Significant Differences in Natural Killer Cell Activity in Children According to Age and Sex

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Summary: Peripheral blood mononuclear cells from 45 children, aged 1 to 19 years were evaluated for natural killer cell cytotoxic activity against K-562 erythroleukemic cells. The results of a ^{51}Cr release assay demonstrated that the NK cell activity of normal children is dependent on age and sex. Younger females, aged 1 to 9 years, were more reactive than elder females aged between 10 and 19 years ($P < 0.001$). Males aged 1 to 19 years were less reactive than adults ($0.02 < P < 0.05$). Among children, the levels of NK cell activity were significantly greater in females than in males ($0.001 < P < 0.01$). In contrast, among elder children, the levels of NK cell activity were significantly greater in males than in females ($0.01 < P < 0.02$). The NK cell activity of elder female children was 60 % and 59 % that of the elder male children, at the two E:T ratios of 50:1 and 100:1, respectively.

Key words: natural killer cell—cytotoxicity—peripheral blood mononuclear cell—age-related difference—sex-related difference

Introduction

The existence of natural killer cell cytotoxicity as a phenomenon was initially disputed, as an artifact of the assay but was finally confirmed as a distinct reproducible form of cytotoxicity mediated by a definable subclass of effector cells. Natural killer cells have the unique ability to recognize, without prior sensitization, nonantibody-coated targets and to spontaneously lyse these allogeneic, syngeneic, and xenogeneic tumor cells (Herberman et al. 1979). The capability to nonspecifically detect and eliminate foreign cells upon in-

itial exposure has led to the proposal that natural killer function is the primary effector of the immunosurveillance (Herberman et al. 1979; Lotzova and McCredie, 1978; Pross and Baines, 1977).

There is increasing evidence that natural killer cells may play an important role in resistance against tumor cell growth and also in bone marrow transplants (Goleik et al. 1982; Warner and Dennert, 1982). In mice and rats, the levels of natural killer cell activities follow a characteristic age-related pattern. The cells possessing natural killing activity first appear at 3-4 weeks of age, reach a peak level between

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The abbreviations used are: NK, natural killer; RPMI, Roswell Park Memorial Institute; FCS, fetal calf serum; MEM, minimum essential medium; ALL, acute lymphoblastic leukemia.

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5-10 weeks of age, and then decline to low levels thereafter. There are also considerable differences in levels of activity among various strains of animals (Stutman, 1980). Reports of levels of human natural killer cell activity according to age and sex are controversial (Forbes et al. 1980; Herberman et al. 1979; Nagel et al. 1981; Penschow and Mackay, 1980) and studies dealing with natural killer cell cytotoxicity in children under 10 years of age are not well documented.

This paper therefore describes the natural killer cell activity of peripheral blood mononuclear cells in normal children and the results are compared with that of normal adult group.

Materials and Methods

Study Population

The Population studied was composed of 45 children (23 females and 22 males) and 10 adults. This group included normal children, children with congenital heart disease (CHD), and healthy adults. The types of CHD were atrial septal defect (ASD), ventricular septal defect (VSD), and patent ductus arteriosus (PDA). Children and adults were screened to exclude those with a history of viral infection less than three weeks prior to testing and those with a personal history of neoplastic or other serious disorders.

Effector Cells

The children with CHD were given cephalixin (50 mg/kg p. o.) one day before cardiac catheterization and hydroxyzine hydrochloride (2 mg/kg i. m.), pethidine hydrochloride (1 mg/kg s. c.) and ketamine hydrochloride (5 mg/kg i. m.) prior to the tests. In children with CHD, blood samples were drawn at the time of pre- or post-operative cardiac catheterization. Peripheral blood mononuclear cells were isolated on a Ficoll-Conray Gradient and washed three

times in phosphate buffered saline (PBS). To remove adherent mononuclear cells, the cell suspension was incubated in plastic tissue culture dishes No. 3002 (Falcon Plastics Co., U.S.A.) for one hour at 37°C in MEM with 10 % heat-inactivated fetal calf serum. Non-adherent cells were washed twice in MEM-10 % FCS and adjusted to final concentrations of 1, 5 or 10×10^6 cells/ml.

Target Cells

K-562 cell line was generously supplied by Dr. Hiromitsu Okano, Kyushu Cancer Center.

