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Radošević-Stašić, Biserka; Udović-Širola, Marija; Stojanov, Lovorka; Ribarić, Ljubomir; Rukavina, Daniel

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Growth of Allogeneic Sarcoma in Mice Subjected to Halothane Anesthesia and/or Surgical Stress

Biserka Radošević-Stašić, MD, PhD, Marija Udović-Širola, MD, PhD,

Lovorka Stojanov, MD, Ljubomir Ribarić, MD, PhD, and Daniel Rukavina, MD, PhD

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The present study was designed to clarify mechanisms involved in suppression of cell-mediated immunity reported in patients undergoing major surgery with general anesthesia by determining the effects of halothane anesthesia with and without surgery on the growth of Sarcoma I (Sa I), a tumor allogeneic to BALB/c mice. Mice were given subcutaneous injections of 5×10^6 tumor cells from A/Jax mice and then immediately exposed to 0.5%–1.0% halothane for 1 hr without surgery ($n = 7$) or with surgery (midline laparotomy; $n = 12$). In control groups mice were also injected with tumor cells but were not exposed to prolonged

halothane anesthesia. Some of them received only Sa I ($n = 6$), while the rest ($n = 7$) were also laparotomized. The rejection time of Sa I in mice exposed to halothane anesthesia was significantly longer (15.4 ± 1.25 days) than in untreated controls (12.0 ± 0.68 days) ($P < 0.05$). In the mice exposed to halothane tumor growth was also greater. Surgical stress per se did not significantly affect growth or rejection time of Sa I (11.0 ± 0.66 vs 12.0 ± 0.68 days). Similarly, the combination of surgical stress with halothane anesthesia did not affect the immunosuppression associated with halothane alone (12.9 ± 1.3 vs 15.4 ± 1.25 ; $P < 0.05$). The results indicate that halothane anesthesia per se may be associated with impairment of cell-mediated immunity under experimental conditions.

Key Words: ANESTHETICS, VOLATILE—halothane. IMMUNE RESPONSE, HALOTHANE. CANCER, HALOTHANE.

It has been suggested that the transient immunodepression that develops after general anesthesia and surgery may contribute to the propagation of infection, impairment of wound healing, and spread or growth of malignancies (1–3). This may reflect the effects of the anesthetics and/or concomitant operative stress on immune response. There is, however, a lack of agreement about the mechanism by which anesthesia and/or surgery may affect host immunocompetence. In our previous study we observed that immunosuppression in postoperative patients is greater after general than after epidural anesthesia. This pointed to direct effects of halothane or to some

participation of stress-induced mechanisms in the observed immunosuppression, since it could be presumed that adrenal stimulation was less with epidural anesthesia because of the blockade of preganglionic sympathetic fibers. In order to obtain more information on mechanisms involved in the development of immunodepression, we attempted in this study to compare the effects of halothane anesthesia alone with the effects of the combination of anesthesia and operation, using as an index of changes of cellular immunity growth and rejection time of Sarcoma I (Sa I), a tumor from A/Jax mice which is allogeneic to the BALB/c mice we used as recipients.

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Address correspondence and reprint requests to Dr. Biserka Radošević-Stašić, Department of Physiology and Immunology, Medical Faculty, University of Rijeka, 51000 Rijeka, Olge Ban 22, Yugoslavia.

Material and Methods

All experiments were done in male BALB/c (H-2^d) mice, aged 2.5–3.5 months. These were inoculated with Sa I tumor cells obtained from ascitic fluid of tumor-bearing A/Jax (H-2^a) mice. A cell suspension was prepared by diluting tumor cells in Hank's

