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Natural Cell-Mediated Cytotoxicity in Cutaneous T-Cell Lymphomas

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Natural cell-mediated cytotoxicity was studied in 24 patients with cutaneous T-cell lymphomas and in 18 age- and sex-matched controls studied concomitantly. Percent cytotoxicity was determined by 4-h ⁵¹Cr release assay using K562 targets at effector to target ratios of 100:1, 50:1, and 25:1. Mean percent cytotoxicity was significantly lower in patients than in controls at an

effector to target cell ratio of 100:1. Likewise, decreased cytotoxicity was found at effector to target ratios of 50:1 and 25:1, although this difference was not significant. When natural killer activity was analyzed separately for males and females, cytotoxicity was lower in both, although the decrease was significant only for male patients. Impairment of natural killer activity did not correlate with blood zinc levels, but appeared to correlate with stage of disease.

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Abbreviations:

ADCC: antibody-dependent cellular cytotoxicity
CTCL: cutaneous T-cell lymphoma(s)
MEM: minimum essential medium
MEM-FCS: MEM with fetal calf serum
NMC: natural cell-mediated cytotoxicity
NK: natural killer
TNM: tumor size, node, metastasis

Immune disturbances have been associated with cutaneous T-cell lymphomas (CTCL). Several studies have reported alterations in cellular [1-5] and humoral [6-8] immunity, although some of the data are inconclusive. Recently, cells mediating antibody-dependent cellular cytotoxicity (ADCC) and natural killer (NK) cells have been gaining attention because of their role in natural host defense mechanisms. Investigation of ADCC in patients with CTCL showed that ADCC was decreased in Sézary syndrome, but normal in mycosis fungoides, suggesting impairment may correlate with severity of disease [9]. In the present study we determined NK activity in patients

with various stages of CTCL and compared this to age- and sex-matched controls tested concomitantly. Since zinc deficiency has been found in various malignancies [10], and may result in reduced NK activity [11], we also analyzed blood zinc and copper levels at the time of study.

MATERIALS AND METHODS

Patients and Controls

Twenty-four patients (16 males and eight females) with histologically confirmed CTCL were studied. Patients ranged in age from 30 to 78 years (mean, 59.9) and duration of disease from time of diagnosis ranged from 1 week to 13 years (mean, 3 years). Eleven patients had received no therapy for at least 1 month prior to study, 3 of whom had never been treated; 10 were receiving local therapy (topical mechlorethamine hydrochloride, ultraviolet A radiation, or electron beam therapy) and 3 were on both local therapy and systemic chemotherapy. Using the TNM (tumor size, node, metastasis) classification, 3 patients had Stage Ia disease and 3 had Ib; 7 patients had Stage IIa and 3 had IIb; 6 patients had Stage IVa and 1 had IVb. Control subjects (12 males and 6 females) consisted of 18 normal volunteers aged 39-88 (mean, 58.3). After obtaining informed consent, all blood samples were drawn between 9:00-10:00 AM.

Total Lymphocyte Count

This was derived from the total leukocyte count and differential white blood cell count on Wright's stained peripheral blood smears.

Isolation of Peripheral Blood Lymphocytes

Heparinized venous blood was mixed with phosphate-buffered saline and layered on a Ficoll-Hypaque gradient. After 30 min of centrifugation at 400 g, interface cells were collected, washed with Eagle's minimum essential medium (MEM), and resuspended in MEM supplemented with 10% fetal calf serum, 200 mM glutamine, 100 μ U/ml penicillin, and 100 μ g/ml streptomycin (MEM-FCS). In the resultant preparations, cell viability was greater than 95% by trypan blue dye exclusion.

