Laboratory Report

Failure of Enflurane and Halothane Anesthesia to Inhibit Lymphocyte Transformation in Volunteers

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Changes in the peripheral blood leukocyte count and in the ability of lymphocytes to transform in response to phytohemagglutinin were studied in healthy volunteers undergoing prolonged enflurane or halothane anesthesia without coincident surgical operation. Anesthesia was associated with a modest leukocytosis that persisted into the first post-anesthetic day, primarily due to an influx of neutrophils into the circulation. There was no significant alteration, either during or following anesthesia, in the ability of the volunteers' lymphocytes to transform in response to phytohemagglutinin when compared with either preanesthetic values or unanesthetized controls. Depression of lymphocyte transformation does not appear to follow prolonged enflurane or halothane anesthesia in the absence of a surgical procedure. (Key words: Immune response, volatile anesthetics: Blood, leukocytes, immune response: Anesthetics, volatile, enflurane: Anesthetics, volatile, halothane.)

The in vitro determination of the ability of lymphocytes to respond to specific antigens or nonspecific mitogens is commonly accepted as a method for assessing immunologic competency.1 Since the lymphocyte plays a central role in specific immunity to foreign antigens, a deficiency in this phase of the immune response suggests a predisposition to malignancy or infection.

Depression of lymphocyte transformation has been reported to occur in man after anesthesia and operation. It is demonstrable within two hours of induction of anesthesia,2 maximal in the immediate postoperative period,3 and persists for as long as three weeks.4 This reduced lymphocyte responsiveness is closely correlated to the extent of surgical trauma,5 the amount of blood lost,6 and the presence of debilitating disease.7,8 It has also been suggested that anesthetic agents and techniques do not affect lymphocyte transformation;9 however, the influence of general anesthesia in the absence of coincidental operation has not been determined. In this report we describe the effects of general anesthesia with enflurane or halothane, administered to healthy volunteers, on lymphocyte transformation and the peripheral blood leukocyte count.

Methods and Materials

Enflurane or halothane anesthesia was administered to unmedicated human volunteers in San Diego. The protocol for these studies was approved by the Human Research Committees of the Veterans Adminis-
Table 1. Effects of Enfuran in Human Volunteers (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Before Anesthesia</th>
<th>Midanesthesia</th>
<th>End of Anesthesia</th>
<th>One Day after Anesthesia</th>
<th>Five Days after Anesthesia</th>
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</thead>
<tbody>
<tr>
<td>Lymphocyte transformation (per cent of pre-anesthetic value)</td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>100 ± 34.6</td>
<td>162 ± 51.4</td>
<td>236 ± 19.8</td>
<td>205 ± 51.0</td>
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<tr>
<td>No anesthesia (Group II)</td>
<td>100 ± 6.5</td>
<td>121 ± 9.7</td>
<td>162 ± 56.3</td>
<td>140 ± 76.8</td>
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<tr>
<td>Mitogenic index</td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>4.1 ± 0.6</td>
<td>8.4 ± 1.4</td>
<td>11.2 ± 1.7</td>
<td>10.1 ± 2.9</td>
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<tr>
<td>No anesthesia (Group II)</td>
<td>4.5 ± 1.0</td>
<td>4.6 ± 0.9</td>
<td>5.9 ± 2.9</td>
<td>11.7 ± 7.5</td>
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</tr>
<tr>
<td>Leukocyte count (× 1000 mm(^3))</td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>7.3 ± 0.4</td>
<td>10.0 ± 0.4</td>
<td>12.0 ± 0.7</td>
<td>9.4 ± 0.5</td>
<td>8.3 ± 0.9</td>
</tr>
<tr>
<td>No anesthesia (Group II)</td>
<td>8.3 ± 0.7</td>
<td>8.7 ± 0.9</td>
<td>8.1 ± 0.9</td>
<td>7.3 ± 0.04</td>
<td>9.5 ± 1.64</td>
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<tr>
<td>Lymphocytes (× 1000 mm(^3))</td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>2.8 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.4 ± 0.4</td>
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<tr>
<td>No anesthesia (Group II)</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.5</td>
<td>3.1 ± 0.4</td>
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<tr>
<td>Hours until into culture</td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>51.5 ± 0.6</td>
<td>50.1 ± 3.0</td>
<td>43.4 ± 0.5</td>
<td>37.0 ± 4.6</td>
<td>43.5 ± 4.5</td>
</tr>
<tr>
<td>No anesthesia (Group II)</td>
<td>42.6 ± 4.3</td>
<td>38.3 ± 4.3</td>
<td>44.2 ± 0.3</td>
<td>39.0 ± 8.91</td>
<td>41.0 ± 11.94</td>
</tr>
</tbody>
</table>

* Significantly different from corresponding anesthetized control value (P ≤ .05).
† n = 2.