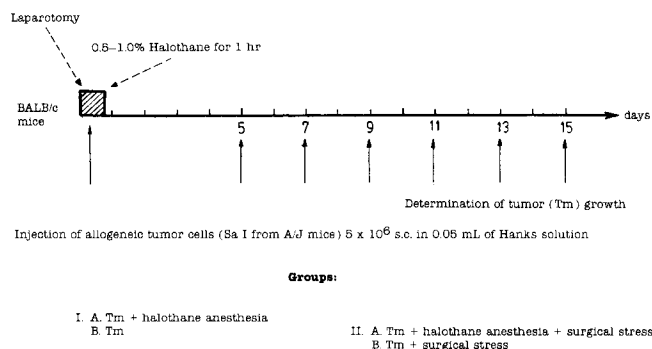


Figure 1. Experimental protocol for the determination of growth and rejection times of an allogeneic tumor in mice

balanced salt solution (HBSS). The viability of cells was always greater than 90%, as determined by exclusion of trypan blue dye. An inoculum of 5×10^6 tumor cells in 0.05 mL of HBSS was injected subcutaneously in the right hind leg of all our mice, after which the animals were divided into two main groups—I and II, which were further subdivided into experimental (A) and control (B) groups (Figure 1). In group IA the effect of halothane alone was studied, while in group IIA the combination of the effects of halothane anesthesia and operation were analyzed.

Anesthetization of mice was performed in a 1-L animal metabolic cage containing soda lime for carbon dioxide absorption. The air with or without halothane (which came from a Fluotec vaporizer) was added to the chamber by the use of a respirator for small animals adjusted for a total flow of 0.4 L/min. Anesthesia with 0.5%–1.0% halothane started immediately after the inoculation of tumor cells (group IA = Tm + halothane), or immediately after the surgery (group IIA = Tm + surgical stress + halothane) and continued for 1 hr. Mice in the control (B) groups were treated identically as those in group A. They were put in the same metabolic cage for 1 hr, but without halothane in the air added to the chamber (IB = only tumor; IIB = Tm + surgical stress). The nonspecific surgical stress consisted of a midline laparotomy. It was performed in all animals in groups IIA and IIB under short (3 min) halothane anesthesia. In group IIA the anesthesia was then administered for another hour; it was immediately discontinued in group IIB. In all mice, tumor growth was determined on the basis of the frequency and time of Sa I rejection, which was estimated for the mean diameter of subcutaneous nodules of the tumor and times of their disappearance (rejection times). The incidence of reappearance of tumor after its regression and death of animals was also recorded.

Statistical analysis was performed by Student's

t-test and χ^2 assay. Values of $P < 0.05$ were considered statistically significant.

Results

Only three out of the 32 animals inoculated with Sa I cells failed to develop tumors, and all except one of these 29 mice that accepted allogeneic tumor were able to reject it. The number of these exceptions ($n = 4$) was too small to be of statistical significance (Table 1).

Statistically relevant differences were, however, found when mean rejection times were compared between the groups (Table 1). Mice given halothane without operation (group IA) tolerated tumors longer than did mice without halothane (group IB) ($P < 0.05$), suggesting that halothane alone has a suppressive effect on cell-mediated immunity. This conclusion is also supported by the fact that in the same group (IA) the mean tumor diameter was the greatest and that one mouse died after the reappearance of sarcoma at the site of inoculation. Although these observations were not statistically significant, it is interesting that they were seen in group A only. Surgical stress provoked by laparotomy did not change the times of the rejection of Sa I (groups IIB vs IB), but the "stress" of laparotomy associated with 1 hr of halothane anesthesia resulted in some impairment of immunosuppression provoked by the halothane anesthesia alone (groups IIA vs IA). Decreases in the size and rejection times of tumors seen in group IIA were, however, not statistically significant, in comparison with values found in group IA.

The results, therefore, point to the immunosuppressive effect of halothane alone (group IA) and to the lack of a synergistic effect of halothane anesthesia and laparotomy, used as a stress event, on cell-mediated immune response.

