Natural Killer Cell Cytotoxicity in the Peripheral Blood, Cervical Lymph Nodes, and Tumor of Head and Neck Cancer Patients

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ABSTRACT

This study evaluated peripheral blood lymphocyte and lymph node lymphocyte natural killer (NK) cell activity in 22 patients with head and neck squamous cell carcinoma and eight patients undergoing surgery for nonmalignant conditions who served as controls. A novel mixed-model analysis of variance was used to analyze the results because of the inherent difficulties in data interpretation among heterogeneous groups when several concurrent variables impinge upon the results. The peripheral blood lymphocyte NK activity of cancer patients was significantly less than controls. In contrast, lytic activity from uninvolved draining lymph nodes of cancer patients was comparable to the activity of control nodes. However, if the node contained a small focus of metastatic tumor, NK activity was significantly diminished relative to uninvolved nodes from cancer patients or to control nodes. The mixed-model analysis of variance was particularly helpful in confirming this finding. Finally, NK lysis by tumor-infiltrating lymphocytes, purified from grossly metastatic nodes, was severely depressed. These data indicate that a spectrum of NK suppression exists in draining lymph nodes of head and neck squamous cell carcinoma patients, and that the level of activity depends upon the degree of nodal tumor involvement.

INTRODUCTION

The existence of immunological defects in patients with H&NSCC3 is well established (1–3). In fact, decreases in T-lymphocyte-mediated immune responses are more pronounced in H&NSCC patients than in patients with other neoplasms (3–5). More recent studies on the PBL NK cell and antibody-dependent cell cytotoxic activity of H&NSCC patients have also demonstrated significant depressions in activity compared to controls (6, 7).

In contrast to the clearly documented immunosuppression in systemic cell-mediated responses, loco-regional immune function in these patients is less well studied. T-lymphocyte numbers in uninvolved (8) as well as metastatic (9) neck lymph nodes are comparable to controls. Furthermore, mitogen-induced proliferative responses of T-cells from cancer nodes are normal (10).

Since activated NK lymphocytes in loco-regional sites may be an important defense mechanism against the growth and spread of tumor cells, the current study investigated NK cytotoxicity of lymphocytes in proximity to the tumors of H&NSCC patients. For these experiments, the NK cytotoxicity of PBL, lymphocytes from tumor-free and focally tumor-containing lymph nodes (LNLs), and TILs was determined. These results were compared with the NK activity of PBL and neck LNL of control patients undergoing neck surgery for nonmalignant conditions.

Interpretation of such data is complex because several coexisting variables could potentially influence the individual data points in such a study. Obvious factors include: (a) the presence or absence of cancer (i.e., whether the tissue sample is from a control or cancer patient); (b) the source of the tissue sample (i.e., whether the NK effectors are PBL, lymphocytes from uninvolved nodes, lymphocytes from normal portions of nodes containing a small metastatic tumor focus, or TILs purified from completely effaced metastatic nodes); (c) whether or not the tissue sample contained cancer; and (d) whether or not the patient had metastatic disease. To allow a simultaneous assessment of these fixed effects which could potentially contribute to the NK activity of the samples, a general mixed-model ANOVA with repeated measures was utilized. This ANOVA is well suited for a study of this nature because it accounts for differences in number and source of samples which vary from subject to subject. In addition, this method of analysis prevents over or under weighting of data obtained from individuals from whom different numbers of samples were obtained.

The results of this study indicate that the PBL NK activity of H&NSCC patients is clearly lower than controls. Furthermore, a spectrum of NK activity exists in draining nodes depending upon the degree or pattern of tumor involvement. This ranged from normal NK lysis in uninvolved nodes to modestly but significantly decreased activity in nodes focally involved with tumor to severely depressed activity in TILs purified from obliterated metastatic nodes.

MATERIALS AND METHODS

Patient Material. Twenty-two previously untreated patients with H&NSCC who underwent a radical neck dissection and resection of their primary tumor were included in the study. Shortly after the induction of general anesthesia, a sample of peripheral blood was taken. Preliminary studies in five patients (data not shown) demonstrated that general anesthesia did not affect PBL NK activity. Upon removal of the surgical specimen, one to five lymph nodes and occasionally metastatic tumors from completely effaced lymph node with extracapsular spread were harvested from the specimen. Also, an effort was made to identify lymph nodes that contained only a small focus of tumor. In these specimens, the tumor focus was trimmed away before the remaining, normal appearing portion of the node was prepared for chromium release assay.

