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Development of hyporesponsiveness of natural killer cells to augmentation of activity after multiple treatments with biological response modifiers

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Summary. *Four biological response modifiers (BRMs), MVE-2 (maleic anhydride divinyl ether), Corynebacterium parvum (C. Parvum), PolyICLC (polyinosinic:polycytidylic acid stabilized with poly-L-lysine), and mouse αβ-interferon (αβ-IFN), were tested to assess whether repeated treatments would repeatedly induce or sustain augmented levels of natural killer (NK) cell activity and/or macrophage (M0)-mediated inhibition of tumor cell growth. In contrast to a significant increase in splenic NK activity obtained with a single treatment with each of the agents, multiple treatments with these BRMs led to a progressive decrease in the degree of augmentation of NK activity. In contrast, multiple injections with these agents resulted in sustained augmentation of M0-mediated reactivity. Separation of the spleen cells by Percoll discontinuous density gradient centrifugation indicated that with mice treated once with each BRM high levels of NK activity were detected in the lower density fractions and that these fractions contained a higher percentage of large granular lymphocytes (LGLs) than that found in comparable fractions from normal mice. In contrast, cells in the lower density fractions from mice that received multiple treatments had decreased NK activity and an appreciably lower proportion of LGLs. These results indicate that the development of hyporesponsiveness to augmentation of splenic NK-cell activity following multiple treatments with BRMs may be attributable to a decreased percentage of LGLs, the effector cell population responsible for NK cell-mediated cytotoxicity.*

Introduction

Natural killer (NK) and macrophage (M0) tumoricidal activity have been reported to be augmented with several chemical and biological agents (biological response modifiers, BRMs) [10] which have shown some antitumor prophylactic or therapeutic efficacy in animal tumor systems [1, 3, 5, 9, 22, 27]. For optimal therapeutic efficacy, sustained or repeated augmentation of effector cell function would probably be needed. However, recent phase-I clinical studies [17, 20] and a preclinical study [3] indicate that multiple treatment with interferon (IFN) failed to boost NK activity. Similarly, while several different molecular weight maleic anhydride divinyl ethers (MVE) have been shown to significantly augment NK-cell- activity [1], we recently reported that multiple treatments with a low-molecular-weight MVE-2 led to the development of hyporesponsiveness to augmentation of NK-cell activity [22].

Although most therapeutic protocols with BRMs involve multiple treatments there is still a paucity of information about the effects of such protocols on effector cell functions. The development of hyporesponsiveness resulting from multiple treatments with two BRMs, IFN, and MVE-2 [3, 17, 20, 22], prompted us to test whether hyporesponsiveness would also occur with other BRMs that have been reported to augment NK-cell activity following a single treatment. Because of the possible important role of NK cells and macrophages in resistance to metastases and in BRM-mediated antitumor therapy [1, 11, 13, 27], it was important to analyze the mechanism of hyporesponsiveness of NK activity that might be induced by BRMs and also to determine whether such hyporesponsiveness occurs with M0 activity.

In this study, four BRMs that have been reported to augment NK-cell and M0-tumoricidal activity [1, 5, 9, 15] (MVE-2, *C. parvum,* PolyICLC, and mouse αβ-IFN) were tested to assess the response of both NK cells and M0s to single versus multiple treatments.

NK-cell activity has been reported to be closely associated with large granular lymphocytes (LGLs) found in mice [14], rats [23], and humans [31]. Santoni et al. (submitted for publication) observed that following a single treatment with MVE-2, PolyIC, *C. parvum,* or αβ-IFN, the observed in vivo augmentation of mouse splenic NK activity was accompanied by a substantial increase in the percentage and absolute number of splenic LGLs, the majority of which had a lower density than that characteristic of LGLs from normal mice. Many of these cells were undergoing DNA synthesis, and it appeared that the augmented NK activity was attributable, at least in part, to an expansion in the LGL population. Along the same lines, Biron et al. [2] reported that virus-activated NK cells undergo blastogenesis. These blast cells, separated in the lower density cell fractions by centrifugal elutriation, were the most efficient at YAC-1 target cell lysis. The blast-size effector cells were identified as NK cells by their antigenic markers as well as their cytolytic activity. Based on these findings, in the present study, we analyzed the size of the LGL population in

