GENERATION OF NATURAL KILLER CELLS:
AN AUTONOMOUS FUNCTION OF THE BONE MARROW*

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Natural immunity against tumors (1), foreign cell grafts (2), and infectious agents (3) is frequently independent of T lymphocytes and requires cell types yet poorly defined. In the mouse, in vivo resistance against transfer of Moloney lymphoma would seem closely associated with the existence of naturally occurring non-T killer (NK) cells in the spleen (1). Mouse strains can be classified as "high" (CBA, C57BL/6, C57L, and B10.A) or "low" reactive (A and A congenics) with regard to their in vitro NK activity (reference 4 and present results). It has been suggested that NK activity might be directed against target cell structures associated with expression of endogenous C-type viruses (5). According to this hypothesis, NK cells would be produced as a result of natural sensitization to antigens associated with ubiquitous, possibly oncogenic viruses. Hence, it would be important to know whether the differences in NK activity observed between inbred mouse strains are determined at the level of the precursor cells in the bone marrow or by other factors of the host environment. To analyze this, we took advantage of the following in vivo transfer system: Pairs of mouse strains "identical" at their \(H-2\) regions but classified as either high or low reactive with regard to NK activity were selected. These mice were used as donors or as irradiated recipients for crisscross transfers of bone marrow cells. The ability of the transferred marrow precursor cells to give rise to high or low NK activity in the spleens of the reconstituted recipients was then investigated. In addition, similar experiments involved transfer of bone marrow cells from young or old mice of the same strain into irradiated recipients of varying ages. The data obtained suggest that the generation of high or low NK cell activity is predetermined at the marrow precursor cell level and does not depend on environmental influences of the host.

Materials and Methods

**Mice.** CBA/H, C57L, and A.BY mice were raised locally. A/J and B10.A mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, by courtesy of Dr. J. Lindenmann, Institute for Medical Microbiology, University of Zürich, Zürich, Switzerland.

**Tumor.** YAC-1, derived from a Moloney virus-induced lymphoma in A/Sn mice (6) was grown in RPMI 1640 medium with 10% fetal calf serum (FCS).

**Production of Chimeras.** 8- to 10-wk old animals were lethally irradiated (800 R) and reconstituted by intravenous inoculation of \(3 \times 10^7\) viable bone marrow or fetal liver cells from sex-matched donors. Polymyxin B (125 mg/liter) was given orally for 10 days. The animals were allowed to recover for 6 wk. Mortality was very low and 80-90% of the chimeras could be used. For

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simplicity, chimeras prepared by transfer of cells from donor A into irradiated B recipients will be referred to as A → B mice.

Cytotoxic Assay. NK activity was determined in a 51Cr-release assay (6) using the YAC-1 in vitro line as target cells. Spleen cell suspensions in RPMI 1640 medium containing 10% FCS, HEPES buffer (pH 7.3), and penicillin-streptomycin were mixed in appropriate numbers with 4 × 10^4 51Cr-labeled target cells. Triplicates were incubated at 37°C in 5% CO₂ for 8–12 h using controls for spontaneous and maximal isotope release. 51Cr release and percent lysis were calculated according to the formula previously described (6). Spontaneous isotope release varied between 11 and 23% of total label under these conditions. Sex of effector cell donors was found unimportant for NK activity and is not recorded.

Results

Transfer of High or Low NK Precursor Bone Marrow Into Irradiated High or Low H-2 Compatible Recipients. Bone marrow exhibits very little NK activity but does contain precursor cells for the NK cells appearing in the spleen. First, radiation chimeras were produced by bone marrow exchange between the two H-2b compatible strains C57L (high) and A.BY (low). Controls consisted of irradiated mice reconstituted with syngeneic marrow or untreated normal animals. Fig. 1 shows the data from one of two comparable experiments. Despite the comparatively large individual variation in lytic activity characteristic for the NK system (6) a clear-cut difference in reactivity between the chimeric groups can be seen. Bone marrow reconstitution restored NK activity in the spleens and rendered the chimeras high or low reactive in complete accordance to the NK characteristics of the bone marrow donor strain and thus independent of the host environment.

To confirm these results in yet another high-low strain combination, chimeras between B10.A and A/J mice were tested (Fig. 2). Again, spleen cells from B10.A → A/J chimeras had a significantly higher killing capacity for YAC-1 (P <
Fig. 2. Donor genotype-dependent transfer of NK activity by bone marrow cells. Individual spleens were tested at two different effector to target cell ratios against YAC-1. Mean percent lysis ± SE is expressed. In brackets, no. of mice per group.