Eight patients who underwent neck surgery for a nonmalignant, noninflammatory disease (parotidectomy for benign disease, 3; carotid surgery, 1; thyroideectomy, 1; laryngeal surgery, 1; branchial cyst excision, 1; thyroglossal duct cyst excision, 1) were included in the study as control patients. During surgery a sample of peripheral blood was taken, and one to five lymph nodes were removed from the surgical field.

In all patients, a portion of each sampled lymph node was submitted for pathological evaluation. This study was undertaken with the approval of the Human Subject Protection Committee of the University of California, Los Angeles, and the Committee on Human Studies at
the Veterans Administration Medical Center, West Los Angeles.

Incubation Medium. Complete medium for these studies was RPMI
1640 (Gibco, Grand Island, NY) supplemented with 10% heat-inacti-
vated fetal calf serum (Reheis, Phoenix, AZ), 1 mM glutamine (Gibco),
100 IU/ml of penicillin (Gibco), 100 IU/ml of streptomycin (Gibco),
and 0.5 mg/ml of Fungizone (Gibco).

Tumor Cell Line. K562 erythroleukemia cells were maintained in
vitro and served as the NK-sensitive target. This cell line has been
continually free from Mycoplasma contamination.

Chromium Release Assay. Cells were assayed for cytotoxic activity
against 51Cr-labeled target cells according to the method of Brunner et
al. (11). Briefly, 5 to 10 x 10^6 K562 cells were incubated with approxi-
ately 0.2 mcCi of chromium-51 (New England Nuclear, Boston, MA)
at 37°C for 90 min in 1 ml of complete medium. After washing, 0.1 ml
of target cells (10^6 cells/ml) was added to 0.1 ml of varying concentra-
tions of effector cells which depended on the desired E:T ratio (10:1,
25:1, 50:1, and 100:1). The cell mixtures were incubated in quadrupli-
cate at 37°C for 3 h in a CO2 incubator. The cells were then centrifuged,
and 0.1 ml of the supernatant was removed for counting in a gamma
counter. Maximal release (>90% of incorporated counts) was deter-
mined by addition of 1% Triton X-100 (Sigma Chemical Co., St. Louis,
MO), and background release was determined by the addition of me-
dium alone (<20% of incorporated counts). The percentage of cell-
mediated lysis is calculated as follows.

\[
\text{Experimental group release – background release} \times 100
\text{Maximal release – background release}
\]

Isolation of Effector Lymphocytes. For PBLs, heparinized blood was
obtained from patients and diluted 1:1 with Dulbecco’s PBS (Gibco).
Mononuclear cells were prepared by the Ficoll-Hypaque (Sigma) den-
sity gradient purification technique (12). The mononuclears in the samples
were consistently less than 6% when assessed by Wright staining.

For LNLs and TILs, specimens of lymph node or tumor that had
been harvested from effaced nodes were minced, teased apart, passed
through a No. 60 stainless steel screen, and washed in PBS by a modifica-
tion of the method of Vose and coworkers (13). Approximately
2 x 10^6 tumor cells or lymph node cells were layered on Ficoll-Hypaque
(specific gravity, 1.077) and centrifuged for 15 min at 800 x g. Tumor
cells and red blood cells were pelleted by centrifugation. Mononuclear
cells recovered at the interface were then thoroughly washed. Viability
was always greater than 90% by trypsin blue exclusion. Satisfactory
separation of TIL and tumor cells was judged by morphological ex-
amination of Papanicolaou- and May-Grünwald Giemsa-stained
smears. In all TIL preparations, tumor cell contamination was less than
5%. The macrophage content of these samples was 5 ± 3%.

Preparation of Enriched Tumor Cells. Enriched preparations of tumor
cells were obtained by a modification of previously described methods
(13-15). Ten million cells from disaggregated tumors were resuspended
in 1 ml of 30% Percoll in PBS and layered in centrifuge tubes on
discontinuous Percoll gradients consisting of 3 ml each of 60%, 50%,
and 40% Percoll (Sigma) in PBS. The tubes were centrifuged at 800 x
g for 30 min at room temperature. Tumor cells were collected above
the 30% Percoll and at the 30 to 40% interface. Satisfactory tumor cell
purification was judged by morphological examination of Papanico-
laou- and May-Grünwald Giemsa-stained smears and was found to be
greater than 90%.