Natural Cell-Mediated Cytotoxicity (NMC)

Target cells were K562 cells, standard targets for NK activity, which were kindly supplied by Dr. Paul L. Black (Department of Microbiology and Immunology, Temple University Health Sciences Center, Philadelphia, Pennsylvania). These cells were carried in vitro in MEM supplemented with 5% fetal calf serum and 200 mM glutamine. K562 cells (5×10^6) were labeled by incubation in 0.05 mCi sodium ^{51}Cr -chromate (1 mCi/ml) for 1 h at 37°C, washed 3 times in MEM-FCS, and resuspended at 10^6 cells/ml.

Peripheral blood lymphocytes (100 μ l) were added to 100 μ l of target cells in 96-well V-bottom microtiter plates (Linbro Chemicals, New Haven, Connecticut) at varying concentrations to give effector to target cell ratios of 100:1, 50:1, and 25:1. All samples were run in triplicate. The cells were centrifuged at 40 g for 2 min, incubated at 37°C in a humidified atmosphere containing 5% CO_2 -95% air for 4 h, then centrifuged at 200 g for 10 min. Supernatant (100 μ l) from each well was aspirated, and experimental ^{51}Cr release determined in a Searle gamma scintillation counter. Spontaneous release was assessed by incubating ^{51}Cr -labeled K562 cells in media without effector cells, and in all experiments was below 10%. Total ^{51}Cr incorporated was determined in 100 μ l of ^{51}Cr -labeled K562 cells and divided by 2. Percent cytotoxicity was calculated as follows:

$$\frac{\text{Mean experimental release} - \text{mean spontaneous release}}{\text{Mean total incorporated} - \text{mean spontaneous release}} \times 100$$

Blood Zinc and Copper Levels

Blood metal levels were analyzed by anodic stripping voltammetry (ESA 2011/PMI1014S voltammeter). Refrigerated 100- μ l blood samples from capillary containers were placed in trace metal-free analysis cells, combined with 2 rinses of 50- μ l aliquots of distilled deionized water and digested to dry white residue at 200°C in 300 μ l of acid reagent (24 parts HNO_3 :24 parts HClO_4 :1 part H_2SO_4). The acid-digested samples were then reconstituted in trace metal-free buffer (1.0 formula weight sodium acetate-0.2 formula weight sodium chloride) and electroplated at -1170/mv for 25 min while nitrogen bubbled through the solutions. The electrodes were subsequently stripped of zinc and copper while

recording amperometric peak heights. Metal contents were calculated from peak height values obtained from concurrently run standards with corresponding concentrations of elements expressed as $\mu\text{g}/100$ ml.

RESULTS

Total lymphocyte count was significantly lower in CTCL ($1393 \pm 101/\text{mm}^3$) (SEM) than in controls ($2010 \pm 164/\text{mm}^3$) ($p < .01$). Mean percent NCMC in CTCL ($47.6 \pm 3.9\%$) was also significantly less than controls ($59.5 \pm 3.4\%$) at an effector to target cell ratio of 100:1 ($p < .05$) (Fig 1). Likewise, decreased NK activity was present at effector to target cell ratios of 50:1 ($42.1 \pm 3.9\%$ vs $53.2 \pm 3.7\%$) and 25:1 ($30.8 \pm 3.8\%$ vs $38.8 \pm 3.5\%$), although this difference was not significant (Fig 1).

