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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.

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

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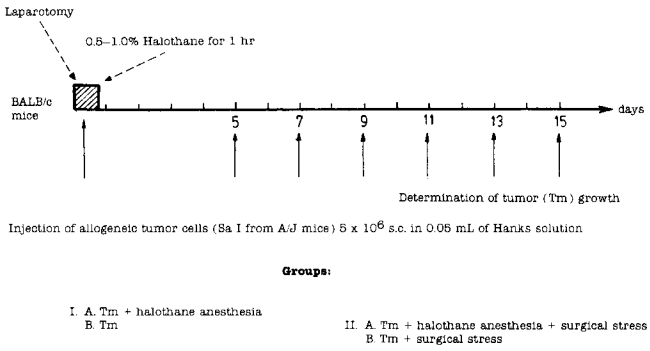

Figure 1. Experimental protocol for the determination of growth and rejection times of an allogeneic tumor in mice

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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