood pressure, cardiac output, and partial pressures of oxygen and carbon dioxide. It is also possible that the use of morphine or spinal block exerted a halothane-sparing effect. Although these differences may affect immunity or tumor cells, we suggest that they had minor impact in the current study. First, compared with undisturbed control rats, anesthesia alone, while still assumed to exert its physiologic effects, had no significant effect on lung tumor retention. Second, a previous study in rats indicated that the suppression of NK cell activity by high-dose narcotic anesthesia was not attenuated by mechanical ventilation, which achieved normal carbon dioxide and oxygen levels.⁴⁴

We previously hypothesized that pain alleviation is a mechanism reducing the neuroendocrine response to surgery and suggested that it underlies the protective effects of systemic morphine.¹⁵ The current study reinforces this notion by showing that spinal blockade, a different method for inducing analgesia, can attenuate the promotion of metastasis by surgery. This method applies a 200-fold lower dose of opiates, refuting alternative hypotheses that morphine induces its beneficial effects by directly acting on the immune system, on tumor cells, or on other peripheral mechanisms affecting metastasis.

In the current study, both systemic morphine and spinal block attenuated the detrimental effect of surgery on lung tumor retention (fig. 2), whereas only spinal block conferred protection in the second experiment, where pulmonary surface metastases were counted (fig. 3). One possible explanation is that spinal block exerted a superior or more prolonged analgesia, as is suggested by the results of the pilot study (fig. 1). However, in previous studies, prolonging the analgesic effect of morphine by adding a postoperative dose of systemic morphine, or by using a slow-release formulation of the drug, was found not to offer any benefit in attenuating the adverse effects of surgery on metastasis.¹⁵ Alternatively, even achieving a similar degree of analgesia, one can expect a more profound suppression of the neuroendocrine stress response to surgery when using neuroaxial local anesthetics compared with systemic or neuraxial opiates.^{10,11} This can probably be ascribed to a more dense and widespread blockade of ascending sensory pathways, as well as to an extensive blockade of descending sympathetic efferents, which is not directly induced by morphine in the spinal cord. It is noteworthy that the beneficial effect of systemic morphine through the attenuation of the neuroendocrine stress response may be reduced by its known dose-dependent negative effects on NK cell activity and resistance to metastasis.^{14,45}

In conclusion, the addition of spinal block in our model has an advantage over the use of general anesthesia alone, and we suggest that it acts by reducing the neuroendocrine response to surgery. In clinical practice, an epidural block can be carried over into the postoperative period. It is thus reasonable to assume, but certainly proof is needed, that the favorable effect of prolonged epidural block on immune function and tumor metastasis will exceed the effect we found using short-term spinal block. The clinical significance of attenuating postoperative immunosuppression by regional anesthesia is still unclear, but some evidence suggests a reduced incidence of postoperative infections.¹² To our knowledge, the present study provides the first experimental evidence that regional anesthesia may reduce postoperative metastatic development. If similar effects are demonstrated in other models of cancer, a controlled clinical study should be considered.

References

1. Tonnesen E: Immunological aspects of anaesthesia and surgery. *Dan Med Bull* 1989; 36:263-81
2. Salo M: Effects of anaesthesia and surgery on the immune response. *Acta Anaesthesiol Scand* 1992; 36:201-20
3. Pollock RE, Lotzova E, Stanford SD: Surgical stress impairs natural killer cell programming of tumor for lysis in patients with sarcomas and other solid tumors. *Cancer* 1992; 70:2192-202
4. Ben-Eliyahu S, Page GG, Yirmiya R, Shakhar G: Evidence that stress and surgical interventions promote tumor development by suppressing natural killer cell activity. *Int J Cancer* 1999; 80:880-8
5. Lundy J: Anesthesia and surgery: A double-edged sword for the cancer patient. *J Surg Oncol* 1980; 14:61-5