The cell line was maintained as a stationary suspension culture in RPMI-1640 medium supplemented with 10 % FCS, 0.1 g kanamycin and 2.0 g sodium bicarbonate. K-562 target cells were washed once in culture medium and the supernatant was aspirated. Tris-phosphate buffer, 0.2 ml of 5 % FCS (pH 7.4) was added to the pellet followed by addition of 0.1 ml $\text{Na}_2^{51}\text{CrO}_4$ solution (1 mCi/ml, specific activity 200 mCi/mg) per 2×10^6 target cells and incubated at 37°C for 45 min. The labelled cells were then washed three times in MEM-10 % FCS with absolute minimum pipetting and finally adjusted to 1×10^5 cells/ml.

Cytotoxicity assay

Radio-labelled target cells (1×10^4) were dispensed into test tubes (Sumitomo Bakelite Co., JAPAN). Three different sets of effector with target cell ratios of 100:1, 50:1 and 10:1 were used and each ratio was run in triplicate. Following the addition of the target cells, the tubes were centrifuged for 3 min at $200 \times g$ and then incubated at 37°C in a humidified atmosphere containing 5 % CO_2 and 95 % air. After 3-hours' incubation, the tubes were centrifuged for 5 min at $600 \times g$. Supernatants were removed, and placed in individual polyethylene culture tubes (12 $\times$ 75 mm) and the radioactivity determined in a

gamma counter (Aloca. Auto Well Gamma System ARC 505 R. SERIAL No. RW-10089).

The percentage of specific ^{51}Cr release was calculated as % of ^{51}Cr release =

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100$$

The above formula indicates that experimental release was determined with the presence of effector cells.

Spontaneous release was determined by incubation of target cells in medium without effector cells resulting in less than 20 % of the maximal release. The maximal release was determined by measuring counts per min (cpm) in the supernatant after detergent lysis of the target cells with 1 % Triton $\times 100$.

Results

Effect of Age and Sex on NK Cell Activity

The NK cell activity of peripheral blood lymphocytes from 45 children against K-562 target cells is shown in Fig. 1. The degree of cytotoxicity of the mononuclear cells obtained from young females, aged 1 to 9 years, was much higher than those of the teenage group. The results were found to be statistically significant ($P < 0.001$). In males, there was no significant difference between the two groups. In children, however, the level of NK cell activity was significantly higher in females than in males ($0.001 < P < 0.01$). In contrast, among teenagers, the level of NK cell activity was significantly higher in males than in females ($0.01 < P < 0.02$). The NK cell activity of teenage females was 60 % and 59 % of that of males, at the two E:T ratios of 50:1 and 100:1 respectively. Males, aged 1 to 19 years, were less reactive than adults ($0.02 < P < 0.05$).

Fig. 1. Geometric mean percentage of ^{51}Cr release at a 50:1 effector:target ratio by age group and sex. Radiolabelled K-562 target cells were incubated 3 hr as described in Materials and Methods. $\square$, Percentage of NK cell activity of males; hatched , Percentage of NK cell activity of females; bars, S.E. The NK cell activity of normal individuals is age and sex-dependent. Number of individuals in each age group is represented by n.

NK Cell Activity of Children with and without premedication

There was no significant difference in NK cell activity between the groups of children with and without premedication, as determined by Student's t-test (Fig. 2).

Longitudinal Follow-up of NK Cell Activity

Fig. 3. displays the results of two separate measurements of NK cell activity on the same individuals taken 2 to 9 (mean, 5.6) months apart. Although the sample size was small ($n=12$), considerable variation among individuals in levels of NK cell activity was found and no statistical difference by paired t-test analysis was noticed between the first and second determinations of NK cell activity.

Fig. 2. NK cell activity of children under 10 years of age with and without premedication.

□, NK cell activity of boys receiving premedication; ▨, NK cell activity of boys without it; ▤, NK cell activity of girls receiving premedication; ■, NK cell activity of girls without it; bars, S.E. By t-test, there were no significant differences in NK cell activity between the group of children with premedication and the group of children without it. Number of individuals in each group is represented by n.