Discussion

Despite the large body of evidence that points to the immunosuppressive properties of both halothane anesthesia and surgical stress (1–3), comparisons between the effects of these two conditions in the same experimental model are infrequent. Humphrey et al. (5) compared the effects of exposure to 0.5% halothane for 1 hr to those obtained after amputation of one hind limb on the humoral immune response of rats. The data showed that both procedures inhibit the generation of antibody-producing cells in spleen to sheep red blood cells (SRBC) given 6–7 days before

Table 1. Growth of Allogeneic Tumor in BALB/c Mice Subjected to Separate or Combined Effects of Halothane Anesthesia and Surgical Stress

Group	Treatment	No. of mice	No. of accepted tumors	Maximal diameter (mm)	Rejection time (days)	Reappearance of Sa I	Death of animals (No.)
I.	A. Tm + halothane	7	6/7	7.36 ± 1.05	15.43 ± 1.25*	1/7	1/7
	B. Tm	6	6/6	6.33 ± 0.73	12.0 ± 0.68	0/6	0/6
II.	A. Tm + operation + halothane	12	10/12	5.0 ± 0.57	12.9 ± 1.3	0/12	0/12
	B. Tm + operation	7	7/7	5.8 ± 0.63	11.0 ± 0.66	0/7	0/7

Tm = Sarcoma I (Sa I) injected subcutaneously immediately before halothane anesthesia (0.5%–1.0% for 1 hr); Operation = midline laparotomy done immediately after the inoculation of Sa I.

Results are expressed as mean ± standard error.

*A statistically significant difference was found only between groups IA and IB ($P < 0.05$).

amputation or exposure to halothane. The observed depression was of similar degree and lasted 48 hr. In a slightly different model we noticed that even shorter exposures of mice to halothane (1 hr after the challenge with SRBC) induce significant suppression of plaque-forming cells generation. Surgical stress which consisted of a midline laparotomy potentiated this immunosuppression, indicating that additional inhibitory pathways were probably activated (6).

Data presented in this study show that halothane anesthesia alone also inhibits the cell-mediated immune reactions responsible for the rejection of allogeneic tumors. Our results, however, differ from those obtained by Cullen and Sundsmo (7) with the same allogeneic tumor but under different experimental conditions. In $C_{57}Bl/6$ ($H-2^b$) mice as recipients, they noticed that anesthesia with 0.5%–0.7% halothane, administered for 5 hr on the day of tumor inoculation and 1 week later, did not influence the time of tumor appearance, rate of tumor growth, or time of tumor regression. The observed difference in results could be caused by reexposure to halothane, which may eliminate and cancel the inhibitory effect of the first exposure to halothane. However, the results of Cullen and Sundsmo (7) demonstrate that such inhibition of immunoresponse to allogeneic tumors does not exist even in the early period of tumor growth. It is therefore likely that BALB/c ($H-2^d$) mice, which we used in our experiments, are more sensitive to the action of halothane. It should also be emphasized that the dynamics of tumor growth in our study differed from that of Cullen and Sundsmo even in the control, unanesthetized mice, with tumor regression times of 20.3 ± 5.5 days reported by Cullen and Sundsmo (7) and 12.0 ± 0.68 days in our present experiments (group IB).

Immunosuppression in our study was a consequence of the relatively short exposure of BALB/c mice to halothane (for only 1 hr after the implantation

of tumor cells). It is therefore likely that halothane anesthesia particularly affects the early postimplantation events, when the process of tumor recognition occurs. A/Jax-derived Sa I ($H-2^a$) is a tumor which expresses K^k , D^d , and L^d antigens but is devoid of class II Ia molecules on the cell surface, as determined by flow cytometric analysis (8). Grafts that differ from the recipient only at class I regions of major histocompatibility complex (MHC) may be rejected by two potential pathways (9,10): (1) through the activation of $Lyt\ 2^+$ T cells (which express cytolytic activity after direct class I antigen recognition); and (2) by the action of $L_3T_4^+$ T (helper) cells which may recognize class I alloantigen in association with self Ia structures. The latter form of T-cell activation may involve processing and presentation of alloantigens on host antigen-presenting cells (APC) or corecognition of class I alloantigen and syngeneic Ia molecules on donor APC (11,12). The primary pathway for the rejection of Sa I tumor in BALB/c mice has been reported (13) to be the direct activation of class I-MHC antigen-reactive $Lyt\ 2^+$ T cells, because it has been found that in vivo depletion of the $L_3T_4^+$ T-cell subset does not prolong Sa I tumor growth, in contrast to the induction of lethal tumor growth of recipient mice in which the $Lyt-2^+$ subset had been depleted (13). $L_3T_4^+$ T cells may, however, on one hand augment the $Lyt\ 2^+$ T-cell response to Sa I (13) and on the other facilitate the response of B lymphocytes in the production of antitumor antibodies, which may promote progressive tumor growth by the production of an immunologic enhancement (14). From this it is apparent that multiple factors and cells are involved in the immune response to Sa I. Halothane may affect all or almost all of them. It is known that halothane anesthesia may inhibit the process of phagocytosis (15,16), mobility of lymphocytes (17), lymphocyte transformation and division (18), and also the process of the killing of tumor cells

