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Evidence for the role of natural immunity in the control of metastatic spread of head and neck cancer*

Stimson P. Schantz[1], Barry W. Brown[2], Ernest Lira[1], Dorothy L. Taylor[1], and Nancy Beddingfield[1]

[1] Department of Head and Neck Surgery,
[2] Department of Biomathematics, The University of Texas, M D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Avenue, Houston, TX 77030, USA

Summary. Deficient natural killer (NK) cell activity may contribute to the development of distant metastases in the head and neck cancer patient. A total of 246 previously untreated patients expressed deficient NK activity against K562 target cells when compared to 110 age-matched healthy controls (70 ± 48 lytic units (LU) versus 95 ± 52 LU) ($P < 0.001$). Some 164 consecutive patients have undergone definitive therapy subsequent to NK cell assessment and have been followed for a minimum of 12 months (median = 16 months), and 23 have developed recurrent disease in distant sites. The risk of subsequently (1) developing distant metastases, (2) developing regional metastases, and (3) dying of progressive cancer was inversely related to pretreatment NK LU values ($P < 0.02$, < 0.02, < 0.005, respectively, by the Cox proportional hazards model). NK cell function within the peripheral blood of the patient with head and neck cancer could be related to the percentage of Leu 11+ NK cell subsets ($P < 0.01$ by linear regression analysis) as determined by both single-parameter and multiparameter flow cytometric assessment. Contrastingly, no relationship could be identified between NK function with the percentage of circulating Leu 7+ cell subsets. In vitro measured NK cell function identifies a population at increased risk for developing distant metastases, thus supporting the role of natural immunity as defense mechanism against blood-borne disease.

Introduction

Animal studies have implicated the natural immune system as a first-line defense against the virally transformed or virally infected neoplastic cell [32, 37]. Within the mouse model, selective abrogation of this system allows blood-borne tumor emboli to escape and increases the probability of subsequent metastatic disease [7, 8, 19].

These experimental observations may be increasingly important in our understanding of squamous cell carcinoma of the upper aerodigestive tract. First, recent seroepidemiologic and genetic hybridization analyses have implicated viral agents as an etiologic factor [5, 27, 28, 39]. Second, with improved local and regional disease control by combination surgery and radiation therapy, the significance of distant metastases in disease progression is becoming more evident [34]. The capacity of the host to recognize the virally transformed neoplastic cell, potentially via the natural killer (NK) cell system, may be an important factor in controlling disease progression following therapy. Such a hypothesis is supported by preliminary evidence suggesting that the level of NK cell cytotoxic capacity of peripheral blood lymphocytes (PBL) has prognostic significance for patients with head and neck cancer [23, 25, 26].

The characteristic properties of the cell populations responsible for the natural immune cytotoxic response have been extensively investigated. Morphologically they are defined as large granular lymphocytes (LGL) [31]. Phenotypically, NK cells are a heterogeneous population in which the near total population expresses the Leu 7+ and/or the Leu 11+ phenotype [1, 16]. Investigators have determined that characterizing antigen expression on the NK cell provides information regarding the cell's relative cytolytic capacity; Leu 11+ cells have the greatest cytotoxic potential [14]. Such results suggest that the quantitation of percentages of NK cell subsets within the peripheral blood may define overall cytolytic capacity against allogeneic targets.

The present prospective clinical study details the relationship between in vitro measured NK cell activity and the probability of subsequent distant metastases in patients with head and neck cancer. We further examined to what extent lytic unit (LU) capacity in these patients can be predicted by quantifying the percentages of phenotypically defined NK cell subsets within the peripheral blood. A positive correlation would serve several purposes. It would support the role of natural immunity as an important antitumor defense system. Additionally, a significant relationship between the number of particular NK cell subsets and cytotoxic function would increase our understanding of factors that contribute to altered natural immune responses in patients with head and neck cancer.

Materials and methods

Patient population. Persons with previously untreated squamous cell carcinoma of the head and neck, as previously described [23] from the basis of this report. Stage of disease was determined for each patient, using criteria of

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Offprint requests to: Stimson P. Schantz, Department of Head and Neck Surgery-69, The University of Texas System Cancer Center, 1515 Holcombe, Houston, TX 77030, USA

the American Joint Committee on Cancer [4]. Disease stage was assigned by members of the Department of Head and Neck Surgery without knowledge of the respective patient's NK cell function. In no patient were distant metastases clinically evident at the time of presentation and NK cell evaluation.