Abbreviations used in this paper: BRMs, biological response modifiers; MVE-2, maleic anhydride divinyl ether of molecular weight 15,500; C. parvum, *Corynebacterium parvum;* PolyICLC, polyinosinic-polycytidylic acid stabilized with poly-L-lysine in carboxymethylcellulose; IFN, interferon; NK cells, natural killer cells; M0, macrophage; LGLs, large granular lymphocytes; PGE, prostaglandin E; FBS, fetal bovine serum; PBS, phosphate-buffer saline composed of 4.86 g NaCl, 0.306 g KH_2PO_4, and 2,417 g $NaHPO_4$ in 100 ml H_2O adjusted to pH 7.2; LPS, lipopolysaccharide

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the spleens of mice treated with multiple doses of BRMs. The results indicated that multiple treatments with each of these BRMs resulted in reduced or undetectable augmentation of NK activity and less or no expansion in the splenic LGL population.

Materials and methods

Mice. [C57BL/6 × $C_3H)F_1$] $B_6C_3F_1$ hybrid mice were obtained from the Animal Production Area, NCI-Frederick Cancer Research Facility, Frederick, Md, USA.

Reagents. Maleic anhydride divinyl ether (MVE)-2) of molecular weight 15,500 was kindly supplied by Dr R. Carrano, Adria Laboratories, Columbus, Ohio, USA. Polyinosinic-polycytidylic acid stabilized with poly-L-lysine in carboxymethylcellulose (PolyICLC) was kindly supplied by Dr H. B. Levy (NIAID, NIH, Frederick, Md,USA). *Corynebacterium parvum (C. parvum)* was obtained from Wellcome Reagents Limited, Wellcome Research Laboratories, Beckenham, England, $\alpha\beta$-Interferon ($\alpha\beta$-IFN), purchased from Enzo Biochem. Inc., NY, USA, was a mixture of α- and β-IFN produced following infection of mouse fibroblasts by Newcastle disease virus (partially purified, with specific activity of 2.3×10^7 U/mg protein).

Tumor cells. The tissue culture cell line of YAC-1, a Moloney virus-induced lymphoma of A/Sn origin, was used as the target for NK activity. MBL-2, a lymphoblastic leukemia cell line (C57BL/6-derived) was used as the target for M0-mediated tumor inhibitory activity. Both cell lines were maintained in tissue culture with RPMI 1640 medium with 2 m*M* L-glutamine (GIBCO), 100 μg/ml gentamicin (Microbiological Associates, Bethesda, Md, USA), and 10% heat-activated fetal bovine serum (FBS) (Associated Biomedic Systems, Buffalo, NY, USA).

Effector cell preparation. Spleen cells were prepared as previously described [7]. Peritoneal exudate cells were collected by lavaging the peritoneal cavity of the mice with 10 ml RPMI 1640 medium. Removal of adherent spleen cells was performed by passage through a nylon wool column according to the method of Julius et al. [12].

NK assay. A conventional ^{51}Cr-release assay was used as previously described [8]. In brief, 1×10^4 radiolabeled tumor cells (200 μCi $^{51}Cr/10^6$ cells, incubated for 1 h at 37° C) in 0.1 ml volume were added to graded numbers of splenic effector cells in round bottom 96-well microtiter plates (Costar, Cambridge, Mass, USA). Triplicate cultures were incubated at 37° C for 4 h in a humidified 5% CO_2-in-air atmosphere. At the end of the incubation period the supernatant was removed by the Titertek automatic harvesting system and counted on a γ-scintillation counter. YAC-1 was used as the target for NK activity. The percent cytotoxicity was calculated from the formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental cpm} - \text{SR cpm}}{\text{MR cpm} - \text{SR cpm}} \times 100,$$

where SR = spontaneous release, as determined by incubation of 1×10^4 tumor cells without effector cells, and MR = maximum release as determined by incubation of 1×10^4 tumor cells with 0.5% sodium dodecyl sulfate.

