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Acute Leukemia and Nonspecific Killer Cells

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A. Introduction

This paper is to give a contribution to two aspects of the study of leukemia: functional characterization of human leukemia cells and cellular mechanisms leading to the killing of leukemia cells. Considering that some populations of nonmatured lymphocytic and myelolytic cells are natural killer (NK) cells or effectors of the antibody-dependent cellular cytotoxicity (K cells), we have been looking for these effector cells in patients suffering from acute lymphoblastic leukemia (ALL) or acute nonlymphoblastic leukemia (ANLL). In cases with high leukemic blast counts the possibility of detection of leukemic NK or K cells may exist. Since animal experiments indicate a bone marrow origin of non-specific killer cells, we directly compared effector cell activities of mononuclear blood leukocytes (PBL) and bone marrow (BM) cells.

With regard to cellular cytotoxicity directed towards leukemia cells we previously described an antibody-dependent cellular cytotoxicity against ALL cells coated with a xenogeneic ALL antiserum (Milleck et al. 1978). In this report we want to provide some data on NK activity against human leukemia cells.

B. Patients and Methods

The ALL and half of the ANLL patients were children aged 0.5–15 years. Control groups consisted of hematologically healthy children and adults. Mononuclear PBL and BM cells were prepared by Ficoll-Visotrast centrifugation, and killer cell activities were estimated by the ^{51}chromium-release technique. Five thousand target cells in 0.1 microliter were incubated with an excess of 50 times the effector cells at 37°C for 4 h. Targets were cells of the K-562 cell line and ALL (REH) cell line (kindly provided by Dr. M. F. Greaves) for NK and mouse leukemia cells coated with antibodies for K estimation.

C. Results and Discussion

As shown in Fig. 1a, most of the ALL and ANLL patients display a very low or no NK or K cell activity, which confirms the findings of Schmidt et al. (1976). There were some patients with quite normal NK or K cell activity. This is not unexpected with patients having low leukemic blast counts in their blood. At a high ratio of blasts [percentage of blasts in PBL/BM: 90/90 (patient I.W.); 88/99 (patient T.O.); 94/n.t. (patient B.G.)] the NK or K cell activity of PBL or BM cells indicates the existence of leukemia cells with killer cell activity. Patient T.O. had a higher NK activity than K activity (Fig. 2); of patients I.W. and B.G. we tested only their K cell activity. A quite normal killer cell activity of leukemia patients was found after blood transfusion (Schmidt et al. 1978). While one patient (I.W.) was given a transfusion, the two others were not.

Some untreated leukemia patients showed a low level of NK or K cell activity in spite of the low peripheral blast counts. Since repression effect not is involved, we have to look for another explanation. It is conceivable that killer cells which have been used up in the blood or died away cannot be replaced because of the lack of supply from the leukemic bone marrow. Furthermore, it is possible that the killer cell activity of the patients involved was low for genetic reasons. This was not true for at