The median age of 246 patients who fulfilled these criteria was 60 years (range 21–85 years). The male:female ratio was 2.5:1. The primary disease included 95 oral cavity lesions, 70 pharyngeal cancers, and 72 laryngeal cancers. In 9 patients either the primary cancer was unknown or multiple primary cancers existed, which precluded defining one particular site of disease.

Details of longitudinal evaluation for disease recurrence have been described previously [26]. Only patients who were rendered disease-free by treatment were eligible for analysis; 13 patients underwent definitive therapy but were never rendered free of disease as determined by the treating physician. Residual disease was determined clinically and in nearly all instances was located at the primary tumor site. Extension of the disease into anatomical locations not amenable to complete surgical resection was the primary determinant of residual disease in this group. Also, 6 patients were treated elsewhere and thus could be not definitively assessed as to disease status, and 1 patient was not considered treatable. The length of the disease-free interval following therapy was calculated in months from the date of the patients's initial treatment to the last noted physician contact at which the patient had no evidence of disease. Only persons treated at least 12 months prior to the present assessment were considered. Of the 246 patients, 20 were never treated or were treated elsewhere and were not considered evaluable for disease recurrence. Also, 62 patients have been treated within the last 12 months; since their follow-up time was less than the 1-year cutoff, they were not considered in the present longitudinal assessment for disease recurrence. Therefore, 164 persons satisfied the criteria for this longitudinal assessment.

Follow-ups occurred at 3-month intervals in the first year, 6-month intervals in the second year, and then annually. Patient disease status was assessed at these intervals by members of the Department of Head and Neck Surgery, again without knowledge of the LU value. The presence or absence of distant metastases was determined by clinical assessment coupled with periodic chest x-ray analysis.

Controls. The control population consisted of 110 age-matched healthy persons. The median age was 60 years (range 41–78 years). The male:female ratio was 1:1.

Reagents. RPMI 1640 medium (Gibco, Chagrin Falls, Ohio) was supplemented with 10% fetal calf serum (Hyclone, Logan, Utah), 1% glutamine, and 2.5% gentamicin and termed supplemented culture median (SCM). Hanks' balanced salt solution (HBSS), used in washing cells, was also obtained from Gibco. Monoclonal antibody labeling of PBL and the washing of antibody-labeled cells employed Dulbecco's phosphate-buffered saline (DPBS) supplemented with 0.2% sodium azide. The monoclonal antibodies, Leu 7 conjugated to fluorescein isothiocynate (FITC) and Leu 11c conjugated to phycoerythrin (PE), were obtained from Becton Dickinson, Mountain View, Calif. The specificity of these antibodies, which define NK cell populations, has been defined previously [1, 18]. Isotype-matched goat-anti-mouse FITC- and PE-conjugates monoclonal antibodies were likewise obtained from Becton Dickinson.

Effector cell preparation. Effector cells (PBL) were prepared as previously described [24]. Briefly, following venipuncture, leukocyte-enriched plasma was obtained by sedimentation (1 g) in 0.6% dextran at 37° C for 1 h. Plasma was centrifuged at 180 *g* for 10 min to remove platelets, and the platelet-containing supernatant was discarded. Cells were subsequently washed twice in HBSS. Lymphocyte-rich mononuclear cells were isolated by centrifugation at 400 *g* for 50 min on a Ficoll-sodium diatrizoate density gradient (sp. gr. 1.077, Litton Bionetics, Charleston, SC). Cells at the interface were collected, washed twice in HBSS, and resuspended in SCM. Monocytes were removed from the mononuclear cell suspension by their characteristic adherence to plastic. Suspensions were incubated for 1 h in 15 ml SCM on 100×15 mm plastic Petri dishes at 37° C. Nonadherent cells were recovered by repeated washing with SCM and resuspended in 20 ml SCM.

Recorded cells were then stored at 4° C for 18 h in each instance prior to testing. Viability of cell fractions following storage was greater than 95% as determined by trypan blue exclusion.

Target cells. The K562 target cells (2×10^6 human erythromyeloid leukemia cells) were labeled with 100 μCi radioative sodium chromate (^{51}sodium chromate sp. act. of 500 Ci/mg, Amersham, Arlington Heights, Ill).

Cytotoxicity assay. Isolated lymphocytes (effector cells) and chromium-labeled target cells were incubated at multiple increments from 6:1 to 100:1 as previously described [24]. After incubation and subsequent centrifugation, a 0.1-ml aliquot was removed and counted in a gamma spectrometer (Packard, Downers Grove, Ill). Percent cytotoxicity was computed according to the following formula:

$$\% \text{ cytotoxicity} = \frac{\text{Experimental cpm} - \text{Spontaneous cpm}}{\text{Maximum cpm} - \text{Spontaneous cpm}} \times 100$$

Spontaneous release of ^{51}sodium chromate, determined by incubating target cells in SCM alone for 6 h, ranged from 6% to 12%. Maximum release was determined by incubating target cells in 10% Triton-X 100 for 6 h at room temperature.