Discontinuous percoll density gradient centrifugation. Spleen cells depleted of adherent cells were fractionated by centrifugation on a discontinuous density gradient of Percoll (Pharmacia Fine Chemicals, Uppsala Sweden), by a modification [14, 18] of the original method of Timonen and Saksala [30]. Growth medium and Percoll were adjusted to 290 m0smol/kg H_2O with sterile distilled water and 10 × concentrated phosphate-buffer saline (PBS) (pH 7.4), respectively. Seven different concentrations of Percoll in medium were prepared, with the top fraction having 38.8% (F.0) Percoll, the next having 47.6% (F.1), and subsequent fractions having graded increases of 4.5%, up to the last fraction (F.6) of 70.1%. After carefully laying the gradients into 15-ml conical test tubes, 5×10^7 nonadherent spleen cells were placed on the top of the gradient and the tube was centrifuged at 300 *g* for 30 min at room temperature. The fractions were collected with a Pasteur pipette from the top and were washed once with RPMI 1640 medium plus 5% FBS.

Cytochemical staining. For the morphologic analysis of LGL and the M0 populations, 1×10^5 cells in 0.1 ml medium were centrifuged for 3 min at 200 *g* onto microscope slides by using a Cytospin centrifuge. Air-dried preparations were fixed for 10 min in methanol and stained for 10 min with 10% Giemsa (Fisher Scientific Company, NJ, USA) diluted in pH 7.4 PBS. Morphologic differential counts were obtained by inspection of the slide by oil immersion microscopy. At least 200 cells were analyzed on each slide.

Assay of M0-mediated inhibition of tumor cell growth. The assay for measuring the ability of agents to induce M0-mediated inhibition of tumor cell growth has already been described elsewhere [1, 26].

Briefly, 4×10^5 peritoneal M0 were seeded onto 48-well culture plates (Costar, Cambridge, Mass, USA) and incubated in a humidified 5% CO_2-in-air incubator at 37° C for 2 h. The monolayers were washed three times with PBS to eliminate nonadherent cells. The resulting population consisted of more than 95% M0 as determined by nonspecific esterase staining and morphological criteria. The remaining adherent monolayers were then overlaid with 4×10^4 viable MBL-2 cells. The ratio of M0 to target cells was 10 : 1 at the beginning of the experiment. LPS 10 ng/ml (*E. coli* 0111 : B4, Dibco Laboratories, Detroit, Mich, USA) was added to the assay medium [21, 28, 29]. All cultures were again incubated at 37° C in humidified 5% CO_2-in-air incubator for 72 h, and viable MBL-2 cells were determined by trypan blue dye exclusion.

The percent inhibition of growth and/or survival of MBL-2 cells due to M0 interaction was calculated by:

$$\% \text{ Inhibition} = 1 - \frac{\text{Number of MBL cells incubated with M0}}{\text{Number of MBL cells incubated without M0}} \times 100.$$

Results

Development of NK-cell hyporesponsiveness to BRMs

As expected, a single treatment with each of the four BRMs resulted in a significant increase in splenic NK activity (Fig. 1). In contrast to the consistent augmentation of NK activity attained following a single treatment with the BRMs, multiple