Determination of Lytic Units. One LU is defined as the number of
effector cells required to lyse either 20% (for PBLs) or 2% (for LNLs
and TILs) of target cells. Because the magnitude of a LU is inversely
related to the cytotoxic activity of a sample, NK cytotoxicity is ex-
pressed as the number of LUs in 10^6 effector cells, thereby establish-
ing a direct relationship. PBL activity is expressed in terms of 20% target
lysis because 20% fails in the mid-portion of the rapid upstroke of PBL
cytotoxicity curves. LUs for LNLs or TILs, which have much less
activity, are expressed in terms of 2% target lysis. The techniques of
Pross and coworkers (16) and Bloom and Korn (17) were used to
compute LUs. As such, LUs are derived from the nonlinear expression

\[
Y = K(1 - e^{-\alpha n})
\]

where Y is the percentage of cell-mediated lysis, K is a theoretical
maximum, \(\alpha\) is a relative measure of rate of change of the model with
increasing E:T ratios, and n is the number of effectors. Data are fit to
this equation using the least-squares nonlinear regression analysis of
the BMDP statistical software package (18) and the parameters K and
\(\alpha\) estimated for each sample. Although LUs for LNL and TIL represent
only 2% specific lysis, it has proven to be a consistent and reliable
index of NK activity because it is established from a nonlinear regres-
sion curve that is derived from the 16 data points which comprise the
assay of a single cell sample. Typically the percentage of specific lysis
for LNL samples at the larger E:T ratios is 5% to 20% allowing for an
accurate derivation of LU for 2% lysis. Ten specimens were initially
assayed at multiple E:T ratios to test the accuracy of using LUs derived
from this exponential curve. In all cases, the derived curve showed an
excellent correlation with the experimental data. An example is shown
in Fig. 1.

Analysis of Variance. To simultaneously analyze the several fixed
and random effects, a general mixed-model ANOVA with repeated
measures is used. In order to prevent undue weighting of data from
patients with many samples and to address within subject variation,
this analysis takes into account repeated measures that may occur
within a single individual. For instance, a single cancer patient may
have as many as eight tissue samples (e.g., PBL, 1 to 6 lymph nodes
that may either be tumor containing or tumor free, and TIL), whereas
other patients may have only one or two samples. The analysis of
variance is based on the model

\[
Y_{\text{glm}} = \mu + a_i + b_j + c_k + d_l + \beta_m + \epsilon_{ijkl}
\]

where \(Y_{\text{glm}}\) is the NK activity measured in log(LU in 10^6 effector cells);
\(\mu\) is the overall mean; \(a_i, b_j, c_k,\) and \(d_l\) are the effects of the fixed factors
disease, source, node, and metastasis; \(\beta_m\) is the between patient vari-
bility, with \(m\) indexing the individual patient; and \(\epsilon_{ijkl}\) is the within
patient variation. The model is expressed here in its complete form,
and portions of the model were eliminated as necessary for the specific
analysis. The fixed factors are defined as “disease,” whether the patient
was a cancer or a control patient; “source,” whether the sample was
PBL, LNL, or TIL; “node,” whether the tissue sample contained tumor
or not; and “metastasis,” whether metastatic disease was histologically
present or absent when the entire neck specimen was examined.

This model assigns an estimate of the additive contribution of each
of these factors to the overall mean NK activity for each group. This
estimate specifies the expected difference from the overall mean for
each factor independently of the other factors. It is an index of the
importance of each factor in determining the NK activity of a particular

Fig. 1. Peripheral blood lymphocytes from a single control subject were
prepared on a Ficoll-Hypaque gradient. NK cytotoxicity was determined in
quadruplicate at each effector cell concentration against 1 x 10^6 chromium-51
loaded K562 target cells in a 3-h cytotoxicity assay. Effector cell number varied
from 1 x 10^4 to 1 x 10^6 cells/well. The continuous curve is derived using a least-
squares nonlinear regression analysis by fitting the data to the expression; per-
centage of lysis = \(Y(1 - e^{-\alpha n})\). Points, mean; bars, SD.
sample group; the larger the estimate, the greater the relative importance of the factor in determining that group’s absolute NK activity. The Z-score (the estimate divided by the standard deviation of the estimate) indicates the number of standard deviations that the estimate is away from zero.

The data from these experiments are highly unbalanced with many data cells missing. An illustration of this imbalance is that it is impossible to have a tumor sample from a control patient. The data were therefore fitted to the general-mixed model with an iterative algorithm from the BMDP statistical package. Because of the imbalance, it is difficult to meaningfully analyze the interaction of factors.