0.001) than cells from A/J → A/J mice (Fig. 2 A). On the contrary, A/J → B10.A chimeras differed significantly from B10.A → B10.A animals (P < 0.001; Fig. 2 B) and were as low reactive as A/J → A/J mice or A/J controls (P > 0.5). It could be argued that NK cells contaminating the bone marrow inoculum rather than true precursor cells could have contributed to the restoration of NK activity. Since liver cells from fetuses are invariably nonreactive in NK tests (unpublished observation) adoptive transfers with such cells were performed. Fig. 3 shows that B10.A fetal liver-reconstituted B10.A or A/J recipients express the same degree of NK activity in their spleens differing significantly from the low activity found in normal A/J control mice (P < 0.001 for both ratios tested).

Transfer of Bone Marrow Cells from Young or Old Mice Into Syngeneic Irradiated Recipients of Different Ages. NK activity develops in relation to age with a relatively late and abrupt onset in the spleen at 3 wk after birth and peaks at around 8 wk of age (5, 7). This typical time-course was suggestive of NK cell-activating factors operative in young but not in older mice. To test whether such age related influences on NK generation indeed existed transfer of syngeneic bone marrow between young and old age groups were done in crossovers patterns. The development of NK activity in the spleens of the reconstituted animals was recorded. Results of two independent experiments are shown in Fig. 4. As expected, control mice over 1 yr of age had lower spleen NK activity than young controls between 7 and 8 wk of age (P < 0.005). Irradiation of old animals followed by transfer of bone marrow from either young or old mice resulted in increased spleen killer cell activity. However, marrow from young mice was always better than "old" marrow in restoring NK capacity in the spleen, a fact which was still more pronounced in the young recipients. Age-related changes in NK reactivity of the spleen cell population thus reflect changes occurring at the NK precursor cell level.

Discussion

We have shown here that an exchange of bone marrow cells between mice sharing the same H-2 locus but differing in the degree of NK expression is
followed by a restoration of spleen NK activity in irradiated recipients according to NK cell levels found in the spleens of the hemopoietic cell donors. This indicates that the activity of NK cells in the spleen would depend entirely on the genotype of the transplanted hemopoietic cells. It is not dependent on the splenic tissue framework and would seem subject to no discernible influences of the host environment. The present findings are compatible with the view that NK precursor cells are generated in the bone marrow (8) followed by emigration to the periphery where they can be detected as NK cells mainly in spleen and peripheral blood.

Our results argue against host C-type viruses and their antigens being responsible for the normal regulation of NK activity. Furthermore, they might be of practical relevance to the choice of bone marrow donors in the treatment of leukemias. Thus, if NK cells indeed are responsible for the major early barrier of resistance to leukemias (1) while "conventional" immune reactions only come into play later (and then mostly too late), the careful selection of a high NK precursor cell donor would be logical and might, according to the present findings, be beneficial for the recipient.

In conclusion, we can thus state that the production of NK cells against mouse Moloney lymphomas is under the seemingly autonomous control of precursor cells in the bone marrow. The precursor cells themselves seem to be governed by their genetic background and seem subject to precommitted changes during aging of the individual.

Summary

Generation of natural killer (NK) cells in spleens from radiation chimeras produced between pairs of histocompatible "high" and "low" NK-reactive mouse strains has been investigated. Spleen cells of high-reactive recipients reconstituted with bone marrow from low-reactive mice were found to be low reactive. Conversely, spleen cells of low mice grafted with bone marrow or fetal liver cells...
Fig. 4. NK activity in spleens from bone marrow-reconstituted young and old CBA mice. Over 1-yr old CBA mice were reconstituted with syngeneic bone marrow cells from young animals between 7 and 8 wk of age and vice versa. Splenic NK activity against YAC-1 was tested at an effector to target cell ratio of 50:1. Mean percent lysis ± SE of two independent experiments, A and B, are shown. No. of mice per group is indicated in brackets. P values refer to comparison between the marrow-reconstituted young or old mouse groups in each experiment.

from high donors were high reactive. Similarly, the age-related changes of NK activity were shown to be expressed at the bone marrow precursor cell level. These results indicate that the generation of natural killer cells is an inborn and autonomous function of the bone marrow and does not depend on the genotype or other influences of the host environment.

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