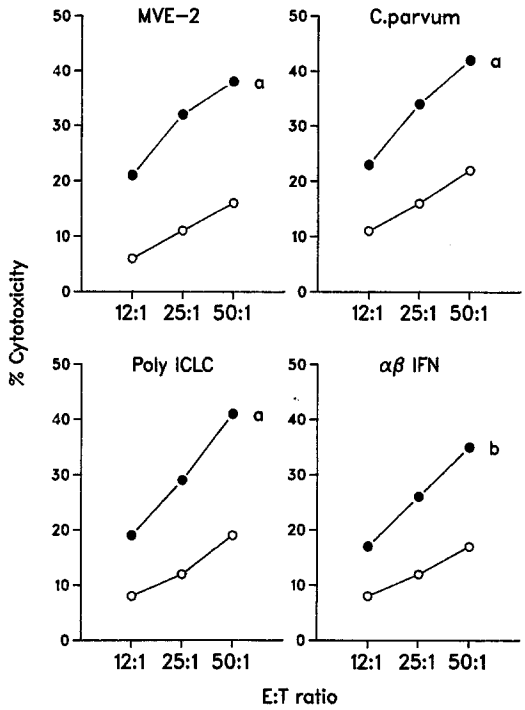

Fig. 1. Augmentation of splenic NK activity by single treatment with MVE-2, *C. parvum*, PolyICLC, or $\alpha\beta$-IFN. $B_6C_3F_1$ mice were injected IP with 25 mg/kg MVE-2, 28 mg/kg *C. parvum*, 1 mg/kg PolyICLC, or 10,000 U/mouse $\alpha\beta$-IFN on day −3. Spleen cells of normal (○) and BRM-treated (●) mice were harvested on day 0 and tested for NK activity. *a* $P < 0.001$; *b* $P < 0.01$ compared with normal control group (E : T = 50 : 1) by Student's *t*-test. Representative results obtained from three separate experiments

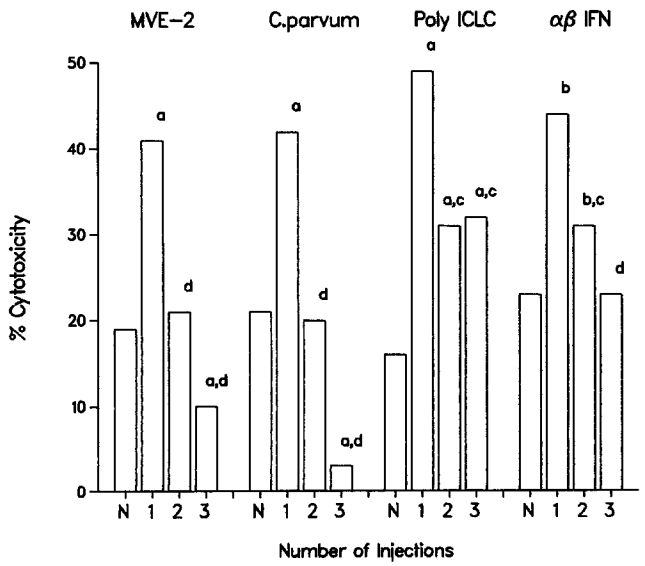

Fig. 2. Development of NK-cell hyporesponsiveness to BRMs. $B_6C_3F_1$ mice were injected IP with 25 mg/kg MVE-2, 28 mg/kg *C. parvum*, 1 mg/kg PolyICLC or 10,000 U/mouse $\alpha\beta$-IFN once (on day −3), twice (on days −6, −3), or three times (on days −9, −6, −3). Spleen cells of normal *(N)* and BRM-treated mice were harvested on day 0 and tested for NK activity (E : T = 50 : 1). *a* $P < 0.001$; *b* $P < 0.01$ compared with normal *(N)* control group; *c* $P < 0.01$; *d* $P < 0.001$ compared with one injection in each group, by Student's *t*-test. Representative results obtained from four separate experiments

treatments resulted in less or no significant augmentation of NK activity (Fig. 2). In fact, three treatments with MVE-2 or *C. parvum* led to a significant decrease of NK activity below that of the normal controls. Two to three treatments with PolyICLC resulted in significantly less pronounced augmentation of NK activity than was induced by one treatment, but NK activity was significantly augmented above control levels by each treatment. Two treatments with $\alpha\beta$-IFN still caused some reduced NK boosting, but three treatments failed to significantly boost NK activity. Thus, two to three treatments with any of the BRMs examined led to hyporesponsiveness to NK boosting, in contrast to the substantial augmentation of the NK activity after a single treatment.