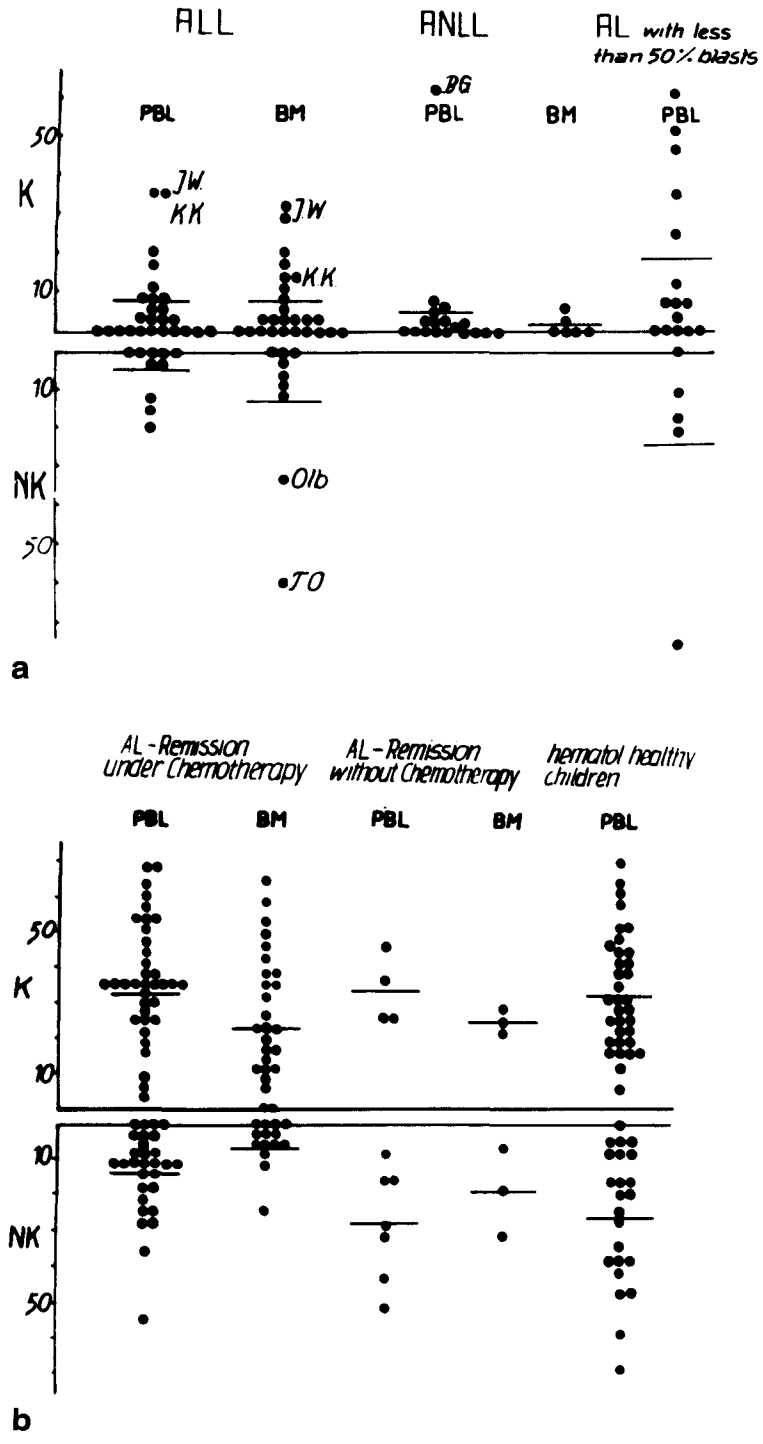

Fig. 1. NK and K cell activities (% specific ^{51}Cr-release) of mononuclear blood leukocytes, *(PBL)* or bone marrow *(BM)* cells. **a** acute non treated leukemia patients; **b** patients in a remission phase and hematologically healthy children. *ALL*, acute lymphoblastic leukemia; *ANLL*, acute nonlymphoblastic leukemia; *AL*, acute leukemia

least two patients, because their killer cell activities increased in a later remission phase.

With remission patients (Figs. 1b and 2) the K cell activity was somewhat lower compared with normal donors. Greater differences appeared in NK cell activity. The patients undergoing chemotherapy had a lower activity than remission patients without therapy or control donors. This finding suggests that chemotherapy has a stronger effect on NK cell activity than on K cell activity.

When comparing the PBL and bone marrow cells of individual hematologically healthy donors, in part coinciding killer cell activities were found, the coincidence being better on the K cell level than on the NK cell level. That PBL and BM cells stem from different sources is revealed by the proportions of E rosette forming cells: PBL: 52%±9% (64 donors) and BM cells: 19%±8% (six donors). With leukemia patients there occurred greater differences in the killer cell activity between PBL and BM cells. Differences were also observable when comparing the NK and K cell activities with each other, which indicates selective inhibition of NK or K cell receptors. But it could also be a hint to the presence of different NK and K cell populations.