6. Stevenson G, Hall SC, Rudnick S, Seleny FL, Stevenson HC: The effects of anesthetic agents on the human immune response. *ANESTHESIOLOGY* 1990; 72: 542-52
7. Klein HG: Immunomodulatory aspects of transfusion. *ANESTHESIOLOGY* 1999; 91:861-6
8. Ben-Eliyahu S, Shakhar G, Rosenne E, Levinson Y, Beilin B: Hypothermia in anesthetized rats suppresses natural killer cell activity and compromises resistance to tumor metastasis: A role for adrenergic mechanisms. *ANESTHESIOLOGY* 1999; 91:732-40
9. Andersen BL, Farrar WB, Golden-Kreutz D, Kutz LA, MacCallum R, Courtney ME, Glaser R: Stress and immune responses after surgical treatment for regional breast cancer. *J Natl Cancer Inst* 1998; 90:30-6
10. Weissman C: The metabolic response to stress: An overview and update. *ANESTHESIOLOGY* 1990; 73:308-27
11. Kehlet H: Multimodal approach to control postoperative pathophysiology and rehabilitation. *Br J Anaesth* 1997; 78:606-17
12. Liu S, Carpenter RL, Neal JM: Epidural anesthesia and analgesia: Their role in postoperative outcome. *ANESTHESIOLOGY* 1995; 82:1474-506
13. Ben-Eliyahu S, Page GG: In vivo assessment of natural killer cell activity in rats. *Prog Neuro Endocrine Immunol* 1992; 5:199-214
14. Page GG, Ben-Eliyahu S, Liebeskind JC: The role of LGL/NK cells in surgery-induced promotion of metastasis and its attenuation by morphine. *Brain Behav Immun* 1994; 8:241-50
15. Page GG, McDonald JS, Ben-Eliyahu S: Pre-operative versus postoperative administration of morphine: Impact on the neuroendocrine, behavioral, and metastatic-enhancing effects of surgery. *Br J Anaesth* 1998; 81:216-23
16. Barlozzari T, Leonhards J, Wiltrout RH, Herberman RB, Reynolds CW: Direct evidence for the role of LGL in the inhibition of experimental tumor metastases. *J Immunol* 1985; 134:2783-9
17. Mestre C, Pelissier T, Fialip J, Wilcox G, Eschaler A: A method to perform direct transcatheter intrathecal injection in rats. *J Pharmacol Toxicol* 1994; 32:197-200
18. Tejwani GA, Rattan A, McDonald JS: Role of spinal opioid receptors in the antinociceptive interactions between intrathecal morphine and bupivacaine. *Anesth Analg* 1992; 74:726-34
19. Shakhar G, Ben-Eliyahu S: In vivo beta-adrenergic stimulation suppresses natural killer activity and compromises resistance to tumor metastasis in rats. *J Immunol* 1998; 160:3251-8
20. Pollock RE, Zimmerman SO, Fuchshuber P, Lotzova E: Lytic units reconsidered: Pitfalls in calculation and usage. *J Clin Lab Anal* 1990; 4:274-82
21. Whiteside TL, Herberman RB: The role of natural killer cells in human disease. *Clin Immunol Immunopathol* 1989; 53:1-23
22. Friberg DD, Bryant JL, Whiteside TL: Measurements of natural killer (NK) activity and NK-cell quantification. *Methods* 1996; 9:316-26
23. Buinauskas P, McDonald GO, Cole WH: Role of operative stress on the resistance of the experimental animal to inoculated cancer cells. *Ann Surg* 1958; 148:642-8
24. Lundy J, Lovett EJ, Hamilton S, Conran P: Halothane, surgery, immunosuppression and artificial pulmonary metastases. *Cancer* 1978; 41:827-30
25. Pollock RE, Babcock GF, Romsdahl MM, Nishioka K: Surgical stress-mediated suppression of murine natural killer cell cytotoxicity. *Cancer Res* 1984; 44:3888-91
26. Colacchio TA, Yeager MP, Hildebrandt LW: Perioperative immunomodulation in cancer surgery. *Am J Surg* 1994; 167:174-9
27. Da Costa ML, Redmond P, Bouchier-Hayes DJ: The effect of laparotomy and laparoscopy on the establishment of spontaneous tumor metastases. *Surgery* 1998; 124:516-25