A probability of \( P < 0.05 \) was considered to be a significant difference between groups. All statistical analyses, including paired t tests and one-way ANOVA, are performed with the BMDP software package (Westwood, CA) on a microcomputer (19). The BMDP programs that were used for these analyses were P3R (nonlinear regression), P3D (paired t tests), P1V (one-way ANOVA), and P3V (general mixed-model ANOVA).

RESULTS

Patient Profile. One-hundred twelve lymph nodes from 22 cancer and 8 control patients were studied. One to five nodes were sampled from each patient. Table 1 demonstrates the clinical characteristics of the patients, as well as the types of nodes removed. An average of four nodes per cancer patient and three nodes per control patient was removed at surgery (discrepancy due to the large tissue specimen available in cancer patients.). While most of the nodes removed from cancer patients were tumor free, six nodes were focally involved and five were completely involved with tumor. The H&NSCC primary tumor sites included tonsil (5 cases), larynx (4 cases), retromolar trigone (4 cases), floor of mouth (4 cases), pyriform sinus (2 cases), based of tongue (1 case), and alveolar ridge (1 case). One patient had an unknown primary. Many of the cancer patients presented with advanced disease (15 of 22 were Stage T3 or T4), and nearly half (9 of 22) had cervical metastases. None of the patients had distant metastasis. A higher proportion of cancer patients were smokers than control patients.

Assessment of PBL Activity. Tables 2 and 3 demonstrate PBL NK lysis from control and cancer patients. The raw group data are shown in Table 2. A conventional method of statistical analysis, shown in the top portion of Table 3, utilizes a simple one-way ANOVA to compare the three groups. The general mixed-model ANOVA is displayed in the bottom portion of the table.

From the one-way ANOVA P matrix (Table 3), it is apparent that the NK activity of control patients is different from cancer patients without metastatic disease. However, it is not clear whether the presence of metastatic disease exerts an independent effect to alter the NK activity of the sample. The general mixed-model ANOVA helps to evaluate the independent effects of the presence or absence of cancer and the presence or absence of metastatic disease in the neck of the cancer patient. The overall sample mean is 1.135 (for LU at 20% target lysis; Table 3). The estimate of contribution of the factor “disease” (i.e., whether the patient has cancer or is a control) is 0.171; this number is positive, indicating that being a control patient contributes to greater NK activity. This is a statistically significant factor with a \( P \) value of 0.038. In contrast, the presence or absence of metastases in the entire neck is not a significant determinant of PBL NK activity (\( P = 0.379 \)). An analysis of the residuals of the logged data from the mixed-model analysis demonstrated a normal distribution. Assessment of the interaction of the factors “disease” and “metastasis” is not possible because of the unbalanced nature of the data. An example of the unbalance is the obvious impossibility of having metastatic disease in a control patient.

Assessment of TIL Activity. Several studies have demonstrated decreased spontaneous cytotoxicity exerted by lymphocytes infiltrating various tumors (20–23). To investigate this issue in H&NSCC, we purified TILs from five completely effaced metastatic nodes. Fractionation of metastatic lymph node cells by density gradient always resulted in a lymphocyte fraction which contained less than 5% contamination with tumor cells. To examine whether this degree of contamination affected lymphocyte cytotoxicity towards K562 targets, tumor cells were purified as described in “Materials and Methods” (>90% tumor cells) and mixed with autologous effector PBLs in an NK cell chromium release assay. As shown in Table 4, the NK activity of autologous PBLs was decreased only when
Table 4 Effect of tumor cells on NK chromium release assay