Expression of LU. Data were expressed as the numbers of LU/1×10^7 PBL, where 1 LU was defined as the number of PBL needed to effect 25% cytotoxicity of 1×10^4 target cells. LU were calculated as previously described [21–24]. The variations in LU values among replicate samples in both our control population and the persons with cancer determined on multiple occasions prior to therapy have been reported elsewhere [26]. NK cell function was determined prior to beginning therapy in all instances.

Immunofluorescence labeling. PBL were labeled with monoclonal antibody according to the manufacturer's specifications [16]. In each instance 1×10^6 PBL were suspended in 50 μl DPBS plus 0.2% sodium azide prior to adding the respective monoclonal antibody. Isotype-matched FITC-

and PE-conjugated antibodies were used as controls to exclude Fc-related binding. All procedures were performed at 4° C. All dilutions and washings were performed with DPBS plus 0.2% sodium azide. After labeling, cells were fixed in a 1% paraformaldehyde/0.85% saline solution and stored at 4° C in the dark until analyzed [15].

Flow cytometric analysis. All analyses were performed using a Coulter Epics V flow cytometer (Hialeah, Fla) interfaced to a Coulter Multiparameter Data Acquisition and Display System Computer with a Tektronix (Beaverton, Ore) printer. FITC and PE fluorochromes were excited by 500 multiwats of a 488-nm argon-laser light beam.

Light emitted from the incidence of the laser beam with the cell stream passed through a 515-nm long-pass barrier filter placed at 90° to that incidence. The light was then split and directed to the respective photomultiplier tubes (PMT) by a 560-nm dichroic short-pass filter placed at 45° relative to the light path. In front of the red (PE) PMT was placed a 575-nm band pass. A 525-nm band pass was placed in front of the green fluorescence (FITC)-sensing PMT.

Optical alignment of the flow cytometer was adjusted using a light-emitting diode placed in the optical path of the PMTs, repositioning the PMTs to optimize sensitivity and resolution. The laser beam and the sample flow column were readjusted prior to each experiment, using 10-μm polystyrene spheres (Coulter).

Data acquisition was based on the color analysis of 10,000 cells in both single-parameter and dual-color immunofluorescence studies. In all instances debris, red blood cells, and platelets were eliminated by "gating" at the appropriate level on both the forward angle and 90° light scatter signals. Peak amplitudes of the electric signals for the green fluorescence and the red fluorescence were converted to an 8-bit binary number (analog-to-digital conversion). All digital signals were assigned into 256 channels by a pulse-height analyzer. Graphics of the data were displayed on a 64×64 channel histogram as dot plots, with four dots per channel.

We determined the percentage of positive cells using control cells without fluorochrome-labeled monoclonal antibody (autofluorescence population), respective fluorochrome isotype controls, and the specific monoclonal antibody-labeled test population. A marker was set on the autofluorescence population such that 1% or less of the cells were to the right of this channel marker. Nonspecific binding by isotype control antibodies was then determined, and in our experiments, averaged 2% for the FITC-isotype control and 6% for the PE-isotype control. By using the autofluorescence control channel marker as a reference point, we determined the percentage of cells positively stained by a specific monoclonal antibody for each test sample.Final percentages for each sample were calculated by subtracting the respective nonspecific binding value from the positively stained test value.

Statistical analysis. Values within the text regarding NK cell activity are expressed as the mean ± SD. For all comparisons of NK activity and NK cell population subsets between groups Student's *t*-test was used. The remaining statistical methods are indicated when appropriate throughout the text.