Anesthesia was induced by inhalation, with end-tidal anesthetic gas concentrations maintained between 1.0 and 2.0 MAC for each anesthetic agent. Total anesthesia time was 5 to 7 hours. Seven volunteers were anesthetized with halothane in oxygen. They received no additional medication and were maintained eupneic by controlled ventilation. Nine volunteers were anesthetized with enfurane in oxygen except for two periods when the carrier gas was changed to 70 per cent nitrous oxide in oxygen. Three of the nine enfurane volunteers each received a single dose of succinylcholine to facilitate endotracheal intubation. While controlled ventilation was used throughout most of the study to maintain Pet\(_{CO_2}\) at normal levels, each enfurane volunteer was allowed to breathe spontaneously for two periods, early and late during the anesthesia, to assess the effects of the anesthetic on respiratory function.

Heparinized blood was obtained from the volunteers and simultaneously from a comparable group of unanesthetized control subjects immediately before, midway through, at the end of, one day after, and five days after the procedure. The samples were airmailed at room temperature to Seattle, where they were put immediately into culture. Lymphocyte transformation in response to phytohemagglutinin (PHA) was assessed by the whole-blood technique of Pauly et al.11 as outlined in detail previously.5 Briefly, the leukocyte count was determined electronically, the percentage lymphocytes counted, and a sample of whole blood diluted in unsupplemented RPMI-1640 culture medium (Grand Island Biological Co.) to a concentration of 10^6 lymphocytes per ml. Three-ml volumes of the suspension were then pipetted into polypropylene tubes (Falcon plastics #2063) and 10 μg of PHA (Burroughs Wellcome) were added to each of seven of the ten tubes prepared for each blood sample. Lymphocytes were incubated for five days at 37°C in an atmosphere of 5 per cent CO\(_2\) in air. Tritiated thymidine (1 μCi) was added to each culture vessel 24 hours prior to the end of the incubation period. The extent of lymphocyte transformation was quantitated in a liquid scintillation counter and expressed.
either as a percentage of the preanesthetic value for each individual or as a mitogenic index where:

Mitogenic index

\[
\text{Mitogenic index} = \frac{\text{DPM of stimulated cultures (PHA added)}}{\text{DPM of unstimulated cultures (no PHA)}}
\]

The lymphocytic response of halothane volunteers was also assessed independently by one of us (R.B.) in San Diego, using the method of Park and Good. These specimens were cultured immediately, avoiding any influence of delays associated with transport of cells to Seattle.

Data were analyzed statistically by Student's t test for paired data, comparing the responses during and after anesthesia with preanesthetic values, and by Student's t test for unpaired data, comparing the response of volunteers with that of simultaneously sampled unanesthetized controls. The level of significance was accepted as \( P < 0.05 \).

Results

Due to mailing difficulties, there was variation in the intervals from drawing the specimens to establishing the cultures. In general, delays were similar for enflurane-anesthetized volunteers and awake controls, but they differed at the initial and mid-anesthesia sampling periods (table 1). In addition, only two specimens were received from unanesthetized controls on days 1 and 5 of the enflurane study, making these results of little value. Similar time-related variability was not found in the halothane study (table 2).

General anesthesia was associated with a slight leukocytosis that persisted into the first postanesthetic day. Although this was not significant for the halothane volunteers, it was valid for those receiving enflurane compared with both preanesthetic values and values for unanesthetized controls. The increased leukocyte count was mainly due to an influx of neutrophils, although lymphocytosis was also apparent in the enflurane volunteers.

Lymphocyte transformation, expressed as percentage of preanesthetic levels, increased during enflurane anesthesia, but was not significant (table 1). Because awake subjects demonstrated a similar change in transformation, a normal diurnal variation is suggested. When expressed as a mitogenic index, there was a similar increased transformation during enflurane anesthesia that was not as obvious in unanesthetized controls. Indeed, the mitogenic index was significantly greater in anes-

### Table 2. Effects of Halothane in Human Volunteers (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Before Anesthesia</th>
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<tr>
<td>Anesthesia (Group I)</td>
<td>100</td>
<td>111.9 ± 18.0</td>
<td>87.9 ± 10.1</td>
<td>87.3 ± 14.5</td>
<td>98.1 ± 23.1</td>
</tr>
<tr>
<td>No anesthesia (Group II)</td>
<td>100</td>
<td>114.0 ± 18.6</td>
<td>126.2 ± 19.6</td>
<td>99.6 ± 10.9</td>
<td>97.8 ± 18.1</td>
</tr>
<tr>
<td><strong>Mitogenic index</strong></td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>3.1 ± 0.5</td>
<td>3.5 ± 0.7</td>
<td>2.8 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>No anesthesia (Group II)</td>
<td>3.8 ± 0.9</td>
<td>4.3 ± 1.0</td>
<td>4.8 ± 0.9</td>
<td>3.5 ± 0.8</td>
<td>3.0 ± 0.8</td>
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<td><strong>Leukocyte count (×1,000/mm³)</strong></td>
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<tr>
<td>Anesthesia (Group I)</td>
<td>6.8 ± 1.0</td>
<td>8.1 ± 0.8</td>
<td>9.0 ± 0.6</td>
<td>8.9 ± 0.9</td>
<td>7.7 ± 2.0</td>
</tr>
<tr>
<td>No anesthesia (Group II)</td>
<td>7.5 ± 0.4</td>
<td>7.5 ± 0.3</td>
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<td><strong>Lymphocytes (×1,000/mm³)</strong></td>
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<td>2.5 ± 0.1</td>
<td>2.9 ± 0.3</td>
<td>2.6 ± 0.2</td>
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<tr>
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<td>38.6 ± 4.5</td>
<td>43.7 ± 3.7</td>
<td>42.6 ± 5.2</td>
<td>39.4 ± 9.5</td>
<td>44.7 ± 7.0</td>
</tr>
<tr>
<td>No anesthesia (Group II)</td>
<td>38.4 ± 4.5</td>
<td>46.9 ± 5.3</td>
<td>39.3 ± 3.6</td>
<td>39.4 ± 9.5</td>
<td>44.7 ± 7.0</td>
</tr>
</tbody>
</table>