| Tissue source | n | NK activity
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>PBL</td>
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<td>1.0000</td>
</tr>
<tr>
<td>LNL</td>
<td>&lt;0.0001</td>
<td>1.0000</td>
</tr>
<tr>
<td>TIL</td>
<td>&lt;0.0001</td>
<td>1.0000</td>
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Table 5 PBL, LNL, and TIL NK activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease</th>
<th>Source</th>
<th>Node</th>
<th>Metastasis</th>
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</tr>
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<tr>
<td>A</td>
<td>Control</td>
<td>LNL</td>
<td>Normal</td>
<td>-</td>
<td>26</td>
<td>0.79 ± 0.44 (10.15)</td>
</tr>
<tr>
<td>B</td>
<td>Cancer</td>
<td>LNL</td>
<td>Normal</td>
<td>+</td>
<td>40</td>
<td>0.86 ± 0.36 (9.90)</td>
</tr>
<tr>
<td>C</td>
<td>Cancer</td>
<td>LNL</td>
<td>Focally positive</td>
<td>+</td>
<td>6</td>
<td>0.80 ± 0.17 (6.70)</td>
</tr>
<tr>
<td>D</td>
<td>Cancer</td>
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<td>Normal</td>
<td>-</td>
<td>35</td>
<td>0.97 ± 0.41 (14.07)</td>
</tr>
<tr>
<td>E</td>
<td>Cancer</td>
<td>TIL</td>
<td>Positive</td>
<td>+</td>
<td>5</td>
<td>-0.07 ± 0.82 (2.83)</td>
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Table 6 PBL, LNL, and TIL NK activity

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Table 7 LNL and TIL NK activity

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Table 8 LNL and TIL NK activity

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The one-way ANOVA P matrix indicates that there is a highly significant difference between each of the three groups (Table 6). Also in Table 6 are the data analyzed by the mixed-model ANOVA. Because there are three levels of "source," PBL, LNL, and TIL, two estimates of contribution are required to derive the predicted means of a sample group. In the case of PBL, the estimate of contribution for PBL is added to the overall mean (1.017 + 0.132). To derive the predicted mean for TIL, the PBL effect and the LNL effect estimate of contributions are subtracted from the overall mean [1.017 - 0.132]. These relationships exist in the formulation of this statistical model because the sum estimates of contribution of PBL, LNL, and TIL are zero. Again the difference between the groups is significant.

Assessment of LNL Activity. Two specific questions were addressed in the assessment of LNL NK lysis of cancer patients: (a) is the activity of uninvolved draining nodes comparable to control patient nodes; and (b) does the presence of a small focus of tumor in the node affect the activity of the rest of the node. The cytotoxicity data required to answer these questions were obtained from five sample groups as shown in Table 7. The data are analyzed by one-way ANOVA in Table 8 by conventional and mixed-model ANOVA techniques.

An interpretation of the effect of each grouping variable by...
one-way ANOVA is exceedingly difficult because each group mean is affected simultaneously by four grouping factors, namely “disease,” “source,” “node,” and “metastasis.” In this analysis, the difference between LNL and TIL NK activity is reinforced with LUs defined at 2% lysis (Group A, B, C, or D versus E). This type of analysis did not detect any significant differences between the activity of uninvolved cancer nodes and control nodes (Group A or B versus C). Nor did it demonstrate a significant difference in NK lysis between uninvolved cancer nodes and focally involved cancer nodes (Group C versus D).

A clearer understanding of the importance of each grouping variable can be ascertained from Table 8 which presents the mixed-model ANOVA. “Disease,” that is, the presence or absence of cancer in a patient, is not a significant factor in determining NK activity of the sample. However, the estimate of contribution for “node,” the presence or absence of tumor in the individual node, is 0.123. This estimate is positive because tumor-free nodes had greater NK activity than nodes which contained a focus of tumor. This is significant with a probability of 0.039. Lastly, the presence of metastatic disease in the entire neck did not exert a significant effect on the NK activity of the sample. Assessment of the interaction of the factors was not possible because of the unbalanced nature of the data. The residuals of the logged data were examined and found to be normally distributed.

The apparent lack of agreement between the two statistical tests regarding the effect of focal nodal tumor involvement on LNL NK activity is of interest. Whether this is due to an insensitivity of the one-way analysis or is due to a peculiarity of the general-mixed model ANOVA was in question. To study this more closely, we examined each cancer patient in whom both positive and negative radical neck lymph nodes were sampled. The data are presented in Table 9. It is apparent that, for each patient, the mean NK activity of the negative lymph nodes is greater than the NK activity of the focally positive lymph nodes, a finding that would support the validity of the mixed-model analysis. In order to verify this apparent difference in NK activity, a paired Student t test was performed. This analysis revealed a statistically significant difference between groups. Therefore, it would appear that the discrepancy in findings between the one-way ANOVA and the general mixed-model ANOVA is due to the insensitivity of the one-way analysis. This is not surprising in view of the great differences in LNL NK activity from patient to patient.

