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SHORT COMMUNICATION

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TNF-α further augments natural killer cells when co-administered with an interferon inducer to irradiated, leukemic, bone-marrow-transplanted mice

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Abstract *Purpose*: We have recently demonstrated that the interferon inducer Poly I:C significantly augments both natural killer (NK) cell numbers and the life span of leukemic, irradiated mice given syngeneic bone marrow transplants (SBMT). The cytokine tumor necrosis factor-α (TNF-α) also stimulates NK cells directly through receptor–ligand mechanisms. We have combined in the present study the NK-enhancing properties of IFN (Poly I:C-induced) and TNF-α by giving Poly I:C to leukemic mice for 8 days after irradiation and SBMT, concomitant with TNF-α during the first 4 days immediately after SBMT. All mice were sampled at day 9 following irradiation, transplant, and treatment. *Methods*: NK cells were identified and quantified by immunoperosidase labeling methods combined with a hematologic staining technique. *Results*: The data reveal that TNF-α, added to the Poly I:C administration protocol, significantly boosted NK cell numbers 2.4-fold over that achieved by Poly I:C alone. *Conclusions*: Since the role of NK cells in the immediate post-transplant period is (a) to destroy residual tumor cells, and (b) to produce hemopoiesis-driving cytokines, it appears that two NK cell stimulants are better than one, at least in the crucial, early post-transplant period.

Key words Bone marrow transplant · Leukemia · Poly I:C · TNF-α

Introduction

TNF-α (tumor necrosis factor-α), a member of a rapidly growing superfamily of cytokines, directly stimulates/activates NK cells [1] to produce interferon. In mice, as in humans, TNF-α is toxic if not administered within a narrow range of dose and exposure time. In the present work we employed our standard irradiation plus SBMT plus Poly I:C methods [2], adding TNF-α to the protocol, in an effort to further boost NK cells.

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Materials and methods

Animals

Six 8-week-old DBA/2-strain male mice, which served as normal SBMT donors as well as leukemic, irradiated, SBMT recipients [2], were housed under supervision of the McGill University Animal Care Facility, which abided by all regulations of the Canadian Council on Animal Care.

In vivo procedures

According to our well-established protocol [2, 3], each mouse was injected with 3×10^6 erythroleukemia (FLV) cells. Poly I:C (125 μg/mouse/day, i.p.), given from day 0–8 after irradiation (^{137}Cs, 450 R × 2 at day 8 of tumor growth) and SBMT (20×10^6 fresh, washed bone marrow cells), boosts new NK cell production from the seeding transplant [2]. Stimulated NK cells also produce a host of hemopoiesis-driving cytokines [4, 5], ensuring rapid engraftment/hemopoiesis from SBMT. Concomitant with the Poly I:C injections for 0–4 days post-irradiation plus SBMT, the mice received subtoxic, i.v. injections (one a day) of (mr)TNF-α (mr: mouse recombinant; 50×10^3 U, sp. act. 6.0×10^7 U/mg, MW 18×10^3), in 0.1 ml HEPES (hydroxyethylpiperazine ethanesulfonic acid) vehicle.

Sampling of host organs and identification and quantification of NK and other hemopoietic cells

Hematologic tetrachrome and immunoperoxidase labeling methods, regularly used in our laboratory, were employed to identify and quantify NK and other hemopoietic cells [2, 3, 6, 7]. The student's t test was used to compare the differences between the means of single treatment (Poly I:C) vs double treatment (Poly I:C + TNF-) for the spleen and bone marrow. It was considered significant if $P < 0.05$.

Results

NK cells in the spleen were significantly augmented by the addition of TNF-α to the Poly I:C treatment

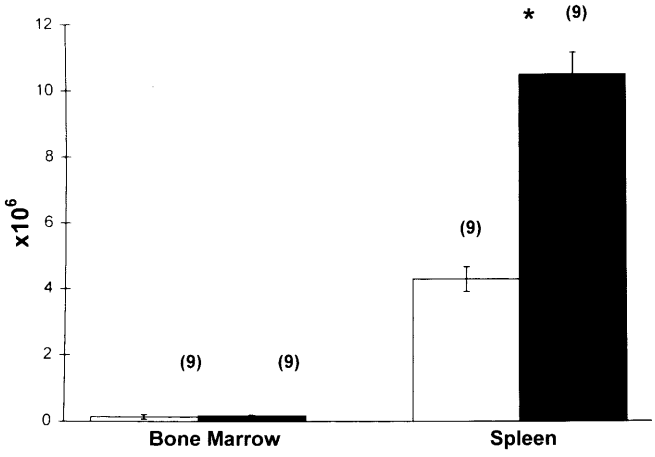

Fig. 1 Absolute numbers of NK cells in the bone marrow and spleen of leukemic mice, 9 days post irradiation + SBMT + Poly I:C + TNF-α (*solid columns*); without TNF-α (*open columns*). Mean ± SE: 9 mice; $*P < 0.000003$

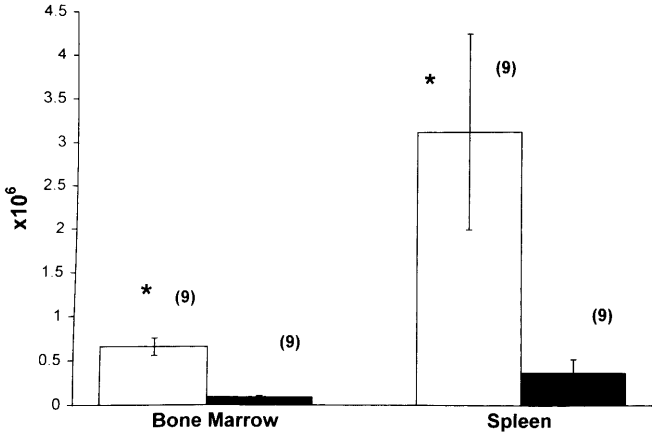

Fig. 2 Absolute numbers of monocytes in the bone marrow and spleen of leukemic mice, 9 days post irradiation + SBMT + Poly I:C + TNF-α (*solid columns*); without TNF-α (*open columns*). Mean ± SE: 9 mice; $*P < 0.04$

protocol (Fig. 1); this contrasted with the effect on monocytes, whose numbers were reduced by the addition of TNF-α (Fig. 2). No other hemopoietic cell lineage (lymphoid, erythroid, myeloid/granulocytic), in any organ, was negatively influenced by the addition of TNF-α to the Poly I:C injection regimen.

Discussion

We previously found that the NK cell numbers in the spleens of irradiated, leukemic mice treated with Poly I:C were significantly increased (2.8-fold) compared to those of the untreated controls [2]. The NK cells of mice treated by the addition of TNF-α to the identical protocol underwent a further 2.4-fold increase compared to those of Poly I:C-treated mice. TNF-α drives NK cell proliferation/development [8], thereby increasing the available, antiresidual-disease (leukemia) immune cell armament. Moreover, upon stimulation with TNF-α, NK cells produce a cascade of hemopoiesis-stimulating cytokines [4, 5]. The negative effect of TNF-α on monocytes may reflect a deficiency in a cytokine that drives monocyte production, that is, GM-CSF (granulocyte-macrophage colony-stimulating factor), not generated by TNF-α administration in vivo, while other cytokines (granulocyte- and peripheral macrophage stimulating) are so generated [9].

In summary, the co-administration of TNF-α with Poly I:C in the crucial early post-transplant days may be of potentially greater therapeutic value than that achieved by Poly I:C alone [2].

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