No value was significantly different from the corresponding unanesthetized control value (\( P \leq 0.05 \)).
thetized than in control subjects at the mid-
anesthesia sampling period. This single sta-
tistically valid difference may be due, in part, to the large variation in times between sam-
ppling and establishing the cultures for the mid-
anesthesia specimens.

Halothane anesthesia was associated with no significant change in lymphocyte transfor-
mation, expressed either as percentage of pre-
anesthetic value or as a mitogenic index (table 2). At the end of anesthesia there was a
tendency toward slight depression of transfor-
mation, which returned to control values by
day 5. A normal diurnal variation was again
seen in control subjects but was not demon-
strable in volunteers given halothane. There
was, however, no statistically significant dif-
ference between the responses of anesthetized
and unanesthetized individuals at any time.

Similar results for halothane volunteers
were obtained in our San Diego laboratories
using the method of Park and Good. There
was no significant alteration in lymphocyte
transformation during or after halothane anes-
thetism when cells were cultured without the
delay associated with mailing to Seattle.

Discussion

Anesthetic agents are capable of many cel-
lar effects, and it is reasonable to implicate
them in postoperative immune deficiency
states. While lymphocyte transformation is in-
hibited by halothane in vitro, the duration of
exposure to clinically relevant concentrations
must exceed 24 hours. The present study
demonstrates that halothane or enflurane anes-
thetism in man is not associated with altera-
tions in lymphocyte transformation in re-
sponse to PHA. These results corroborate
similar observations by Kanto and Cullen,
who observed no abnormality of lymphocyte
function when patients underwent minor oper-
ations with brief anesthetics.

Although the response of volunteers anes-
thetized with either agent was not associated
with any significant difference from the re-
sponse of unanesthetized controls, the data
suggest that enflurane tends to augment trans-
formation and halothane to reduce transform-
ation. Assuming that inhibition of lymphocyte
transformation is harmful, these differences
suggest that enflurane may be more benefi-
cial than halothane. However, the data do
not allow valid comparisons of this type
since the volunteers in the two studies were
not the same and the experimental protocols
differed slightly. First, total anesthetic ex-
posure was greater for the halothane volun-
teers (mean ± SEM 13.7 ± 0.8 MAC-hours)
than for the enflurane volunteers (9.6 ± 0.4
MAC-hours). Second, the enflurane volunteers
were subjected to periods of inhalation of ni-
trons oxide with a reduced inspired oxygen
tension. Nitrons oxide has been associated
with reduced lymphocyte transformation in
human volunteers but not in vitro. However,
the reduction in vitro correlated with the adren-
al cortical response to light anesthe-
sia rather than a direct effect of nitrons
oxide. Third, volunteers receiving enflurane
were subjected to two periods during which
their Pao2 rose to more than 70 torr. This
stress was not given to the subjects receiv-
ing halothane. Finally, the time from blood
collection to placement of cells into culture
was more varied in the enflurane study.

Although lymphocytes remain viable for at
least 57 hours at room temperature with
little effect on T-cell responsiveness, subtle
differences in lymphocyte reactivity at the ex-
tremes of acceptable time intervals may, in
part, account for the different trends with
different drugs. However, the similarity of
results when cells cultured in Seattle were
compared with those cultured in San Diego
suggests that the delay in mailing samples
was not a significant factor.

In conclusion, we feel that no significant
immunosuppression, as assessed by leukocyte
count and by PHA-induced lymphocyte trans-
formation, has been demonstrated in volun-
teers anesthetized with either halothane or
enflurane in the absence of operation. The
impaired lymphocytic responsiveness associ-
ated with operation must therefore be second-
ary to other aspects of the procedure, most
probably neurohumoral responses to surgical
stress. The anesthetic drugs appear to affect
immune competence more by modifying the
response to this surgical stress than by any
direct effect on immunocompetent lympho-
cytes.

The authors are indebted to Mr. Scott Matthias,
Mr. Larry Ray-Kel, and Mr. Howard Prestidge
for their technical assistance.
References


17. Bruce DL: Failure of nitrous oxide to inhibit transformation of lymphocytes by phytohaemagglutinin. ANESTHESIOLOGY 44:155-156, 1976