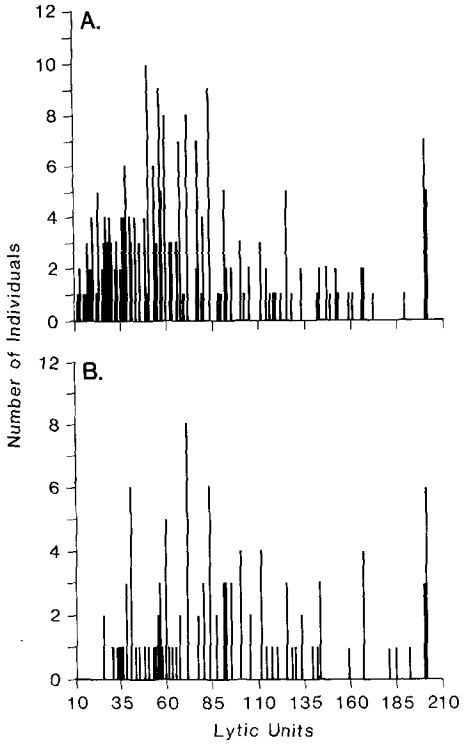

Fig. 1. A comparison of the frequency of individual LU values between 246 patients with head and neck cancer and 110 healthy age-matched controls. One LU is the number of PBL required to lyse 25% of K562 target cells in a 6-h chromium release assay. Individual NK activity is expressed as the number of LUs per 1×10^7 PBL. **A** Head and neck cancer patients. **B** Healthy age-matched controls. The difference in mean NK cell activity between the two groups was significant ($P < 0.001$)

Results

The overall mean NK cell activity of 246 patients with squamous cell carcinoma of the upper aerodigestive tract was 70 ± 48 LU, significantly less than the 95 ± 52 LU expressed by the 110 healthy age-matched controls ($P < 0.001$). The distribution of individual LU values is shown in Fig. 1. The broad overlap of values between the two populations is evident.

Table 1 demonstrates LU values expressed by patients with cancer within the upper aerodigestive tract as a function of site and stage of disease. Using pairwise comparisons within each disease site, significance could be revealed only when comparing stage IV laryngeal cancer patients with stage III laryngeal cancer patients. When assessing all patients by stage of disease independent of site of primary, we found no significant differences between the groupings. Consistent with our previous reports, NK activity is not a reflection of underlying stage of disease as defined by American Joint Committee parameters [23, 26].

NK cell activity and recurrent disease

We examined the relationship between length of survival and pretreatment levels of LU. Using the Cox proportional hazards model, we determined that lessened LU function was predicitve of an increased risk of dying from head and neck cancer despite therapeutic intervention ($P < 0.005$). Based upon previous findings [26], we arbitrarily divided patients into groupings by LU values, i.e., those with values greater than 72 LU, 50–72 LU, and less

Table 1. The relationship of stage and site of disease to natural killer (NK) activity in patients with head and neck cancer

Disease site	Disease stage[a]				All patients (by site)
	I (n)[b]	II	III	IV	
Oral cavity	68[c] (23)	80 (18)	70 (26)	66 (26)	71 (95)
Pharynx	44 (6)	56 (11)	68 (22)	60 (25)	64 (70)
Larynx	75 (9)	70 (17)	80 (31)	50[d] (15)	71 (72)
All patients (by stage)	66 (38)	71 (46)	73 (79)	64 (70)	69 (237)

[a] Disease stage was determined using American Joint Committee staging parameters [16]
[b] (n) = number of patients
[c] Mean NK cell activity expressed as the number of lytic units (LU) per 1×10^7 peripheral blood lymphocytes (PBL) at 25% cytotoxicity of K562 target cells in a 6-h ^{51}chromium release assay
[d] $P < 0.05$ as compared to patients with stage III laryngeal cancer

than 50 LU. This grouping in previous studies collapsed patients into three equally numbered populations to make comparisons regarding disease-free status [26]. Of the 164 patients evaluated, 58 had LU values greater than 72 LU; 4 patients (2 stage III and 2 stage IV patients) in this group have died of disease. Of 49 patients with LU values between 50 and 72, 8 (3 stage II, 3 stage III, and 2 stage IV patients) have died. In the group with the lowest values (less than 50 LU), 19 of 57 have died with progressive cancer. In this latter group of patients who died with disease, 10 had stage III disease and 3 had stage I, II, and IV disease, respectively. The difference in survival duration of the three groups was assessed by the log rank test; overall differences among the three groups was significant ($P < 0.005$). Comparing any two populations revealed significant differences between the less than 50 LU population with the 50–72 LU population; ($P < 0.05$); and the less than 50 LU population with the greater than 72 LU population ($P < 0.005$). No significant differences between the 50–72 LU population and the greater than 72 LU group were noted ($P = 0.25$). Kaplan-Meier estimates of the survival curves for the three groups is shown in Fig. 2.