Discussion

In the present study, the effect of age and sex on levels of cytotoxic NK cell activity in peripheral blood mononuclear cells was studied. The results obtained indicate that the degree of NK cell activity against the K-562 cell line is age and sex dependent. The NK cell cytotoxicity of female children was high, and then declined to lower values following an increase in age, while in males, the level of NK cell activity reached the highest levels at over 20 years of age. Herberman et al. (1979) have reported that NK cell activity was

Fig. 3. Percentage of ^{51}Cr release at a 50:1 effector: target ratio of cells. The same individual was tested on 2 separate occasions. Mean interval between the tests was 5.6 months (range, 2 to 9 months). By paired t-test, there were no significant differences in NK cell activity in this time interval.

relatively stable among various age groups and Nagel et al. (1981) also reported a lack of significant age-related difference in NK cell activity against K-562 cells. Penshow and Mackay (1980), utilizing the Chang cell-line as target, reported that NK cell activity was greater in youth, ages 20 to 39, and Forbes et al. (1980) using K-562 cell line as target, reported greater NK cell activity in young males, aged 11 to 20 years, than in males of 21 to 50 years of age. However, most published data have dealt with either adults or children over 10 years of age, although the results of previous reports vary.

The NK cell cytotoxicity of the females, ages 20 to 39, was 39% and 43% that of the males with in the same age group at E:T ratios of 50:1 and 100:1, respectively, and this difference was found to be statistically significant ($0.02 < P < 0.05$). The NK cell activity of teenage females was

60 % and 59 % that of teenage males, at E:T ratios of 50:1 and 100:1, respectively. In contrast, the NK cell activity of male children was 48 % and 62 % that of the female children, at E:T ratios of 50:1 and 100:1, respectively.

The results suggest that sex hormones may have some influence on the effector cell activity in normal individuals. Estrogen is also one of the cAMP elevating agents and the hormone may act as a suppressive agent of NK cell activity (Roder and Klein, 1979). The estrogen level increases after approximately 11 years of age in females, but in males there is no increase during puberty (Sizonenko, 1978). Our present data seem to be compatible with those physiological conditions based on sex. Santoli et al. (1976) reported that lymphocytes from male donors were about twice as effective as those from female donors in NK cell activity. Forbes et al. (1980) reported that among an older age group, males were slightly more reactive than females, but there were no statistically significant difference in cytotoxicity associated with sex. Nagel et al. (1981) reported the absence of a statistically significant sex-related difference in NK cell activity against the K-562 cell line.

Many reports dealing with human NK cell activity according to age and sex have been reported, however, the results have varied significantly and the level of human NK cell activity among normal subjects within the same age group has been variable. In addition, the laboratory procedures for assessment of NK and K cell activities were far from standardised and methodological variability including the target cell and E:T ratio used could invalidate comparisons of published data. Failure to dissociate effects attributable to NK and K cell cytotoxicity, the influence of sex and age and other factors affecting NK cell activity must also be considered. In addition the activity has been reported to be influenced by HLA haplotype, viral and

other infections (Hauser et al. 1982; Herberman and Holden, 1978; Petranyi et al. 1974). Interferon is known to mediate the immune modulation of NK cell cytotoxic activity.

NK cells may be augmented by interferon via recruitment of pre-NK cells (Saksela et al. 1979) or interferon may act mainly to accelerate the rate of lysis by activated NK cells.

In immunosurveillance, NK cells may play an important role in resistance against tumor growth in the early stages. Macrophages are also known to elicit spontaneous cytotoxic activity against tumor cells (Herberman et al. 1979). T-cell immunity may be a relatively late event and play a more important role in further resistance to progressive tumor growth (Herberman et al. 1979).

In recent years, several investigators reported NK cell activity in a small number of children with acute lymphoblastic leukemia and the results were compared with a normal control group (McGeorge et al. 1982; Oldham et al. 1976; Tanaka, 1981). In acute leukemia, approximately 40 % of the cases occurred in children between 3 and 5 years of age and Cook's series revealed a predominance of males over females in all except the first year of the life (Cooke, 1942). In our study, female children were found to have greater NK cell cytotoxicity than male children. This difference in NK cell activity of children under 10 years of age may have some influence on the incidence of acute leukemia. In experimental studies, the incidence of radiation-induced thymic leukemia in C57BL/6 mice was dramatically reduced by intravenous administration of syngeneic cloned NK cells (Warner and Dennert, 1982) and neuroblastoma spontaneously regressed in 5-10 % of cases. In routine autopsies of infants under 3 months of age, neuroblastoma in situ in the adrenal is seen 40 times more frequently than expected, suggesting a high rate of spon-

tanous regression or differentiation (Beckwith and Perrin, 1963).

It would be of interest to examine the levels of NK cell activity of normal children under 10 years of age and in children with acute leukemia or neuroblastoma.

To clarify NK cell activity according to age and sex, more extensive and long-term observations are needed.

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