by peritoneal exudate cells (19). During the early postoperative period, the generation of cytotoxic T cells is also depressed, because of the presence of suppressor macrophages (20). It has been reported that antibody-dependent cellular cytotoxicity of patients' lymphocytes against coated tumor cells decreases after minor surgery and anesthesia (21). This suggests that the prolonged time of tumor rejection in our study may be related also to changes in the activity of NK or K cells after exposure to halothane. Although it is reported that suppression of NK-cell cytotoxicity (NKCC) is mainly related to the effects of surgical stress (20,22,23), it has been shown that even short periods of anesthesia with pentobarbital may result in an early decrease in NKCC (23). An additional support for the immunosuppressive properties of halothane came from data showing that exposure of fibrosarcoma-bearing mice to 1.5% halothane for 1.5 hr may activate the suppression of the cell-mediated immunity of tumor-bearing animals (24).

Halothane may also have direct toxic effects (25) on several cells participating in the immune response to Sa I. At the same time halothane anesthesia produces homeostatic changes, with a disarrangement of many of the feedback pathways that exist between the neuroendocrine and immune systems (26). Halothane may apparently affect both the afferent and the efferent limbs of these loops. Because of the suppressive action of halothane on mononuclear phagocytes, it can be presumed that the production of interleukin-1 (IL-1) is also depressed. Recent evidence (27) shows that IL-1 not only mediates host responses to infection and alloantigens but can also stimulate the secretion of ACTH from the anterior pituitary gland, acting directly on normal pituitary cells or by stimulating the release of a corticotropin-releasing factor from the hypothalamus. The production and secretion of glucocorticoid hormones themselves can regulate the intensity of immune reaction. It is likely that halothane affects these events; proof of this, however, is lacking. Of particular importance among the efferent pathways known to be affected by halothane anesthesia are those related to dopaminergic, serotonergic, and the autonomic nervous systems. These systems are immunomodulatory (28,29), with effects mediated through the hypothalamo-hypophyseal-adrenal axis (30) or by direct neural contact with lymphatic organs and cells (31). Our knowledge of the relationship of halothane anesthesia to the extensive network of other immunomodulatory substances (neurotransmitters, endorphins, enkephalins, etc. [32]) is, however, still scanty. Although the mechanism of its induction remains unknown, our data

show that halothane anesthesia by itself leads to the development of immunosuppression.

In clinical practice halothane anesthesia is usually followed by operative stress. In this study we intentionally used as "stress" a laparotomy, an operation which mimics the clinical situation. Other forms of experimentally induced stresses are known to affect immune functions (22,23,33). In our experiments laparotomy without prolonged halothane anesthesia (group IIB) did not affect the growth of Sa I, and even in the combination with halothane anesthesia (group IA) surgical stress did not potentiate the immunosuppression produced by halothane alone. These results differ from those found in the same experimental model when the humoral immune response was tested (6). There laparotomy potentiated the immunosuppression provoked by halothane anesthesia. The failure of laparotomy by itself to affect the cellular type of immune response is probably related to the fact that a certain degree of tissue destruction is needed to trigger suppressive mechanisms in the rejection of allogeneic tumor cells. Accordingly, major operations are usually followed by an increased postoperative depression of cell-mediated functions (4,34,35) and longer impairment of IL-2 production of peripheral blood mononuclear cells (36). Although in these situations the effects of prolonged anesthesia cannot be ruled out, experimental models confirm that greater surgical stress (like amputation of one hind limb) may alone be followed by the generation of suppressor cells (22) and impairment of NKCC (23). Another possible explanation of the lack of any additional immunosuppressive effect of stress in combination with halothane anesthesia in our present experiments is the fact that laparotomy was used as a stressor. This type of "stress" is followed by reparatory processes that usually trigger marked changes in the immune homeostasis, owing to the mobilization of cells with morphogenetic functions (37). During wound healing and regenerative growth, the expression of class II antigens on multiple cells occurs (38), enabling them to act as new APC. Owing to the previously described possibility that host APC potentiate through the activation of T helper cells the effects of cytolytic T cells on Sa I (11-13), it is apparent that laparotomy may even facilitate the process of tumor rejection. Our results, in group IIA, showing that laparotomy may minimize the immunosuppressive effect of halothane point to such a possibility.