Since NK activity has been reported to be higher in males than females [12], we analyzed our results for each sex separately. Percent cytotoxicity was $46.5 \pm 5.1\%$ in males with CTCL compared to $62.3 \pm 4.1\%$ in male controls at an effector to target cell ratio of 100:1 ($p < .05$) (Fig 2). Similarly, decreased NCMC was present in males at effector to target cell ratios of 50:1 ($40.6 \pm 5.1\%$ vs $56.9 \pm 3.9\%$) ($p < .05$) and 25:1 ($29.2 \pm 4.9\%$ vs $41.7 \pm 3.6\%$) ($p < .10$), respectively. The 6 male patients with values below the mean consisted of 4 patients with intracutaneous and 2 patients with extracutaneous disease. Four patients were on no systemic chemotherapy at the time of study, while 2 had received their last dose of single agent chemotherapy 3 weeks and 1 week prior to study, respectively. Absolute lymphocyte count was below control mean in 5 of the 6 patients, and at the mean in the 1 patient whose NCMC was closest to the mean. However, NK activity in females with CTCL ($48.5 \pm 6.2\%$), was only slightly lower than female controls ($53.8 \pm 5.8\%$) at an effector to target cell ratio of 100:1 (Fig 2). This was apparent also at effector to target cell ratios of 50:1 ($43.3 \pm 6.1\%$ vs $45.8 \pm 7.5\%$) and 25:1 ($31.7 \pm 5.9\%$ vs $33.1 \pm 7.7\%$), respectively.

Since a correlation between severity of CTCL and immunologic abnormalities has been reported [3-5,9], NK activity was also analyzed according to stage of CTCL. NK activity in 7 patients with extracutaneous disease ($39.9 \pm 9.1\%$) was lower than in 17 patients with only intracutaneous involvement ($50.2 \pm 4.1\%$) at an effector to target cell ratio of 100:1, but this difference was not significant. Likewise, using either the TNM

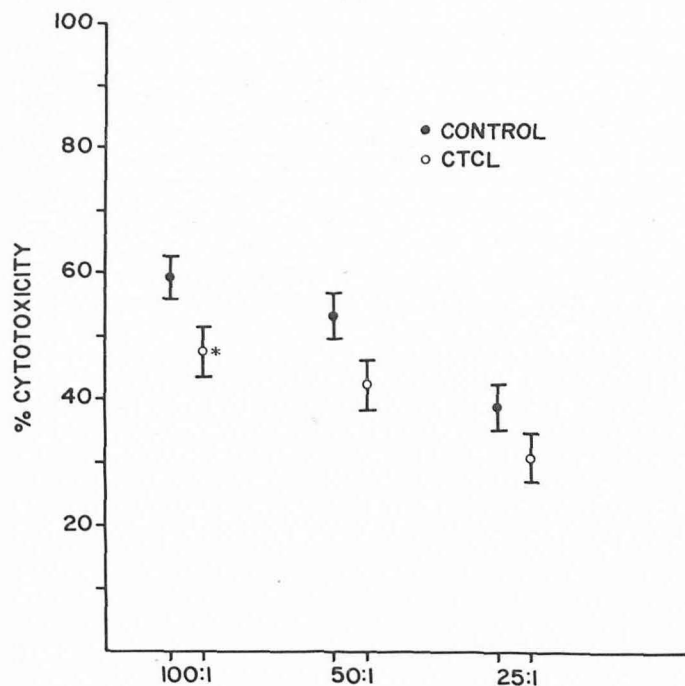


FIG 1. Natural cell-mediated cytotoxicity in CTCL and in controls. Values plotted are mean \pm SE. Effector to target ratios are shown on the abscissa. * = $p < .05$.