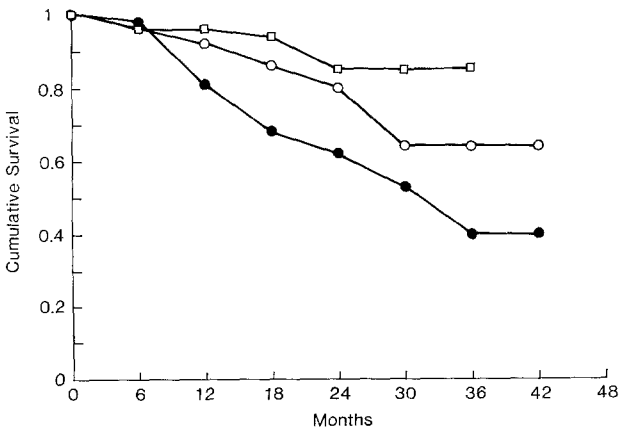

Fig. 2. The relationship between pretreatment NK cell activity and disease-free survival time in patients with head and neck cancer. All patients underwent curative therapy following assessment of NK cell function. Patients were divided into three groups based upon LU value: [] = <72 LU; 0=50–72 LU; ● <50 LU. Differences in survival duration between the three groups were significant ($P < 0.005$) by log-rank testing

We similarly assessed for survival as a function NK activity independent of arbitrary groupings. Figure 3 illustrates the relationship between LU value and survival duration. To construct Fig. 3, 20 LU values spread evenly across the complete range of LU values were used. For each LU value, a survival curve was estimated by the Kaplan-Meier method, using the 20 patients with LU values nearest the one being considered. The survival curve so constructed is displayed at the abscissa LU value used to obtain the patient sample.

The relationship between LU value and type of disease recurrence (local, regional, or distant) was analyzed. Again utilizing a Cox proportional hazards model, we found the most significant metastastic and distant metastatic disease. Following treatment in which all clinically apparent disease had been removed, those persons with lower LU values had the highest risk of disease recurrence within regional lymph nodes ($P < 0.02$) or at distant sites ($P < 0.02$). No relationship between local recurrence and LU was identified. Figure 4 depicts the critical levels of NK activity that were associated with the shortest interval to disease recurrence at a given site (i.e., local, regionally metastatic, and distant metastatic). The maximum change in probability occurred between 45 LU and 60 LU. Of those patients with LUs below 45, 20% died of disease be-

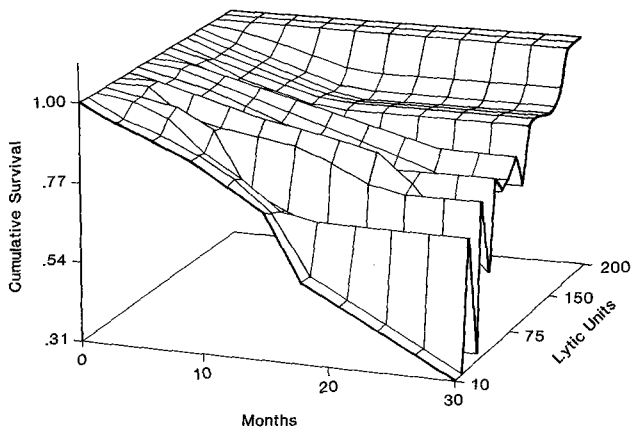

Fig. 3. The relationship between pretreatment NK cell activity and disease-free survival in patients with head and neck cancer. All patients underwent curative therapy following assessment of NK cell function. Patients were evaluated by both time until death and by LU value. Results were significant by the Cox proportional hazards model ($P < 0.005$)

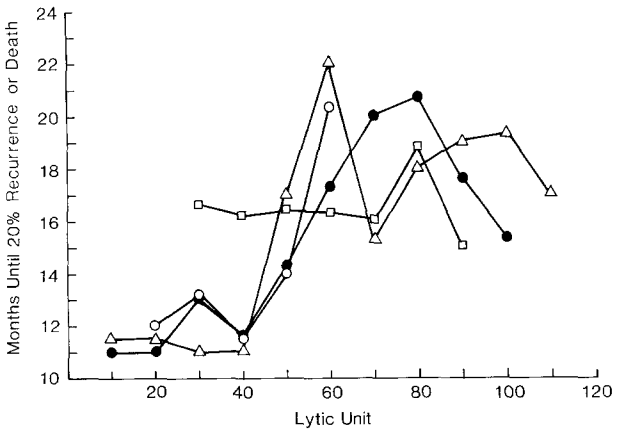

Fig. 4. The length of time in months until 20% of the patients with a designated LU value developed local recurrence, regional recurrence, distant metastases, or death due to disease. ● = death due to disease; 0 = distant metastases; = regional recurrence; [ ] = local recurrence. The maximum rate of change in length of time until 20% recurrence as a function of NK cell value occurred between 45 LU and 60 LU