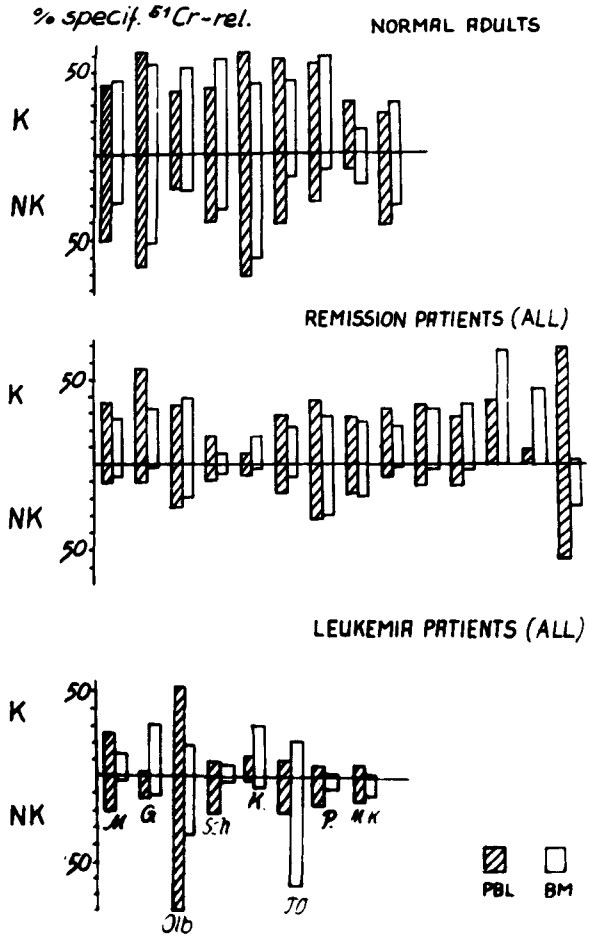

Fig. 2. Comparison of the killer cell activities between mononuclear blood leukocytes *(PBL)* and bone marrow *(BM)* cells of single acute lymphoblastic leukemia *(ALL)* patients and hematologically healthy adults

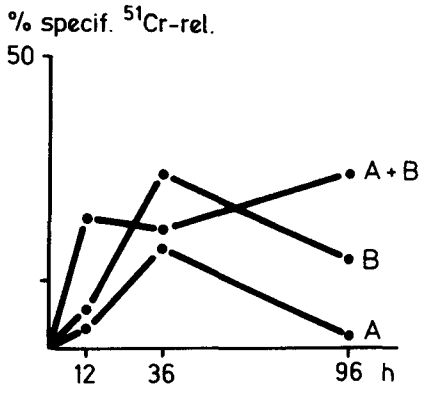

Fig. 3. Induction of NK cell activity against acute lymphoblastic leukemia *(ALL)* (REH) target cells in a mixed culture of PBL of the allogeneic donors *A* and *B*

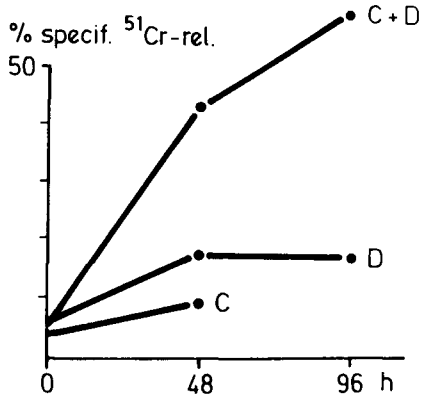

Fig. 4. Induction of NK cell activity in a mixed culture of lymph node cells from the donors *C* and *D* (intestinal lymph node cells from tumor patients)

D. In Vitro Induction of NK Cells Against Leukemia Cells

Unlike the K cell activity against leukemia cells (Milleck et al. 1978) the NK activity is mostly low. However, immunological stimulation may give rise to NK cells destroying leukemia target cells that have not been attacked before the stimulation (Zarling et al. 1979). Figure 3 shows the induction of NK cells against the ALL (REH) cell line in a mixed culture of allogeneic PBL. Such NK cells already occurred on cultivation of the cells from the individual donors, but here the activity abated more rapidly than in the mixed culture. It is interesting to note that the NK cell induction had already occurred within a short span of time. It may be that the decrease in leukemic blasts in the peripheral blood which is sometimes observable one day after blood transfusion is attributable to similar NK cell effects. NK cells have so far been found mainly in blood, bone marrow, and spleen. Figure 4 shows that NK cells may form also in a mixed culture of lymph node cells. This shows that lymph nodes, which have so far been considered as largely inactive, harbor potential NK cells. No K cells appeared in the cocultivation of lymph node cells. This is further evidence that NK and K cells could be different cell populations.

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