In conclusion, our evidence indicates that halothane anesthesia alone induces immunosuppression, which may enhance the growth of allogeneic sarcoma in mice. Stress by laparotomy does not potentiate

immunosuppression, but may rather act as a factor that abrogates the immunosuppressive effect of halothane.

Although more evidence is needed in order to clarify the significance of halothane-induced suppression of the mechanisms involved in tumor rejection, it is clear that the findings obtained here have important clinical implications.

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References

- Walton B. Effects of anaesthesia and surgery on immune status. *Br J Anaesth* 1979;51:37-43.
- Graham CW. Immunological and carcinogenic side effects of anesthetics. *Int Anesthesiol Clin* 1980;18:173-86.
- Gupta S. Immune response following surgical trauma. *Crit Care Clin* 1987;3:405-15.
- Udović-Širola M, Radošević-Stašić B, Ribarić L, et al. The effect of general endotracheal and epidural anaesthesia on the immune status. *Period Biol* 1983;85:115-7.
- Humphrey LJ, Wingard DW, Lang R. The effect of surgery and anesthesia on the immunologic responsiveness of the rat. *Surgery* 1969;65:946-51.
- Radošević-Stašić B, Udović-Širola M, Polić L, et al. Humoral immunity of mice subjected to isolated or combined effects of halothane and operative stress. *Period Biol* 1986;88:24-6.
- Cullen BF, Sundsmo JS. Failure of halothane anesthesia to alter growth of sarcoma in mice. *Anesthesiology* 1974;41:580-4.
- Dallman MJ, Mason DW, Webb M. The role of host and donor cells in the rejection of skin allografts by T cell-deprived rats injected with syngeneic T cells. *Eur J Immunol* 1982;12:511-8.
- Rock KL, Barnes MC, Germain RN, et al. The role of Ia molecules in the activation of T lymphocytes: II. Ia restricted recognition of allo K/D antigens is required for class I MHC stimulated mixed lymphocyte responses. *J Immunol* 1983;130:457-62.
- Mizouchi T, Golding H, Rosenberg AS, et al. Both L₃T₄⁺ and Lyt-2⁺ helper T cells initiate cytotoxic T lymphocyte responses against allogeneic major histocompatibility antigens but not against trinitrophenyl-modified self. *J Exp Med* 1985;162:427-43.
- Singer A, Kruisbeek AM, Andrysiak PM. T cell-accessory cell interactions that initiate allospecific cytotoxic T lymphocyte responses: existence of both Ia-restricted and Ia-unrestricted cellular interaction pathways. *J Immunol* 1984;132:2199-209.
- Goldin H, Singer A. Role of accessory cell processing and presentation of shed H-2 alloantigens in allospecific cytotoxic T lymphocyte responses. *J Immunol* 1984;133:597-605.
- Smith DM, Stuart FP, Wemhoff GA, et al. Cellular pathways for rejection of class I-MHC-disparate skin and tumor allografts. *Transplantation* 1988;45:168-75.
- Kinsky R, Duc H, Chaouat G, et al. Involvement of suppressor cells in active enhancement of allografted tumors. In vivo (adoptive transfer) and in vitro (MLR) evaluation. Synergy with enhancing antibodies. *Cell Immunol* 1981;58:107-23.
- Bruce DL. Effect of halothane anesthesia on experimental Salmonella peritonitis in mice. *J Surg Res* 1967;7:180-5.