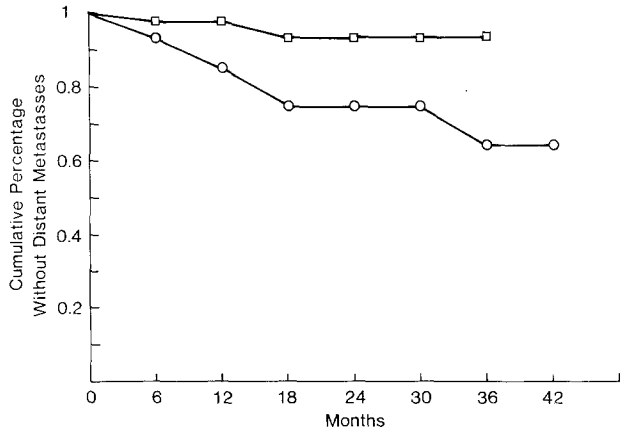

Fig. 5. A Kaplan-Meier analysis of the incidence of distant metastases in patients with head and neck cancer stratified by LU value. All patients were assessed for LU values prior to therapy and were then followed prospectively for the development of distant metastases. [ ] ≥60 LU; 0 <60 LU. Differences in the incidence of distant metastases were significant ($P < 0.005$), by log-rank testing

tween 11 and 13 months. Contrastingly, 20% of those whose LU values were greater than 60 died between 17 to 22 months. In the group of patients with LU values greater than 110, the 20% death rate has not yet been reached. Therefore, no data points can be plotted for them. The relationship between death due to disease and LU value was paralleled by the length of time to regional and distant disease. No correlation between local recurrence and either death due to disease or LU value could be identified.

These data demonstrate that the hazard of dying from head and neck cancer in these 164 patients increased with time and is primarily a function of progressive metastatic disease uncontrolled by therapy. The likelihood of such metastases occurring despite treatment increases significantly in patients whose pretreatment NK cell activity was below the range of 45–60 LU. In the remainder of this report we refer to patients with LU values below 60 as being at high risk for distant metastases and death from head and neck cancer. Figure 5 demonstrates a Kaplan-Meier curve demonstrating the risk of distant metastases in patients with LU values less than 60 LU.

NK Cell activity and NK population subsets

Since May 20, 1985, 84 patients and 53 controls have been randomly selected in whom to quantitate numbers of LGLs and single-parameter determined Leu 7^+ and Leu 11^+ NK population subsets, both in percentages of PBL and in absolute numbers. The distribution of LU values in this group was similar to those in both the entire population and the population followed for disease recurrence (data not shown). Table 2 presents the results of NK population quantitative analyses. The percentage of Leu 7+ cells was higher in the population with head and neck cancer than in age-matched controls. Conversely, the percentage of Leu 11^+ cells in the cancer population was slightly lower. These differences were not significant. Overall absolute numbers of LGLs, Leu 7+, and Leu 11+ cells were not significantly different. A total of 57 patients were as-

Table 2. A comparision of NK populations within the peripheral blood of patients with head and neck cancer and normal controls as determined by single-parameter flow cytometric analysis

	Patients (*n*)[a]	Controls (*n*)	*P* value[b]
Large granular lymphocytes			
Percentage[c]	15 ± 8 (84)	13 ± 5 (53)	0.12
Absolute[d]	32 ±26 (82)	30 ±18 (53)	0.55
Leu 7+ NK cells			
Percentage	16 ±11 (70)	13 ± 8 (43)	0.06
Absolute	36 ±30 (69)	31 ±27 (42)	0.40
Leu 11+ NK cells			
Percentage	15 ± 9 (64)	17 ± 7 (47)	0.23
Absolute	31 ±21 (62)	37 ±22 (47)	0.16
Ratio Leu 7+/Leu 11+[e]	1.5± 1.5 (57)	0.9± 0.7 (40)	0.02

[a] (*n*) = number of individuals
[b] *P* value determined using Student's *t*-test
[c] Mean percentage ± SD of the total PBL
[d] Absolute number of PBL X 10^6 ± SD isolated from 30 ml peripheral blood
[e] The ratio of Leu 7+ to Leu 11+ NK cells within the peripheral blood. Values are expressed as the mean ± SD for the designated population

Table 3. The correlation of NK cell subsets to NK cell activity in normal controls and patients with head and neck cancer

	Single-parameter analysis		Multiparameter analysis		
	Leu 7+	Leu 11+	Leu 7+11−	Leu 7+11+	Leu 7−11+
Patients LU[a]	0.077[b]	0.540[c]	−0.090	0.366	0.571[c]
Controls LU	−0.139	0.238	−0.120	0.016	0.275