DISCUSSION

The major findings of this study are: (a) PBL NK activity of H&NSCC patients is lower than control patients undergoing neck surgery for a noninflammatory nonmalignant condition; (b) the NK activity of TILs is considerably less than LNLs or PBLs (from the same cohort of patients or from control patients); (c) the NK activity of uninvolved lymph nodes is comparable to nodal NK activity of normal controls; and (d) the presence of a metastatic focus in a draining cervical node is an important suppressive influence on the normal portion of the node’s NK activity.

The observation of depressed PBL activity in H&NSCC cancer patients is consistent with a previous study of pharyngeal cancer patients (24). In contrast, Wustrow and Zenner (25) found comparable PBL NK activity in control and H&NSCC patients. The latter study compared groups at a single E:T ratio instead of using LUs which are derived from many E:T ratios. This difference in data analysis may explain the discrepancy between studies.

Severely depressed NK activity in TIL has also been noted in patients with breast (26) and lung (20) cancer. While the decrease in NK activity in TIL is striking, it might be argued that the low levels are not due to a property of the tumor, but simply reflect the normal distribution of NK cells. That is, NK cells are not indigenous to tumors. However, the finding, that lymphocytes isolated from the histologically normal portion of lymph nodes that have a small metastatic focus of tumor have a significant negative estimate of contribution, provides strong evidence that the tumor itself, either directly or indirectly, inhibits NK activity.

Some investigators have attributed decreases in NK activity in cancer patients to decreases in the number of effectors (20, 27), whereas others have attributed this decreased activity to decreases in the cytotoxic potential of existent cells (21). Preliminary studies with conditioned medium generated from TIL and from tumor cells suggest that both are capable of producing a soluble suppressor substance that inhibits NK cell activity of naive donor PBL. Support for this hypothesis is also found in murine studies where tumor bearers have been found to have splenic (28–30) and tumor-infiltrating (22) suppressor cells which inhibit NK activity.

We have elected to express the NK activity for these studies in terms of LUs derived using a nonlinear regression least-squares analysis following the method of Pross et al. (16). Through the mathematical derivation of an exponential curve which represents a cell number-NK activity function, LUs can be much more accurately and reproducibly derived than with techniques that attempt to approximate the slope of the linear portion of the curve. Furthermore, a single number is derived from the entire data set to represent the activity of the sample, substantially facilitating statistical analysis while taking advantage of all the available data. This method of data expression is particularly useful for samples with low levels of NK activity, such as LNL and TIL, where there is inherently more variability from determination to determination.

A unique feature of our study was the use of a general mixed-model ANOVA with repeated measures for the statistical analysis of these experiments. This technique was chosen over more conventional analyses primarily because it addresses several of the problems inherent in human immunological studies. These include the existence of several simultaneous factors which potentially contribute to an observed biological effect, variation in the number of samples which could be obtained from a single subject, and between subject differences in biological activity.

Specifically, this general mixed-model analysis allowed for assessment of the contribution of coexistent factors which could affect NK lysis. In studies such as this, it is often difficult to rigorously define the experimental conditions a priori, in order
to limit study groups to a single set of experimental variables. For instance, we could not be certain that a patient who was clinically free of neck metastatic disease would subsequently within and between subject variation. One possible reason for this could vary in both number and source) and also accounts for we could not be sure that a uniform number of lymph nodes that were not sampled in the experiment but were evaluated at pathological examination of the entire surgical specimen. In addition, through the use of a random variable (the individual subject) and repeated measures, this statistical method allows for variations in the number of samples from subject to subject (which could vary in both number and source) and also accounts for within and between subject variation.

An example of the usefulness of the mixed-model ANOVA was in assessment of activity from nodes with a small focus of tumor. The conventional one-way ANOVA would not have demonstrated the depressive effect of such a focus because of the small sample size, the wide variation in LNL NK activity from patient to patient, and the coexistent influences from other factors. The mixed-model ANOVA, by allowing the use of all data points and assessing the specific contribution of having a tumor focus in the sample to the overall NK activity of the group, clearly demonstrated the significance of this factor. H&NSCC is an excellent model for studying regional alterations in immune function in the cancer patient. Because the neck is a rich source of lymph nodes, regional lymphatics which have been resected en bloc with tumor are readily available for study in the surgical specimens. Additionally, patients frequently undergo neck surgery for nonneoplastic conditions, thus becoming available as donors of control tissue.

In summary, we have shown that there are systemic and regional alterations in NK activity in H&NSCC patients. It is apparent that the tumor itself is capable of depressing NK activity in an area where NK cells are normally existent. Further studies are being undertaken to investigate the mechanism(s) by which these alteration occur.

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