- Saba TM. Mechanism mediating reiculoendothelial system depression after surgery. *Proc Soc Exp Med Biol* 1970;133:1132-6.
- Moudgil GC, Allan RB, Russel RJ, et al. Inhibition by anaesthetic agents of human leucocyte locomotion towards chemical attractants. *Br J Anaesth* 1977;49:97-105.
- Cullen BF, Sample WF, Chretien PB. The effect of halothane on phytohemagglutinin-induced transformation of human lymphocytes in vitro. *Anesthesiology* 1972;36:206-12.
- Cullen BF, Duncan PG, Ray-Keil L. Inhibition of cell-mediated cytotoxicity by halothane and nitrous oxide. *Anesthesiology* 1976;44:386-90.
- Miyazaki S, Akiyoshi T, Arinaga S, et al. Depression of the generation of cell-mediated cytotoxicity by suppressor cells after surgery. *Clin Exp Immunol* 1983;54:573-9.
- Vose BM, Moudgil GC. Postoperative depression of antibody-dependent cellular cytotoxicity following minor surgery and anesthesia. *Immunology* 1976;30:123-8.
- Wang BS, Heacock EH, Wu AVO, et al. Generation of suppressor cells in mice after surgical trauma. *J Clin Invest* 1980;66:200-9.
- Pollock RE, Lotzova E, Stanford SD, et al. Effect of surgical stress on murine natural killer cell cytotoxicity. *J Immunol* 1987;138:171-8.
- Lewis RE, Cruse JM, Hazelwood J. Halothane-induced suppression of cell-mediated immunity in normal and tumor-bearing C₃H₁/H₆ mice. *Anesth Analg* 1980;59:666-71.
- Kusyk CJ, Hsu TC. Mitotic anomalies induced by three inhalation halogenated anesthetics. *Environ Res* 1976;11:366-70.
- Blalock JE, Smith E. A complete regulatory loop between the immune and neuroendocrine system. *Fed Proc* 1985;44:108-11.
- Lumpkin MD. The regulation of ACTH secretion by IL-1. *Science* 1987;238:452-4.
- Hadden JW. Neuroendocrine modulation of the thymus-dependent immune systems. *Ann NY Acad Sci* 1986;496:39-48.
- Paavonen T. Hormonal regulation of lymphocyte functions. *Med Biol* 1987;65:229-40.
- Devoino L, Idova G, Alperina E, et al. Distribution of immunocompetent cells underlying psychoneuroimmunomodulation. *Ann NY Acad Sci* 1986;496:292-300.
- Hall NR, Goldstein AL. Neurotransmitters and the immune system. In: Ader R, ed. *Psychoneuroimmunology*. New York: Academic Press, 1981:521-43.
- Morley JE, Kay NE, Solomon GE, et al. Neuropeptides: conductors of the immune orchestra. *Life Sci* 1987;41:527-44.
- Riley V. Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science* 1981;212:1100-9.
- Berenbaum MC, Fluck PA, Hurst NP. Depression of lymphocyte responses after surgical trauma. *Br J Exp Pathol* 1973;54:597-607.
- Keller SE, Weiss JM, Schleifer SJ, et al. Suppression of immunity by stress: effect of a graded series of stressors on lymphocyte stimulation in the rat. *Science* 1981;213:1397-1400.
- Akiyoshi T, Koba F, Arinaga S, et al. Impaired production of interleukin-2 after surgery. *Clin Exp Immunol* 1985;59:45-9.
- Burch PRJ. Is normal growth regulated by a central system? In: MTP Press, ed. *The biology of cancer—a new approach*. Lancaster: St-Leonard's House, 1976:17-40.
- Jonjić S, Radošević-Stašić B, Čuk M, et al. Class II antigens induction in the regenerating liver of rats after partial hepatectomy. *Transplantation* 1987;44:165-8.