[a] NK cell activity is expressed as the numbers of LU/1 × 10^7 PBL
[b] The *r* value in a Pearson's correlation analysis correlating the percent of the indicated NK cell subset within the PBL population to the respective individual's NK LU value
[c] $P < 0.01$

Table 4. The phenotypic characteristics of NK cell populations in patients at high risk for distant metastases[a] of head and neck cancer

	Patients		Controls	
	<60 LU[b] ($n = 38$)[c]	≥60 LU[b] ($n = 19$)	<60 LU ($n = 16$)	≥60 LU[b] ($n = 25$)
Percentage[d]				
Leu 7+	15±10	20±11	11± 7	14± 9
Leu 11+	12± 7[e]	20± 9	14± 7	17± 6
Numbers absolute[f]				
Leu 7+	35±28	39±30	22±22	35±31
Leu 11+	28±19	35±19	30±26	40±21

[a] Patients considered at high risk for distant metastases have LU values <60 (see Figs. 3 and 4)
[b] NK cell activity within the peripheral blood expressed in LU/1 × 10^7 PBL
[c] n = number of persons evaluated
[d] Percentage of total PBL as determined by single-parameter flow cytometric analysis and expressed as mean ± SD
[e] $P < 0.01$ in comparison with patients with ≥ 60 LU
[f] Absolute number of PBL × 10^6 ± SD isolated from 30 ml of venous blood

sessed for both Leu 7+ and Leu 11+ by single-parameter analysis, and the ratio of Leu 7+ to Leu 11+ cells was determined. The patients with head and neck cancer had a higher ratio of Leu 7^+ Leu 11^+ within the peripheral blood than healthy age-matched controls ($P = 0.02$).

A total of 45 patients underwent both single-parameter and two-parameter flow cytometric analysis, employing the Leu 7 and Leu 11 monoclonal antibodies. Correlations were calculated relating NK phenotypically defined subsets to LU function. As shown in Table 3, in single-parameter analysis only the percentage of Leu 11+ cells was related to LU function in these 45 patients ($P < 0.01$ by Pearson correlation coefficient analysis). Using multiparameter measurements of subsets, a relationship between LU function and NK phenotype was demonstrated only between cells that expressed the Leu 11^+ 7-phenotype. In the control population, no relationship between any NK cell population subset and LU function could be identified. Likewise no relationship between the percentage of cells expressing Leu 7+ versus cells expressing Leu 11+ phenotype could be identified (data not shown). Of note is that two-parameter analysis failed to provide any information additional to that expressed by single-parameter methods in this group of patients regarding LU function.

On the assumption that persons with head and neck cancer who have less than 60 LU have poor prognoses, we examined the NK population subsets comprising these particular patients' peripheral blood stratified by LU value (Table 4). In 57 patients both Leu 7+ and Leu 11+ NK phenotypes were determined. Patients with NK activity less than 60 LU had a significantly lower percentage of Leu 11+ NK cell subsets within the peripheral blood than did the remaining patients. Absolute numbers of Leu 11+ cells were not, however, different. Percentage of Leu 7+ populations in the less than 60 LU, although lower, were not significantly different. Patients at increased risk for distant metastasis and death due to progressive disease (i.e., those with NK cell activity below 60 LU) had diminished percentages of Leu 11+ NK subsets within the peripheral blood.

Longitudinal evaluation of patients in whom NK population subsets were quantitated was limited (median disease-free follow-up = 6 months). Thus, no conclusions regarding the prognostic significance of quantitated levels of Leu 11+ NK cell subsets within the peripheral blood can be stated.

Discussion

Natural killer cell function as determined in vitro against K562 target cells has a prognostic implication for the patient with squamous cell carcinoma of the upper aerodigestive tract. Those persons with diminished lytic capacity have a higher probability of dying of progressive disease despite attempts at curative therapy. Furthermore, NK LU function defines patterns of tumor recurrence. Those patients whose LU function is low develop metastatic disease. The findings from this study support the role of the NK cell system as a front-line defense against blood-borne tumor cells. The study extends observations first established in animal models by Hanna, Gorelik, and others, who demonstrated that selective abrogation of NK cell

populations allows survival of i.v. injected tumor cells and the subsequent generation of pulmonary metastases [3, 7, 8, 19].