28. Lange P, Hekmat K, Bosl G, Kennedy B, Fraley E: Accelerated growth of testicular cancer after cytoreductive therapy. *Cancer* 1980; 45:1498-506
29. Fielding L, Wells B: Survival after primary and after staged resection for large bowel obstruction caused by cancer. *Br J Surg* 1974; 61:16-8
30. Shirakawa T, Tokunaga A, Onda M: Release of immunosuppressive substances after gastric resection is more prolonged than after mastectomy in humans. *Int Surg* 1998; 83:210-4
31. Holmgren L, O'Reilly M, Folkman J: Dormancy of micrometastases: Balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nat Med* 1995; 1:149-53
32. Foss OP, Brennhovd IO, Messelt OT, Efskind J, Liverud K: Invasion of tumor cells into the bloodstream caused by palpation or biopsy of the tumor. *Surgery* 1966; 59:691-5
33. Terman GW, Shavit Y, Lewis JW, Cannon JT, Liebeskind JC: Intrinsic mechanisms of pain inhibition: Activation by stress. *Science* 1984; 226:1270-7
34. Schedlowski M, Falk A, Rohne A, Wagner TO, Jacobs R, Tewes U, Schmidt RE: Catecholamines induce alterations of distribution and activity of human natural killer (NK) cells. *J Clin Immunol* 1993; 13:344-51
35. Irwin M: Stress-induced immune suppression: Role of the autonomic nervous system. *Ann N Y Acad Sci* 1993; 697:203-18
36. Pflug AE, Halter JB: Effect of spinal anesthesia on adrenergic tone and the neuroendocrine response to surgical stress in humans. *ANESTHESIOLOGY* 1981; 55:120-6
37. Rutberg H, Hakanson E, Anderberg B, Jorfeldt L, Martensson J, Schildt B: Effects of the extradural administration of morphine, or bupivacaine, on the endocrine response to upper abdominal surgery. *Br J Anaesth* 1984; 56:233-7
38. Tonnesen E, Huttel M, Christensen N, Schmitz O: Natural killer cell activity in patients undergoing upper abdominal surgery: Relationship to the endocrine stress response. *Acta Anaesthesiol Scand* 1984; 28:654-60
39. Koltun WE, Bloomer MM, Tiberg AF, Seaton JF, Ilahi O, Rung G, Gifford RM, Kauffman GL: Awake epidural anesthesia is associated with improved natural killer cell cytotoxicity and a reduced stress response. *Am J Surg* 1996; 171:68-73
40. Hole A, Unsgaard G: The effect of epidural and general anaesthesia on lymphocyte functions during and after major orthopaedic surgery. *Acta Anaesthesiol Scand* 1983; 27:134-41
41. Rem J, Brandt M, Kehlet H: Prevention of postoperative lymphopenia and granulocytosis by epidural analgesia. *Lancet* 1980; 1:283-4
42. Ryhanen P, Jouppila R, Lanning M, Jouppila P, Hollmen A, Kouvalainen K: Natural killer cell activity after elective cesarean section under general and epidural anesthesia in healthy parturients and their newborns. *Gynecol Obstet Invest* 1985; 19:139-42
43. Le-Cras AE, Galley HF, Webster NR: Spinal but not general anesthesia increases the ratio of T helper 1 to T helper 2 cell subsets in patients undergoing transurethral resection of the prostate. *Anesth Analg* 1998; 87:1421-5
44. Beilin B, Shavit Y, Cohn S, Kedar E: Narcotic-induced suppression of natural killer cell activity in ventilated and nonventilated rats. *Clin Immunol Immunopathol* 1992; 64:173-6
45. Beilin B, Shavit Y, Hart J, Mordashov B, Cohn S, Notti I, Bessler H: Effects of anesthesia based on large versus small doses of fentanyl on natural killer cell cytotoxicity in the perioperative period. *Anesth Analg* 1996; 82:492-7