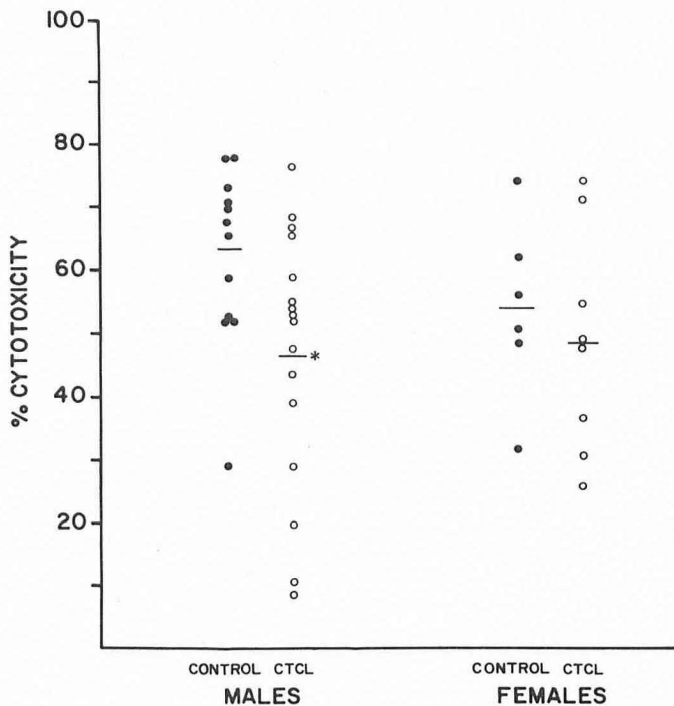


FIG 2. Natural cell-mediated cytotoxicity in CTCL (○) and in controls (●) shown separately for males and females. NCMC was determined by 4 h ^{51}Cr release assay using K562 targets at effector to target ratio of 100:1. Individual values and mean of each group are shown. * = $p < .05$.

staging system for CTCL or classification into premycotic, plaque, tumor, or erythroderma stages, NK activity was always lower in advanced stages of CTCL, although again this decrease was not significant.

No significant difference was observed in blood zinc levels in CTCL ($51.4 \pm 9.7 \mu\text{g}/100 \text{ ml}$) and in controls ($58.5 \pm 10.5 \mu\text{g}/100 \text{ ml}$), or in blood Cu levels in CTCL ($90.7 \pm 3.3 \mu\text{g}/100 \text{ ml}$) and in controls ($91.4 \pm 3.5 \mu\text{g}/100 \text{ ml}$). Furthermore, there was no significant correlation between zinc levels and NCMC in CTCL ($r = -.2$).

DISCUSSION

The results of our study show reduced NK activity in patients with CTCL. This is not explained by the decrease in total lymphocytes in CTCL, since the same ratio of lymphocytes to target cells is used in the NCMC assay. In addition, the impairment in NK activity was not related to blood zinc levels. An attractive hypothesis might be that increased levels of thymic factor found in CTCL [13] depress NK activity, since *in vitro* treatment of lymphocytes with human thymosin lowered NK activity [12]. Alternatively, the decrease in NK activity might be related to treatment of CTCL. Radiation, cyclophosphamide, and steroids have all been associated with reduced NK activity [12]. Although NCMC in 13 patients who were receiving either local or systemic therapy at the time of study was lower than in 11 patients who had received no treatment for at least 1 month prior to study, the difference was not significant. However, whether impairment of NK activity is secondary to decreased numbers of NK cells or reduced function was not assessed in this study. It is also possible that antibodies against

NK cells may be present in the sera of patients with CTCL, similar to systemic lupus erythematosus [14].

When NK activity was analyzed separately for males and females, the reduction in NCMC in male patients compared to male controls was significant, whereas for females the decrease in NCMC was not significant. It is possible that this apparent difference is related to the smaller number of females studied. However, it could be postulated that CTCL occurs more commonly in males than in females because of impairment of NK activity.

The depression of NK activity seen in CTCL appears to be greater in more advanced stages of CTCL. Similarly, defective cell-mediated immunity [3-5] and ADCC [9] were found to correlate with stage of disease.

Our findings are consistent with a recent letter to the editor, reporting decreased NK activity in 10 patients with CTCL [15]. In addition, NK activity was found to be significantly decreased in 59 untreated patients with malignant lymphoma ($27.5\% \pm 19.1\%$) when compared to healthy controls ($43.7 \pm 17.3\%$) and patients with nonlymphoid tumors ($46.3 \pm 16.9\%$) [16]. Although the physiologic significance of this impairment is uncertain, it is possible that decreased NK activity may contribute to the development of lymphomas, including CTCL. Further studies of the mechanisms responsible for this alteration of NK activity in CTCL are indicated.

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