Several studies in humans have documented the relationship between NK cell activity and distant disease [10, 32, 20, 29, 30]. Takasugi and colleagues in 1977 first revealed a correlation between natural immune status and progressively advanced cancer stage [30]. Levels of in vitro measured NK cytotoxicity were lower in patients with stage IV disease than in patients with earlier stages of cancer. No details were provided in that report as to whether all patients with stage IV patients had distant metastatic deposits at the time of assessment. Stage IV disease does not necessarily imply the presence of distant metastases. Steinhauer et al. demonstrated that persons with far advanced breast cancer had lower NK function than patients whose disease remained confined to local sites [29]. Their study examined persons with already established metastatic patterns, but could not determine whether NK function was a cause or a result of these patterns. The present study assessed only patients who were clinically free of distant disease prior to therapy. Our previous reports have detailed the relationship between tumor burden, disease staging, and NK cell LU function in patients with head and neck cancer [23, 26]. We have been unable to identify any relationship between stage, volume of disease at the primary site, or volume of nodal metastases when the variable of primary tumor burden was controlled. Thus, given that tumor burden, whether it be local, regional, or distant, is not the critical variable in defining NK function in the patient with head and neck cancer, and given the observation that NK function was diminished before distant metastases were clinically definable suggest that diminished NK cell activity seems to be a contributing factor to metastatic disease rather than a result. Additional evidence in humans that NK function has biologic relevance in controlling blood-borne disease requires prospective studies that selectively enhance NK function, either through adoptive transfer of NK populations or selective enhancement of lytic function through biologic response modification.

Factors that contribute to diminished NK cell function in the patient with head and neck cancer may be multifold. These patients are characterized by their high levels of tobacco use and alcohol consumption, and both factors are associated with altered natural immune response [6, 22]. A high level of circulating IgA is also characteristic of patients with head and neck cancers [12, 36], and elevated IgA titers have an adverse prognostic implication for them [12]. Furthermore, more recent studies have shown that circulating IgA in these cancer patients is in the polymeric form (pIgA) which can be coupled with the secretory component (SIgA) [36]. Since both pIgA and SIgA, distinct from monomeric IgA, diminish NK responsiveness [13], these patients' diminished NK activity may be related to the increased levels of circulating SIgA or pIgA. Patients with head and neck cancer are, likewise, characterized by having elevated levels of circulating immune complexes, acute-phase proteins, and prostaglandin E_2-secreting monocyte populations [2, 33, 38], each of which contributes to diminished NK cell function [11, 14, 17].

We initially addressed the issue of factors contributing to altered NK cell responsiveness within the head and neck cancer patient by examining the total NK cell population and those population subsets that express either the Leu 7+ or Leu 11+ phenotype. The basis of this approach is drawn from Lanier et al. who demonstrated the differential capacity of NK cell subsets to lyse allogeneic targets [16]. The greatest lytic potential existed within cells expressing Leu 11+ 7− antigens. We reasoned that deficient activity in these patients may relate to diminished numbers of specific NK cell subsets. Consistent with the observations of Steinhauer's group, we could identify no relationship between NK cell function and levels of LGLs. As a population persons with head and neck cancer, despite their diminished NK cell function, had LGL populations, both in absolute numbers and in percentages of PBL, equivalent to those of healthy controls. Most significantly, LU function in these patients correlated directly with circulating percentages of Leu 11+ NK cell subsets. Those patients determined to be at increased risk of distant metastases as defined by LU value expressed significantly lower levels of Leu 11+ NK cells than the remaining population. No relationship between NK function and Leu 7+ NK cells could be identified. Whether the quantitation of Leu 11+ NK cells has independent prognostic significance regarding distant metastases cannot be stated at this time. Longitudinal evaluation of this group of patients is limited.

Further investigations examining the relevance of natural immunity in patients with head and neck cancer will require several approaches. The population reported here could be described as a "training" population [35]. Given the definitions provided by this study, i.e., that patients with LU values less than 60 are at significantly increased risk of progressive disease despite therapy, we plan to examine a "test" population to determine if these definitions remain valid. Multivariate analysis employing commonly accepted clinical factors that indicate risk of distant metastasis, such as the extent of the primary, site of the primary, the extent of regional nodal disease, and NK function will be performed on larger populations. We will determine whether NK cell function has independent prognostic significance. Investigations will attempt to establish the relationship of NK activity to cell-mediated lysis of autologous or allogeneic squamous cell cancer. Results will indicate whether or not NK function as measured in vitro is merely reflective of potentially more relevant underlying host immunologic factors. At present we can, however, reach the following conclusions: (1) based upon our previous observations [23, 26], NK cell function assessed prior to treatment is not reflected in current staging systems; and (2) the results of the present study demonstrate that NK activity as measured against the K562 target cell has prognostic implications regarding the probability of subsequent metastatic disease and death despite definitive treatment. The end results of our investigation suggest that understanding the role of natural immunity in the patient with head and neck cancer will have significant clinical implications.

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