Studies were conducted to assess whether the decreased augmentation of NK activity was related to a reduction in splenic mononuclear cells resulting from the multiple doses of BRMs (Table 1). However, multiple treatments resulted in a substantial increase in the number of mononuclear cells. Thus, the effects of multiple treatments of the BRMs on the NK activity could not be attributed to an overall toxic effect on splenic mononuclear cells. Also, the magnitude of the increase in cell numbers after multiple treatments relative to values seen after one treatment was not sufficient to attribute the strongly reduced NK activity to a dilution of NK-active cells by an increased number of unrelated cells.

Distribution of NK activity on percoll gradients after single or multiple treatments with BRMS

Separation of nonadherent spleen cells from normal mice on discontinuous Percoll density gradients consistently results in a considerable enrichment of NK activity in lower density fractions and depletion of NK activity in higher density fractions [14].

Table 1. Number of splenic mononuclear cells after treatment with BRMs

BRMs	Day of treatment[a]	No. of mononuclear cells per spleen ($\times 10^{-7}$)[b] (mean ± SE)
	None	7.1 ± 0.5
MVE-2	D−3	8.1 ± 0.6[c]
(25 mg/kg IP)	D−6, −3	12.7 ± 0.3[e]
	D−9, −6, −3	15.7 ± 0.7[e]
C. parvum	D−3	8.9 ± 0.9
(1 mg/kg IP)	D−6, −3	10.4 ± 0.7[c]
	D−9, −6, −3	14.5 ± 1.2[d]
PolyICLC	D−3	7.5 ± 0.4
(28 mg/kg IP)	D−6, −3	10.3 ± 1.1[c]
	D−9, −6, −3	11.1 ± 0.6[d]
$\alpha\beta$-IFN	D−3	8.5 ± 0.8
(10 000 units/mouse IP)	D−6, −3	11.8 ± 0.5[d]
	D−9, −6, −3	11.7 ± 0.3[d]

[a] $B_6C_3F_1$ mice were injected with BRMs on the days indicated, and the number of spleen cells was determined on day 0

[b] Representative results were obtained from three separate experiments

[c] $P < 0.01$; [d] $P < 0.01$; [e] $P < 0.001$, compared with normal (none) control group

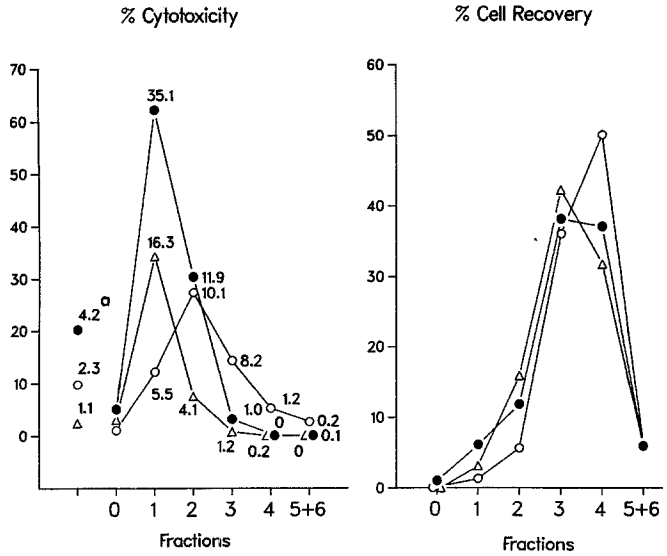

Fig. 3. Distribution on Percoll gradient of NK activity and percentage of LGLs after single or multiple treatments with MVE-2. $B_6C_3F_1$ mice were injected IP with 25 mg/kg MVE-2 once (on day −3) or three times (on days −9, −6, −3). Spleen cells of normal mice (○), mice treated with single injections of MVE-2 (●), and mice treated with three injections of MVE-2 (△) were harvested on day 0 and tested for NK activity (E : T = 10 : 1). *Left panel* distribution of NK activity and percentage of LGLs in fractions. *Symbols* to *left* indicate levels of cytotoxicity of nylon wool-non-adherent spleen cells prior to gradient separation. (E : T ratio = 10 : 1). *a* Percentage of LGLs; *right panel* recovery of cells in each fraction. There were mostly dead cells in fraction 0. Representative results obtained from three separate experiments

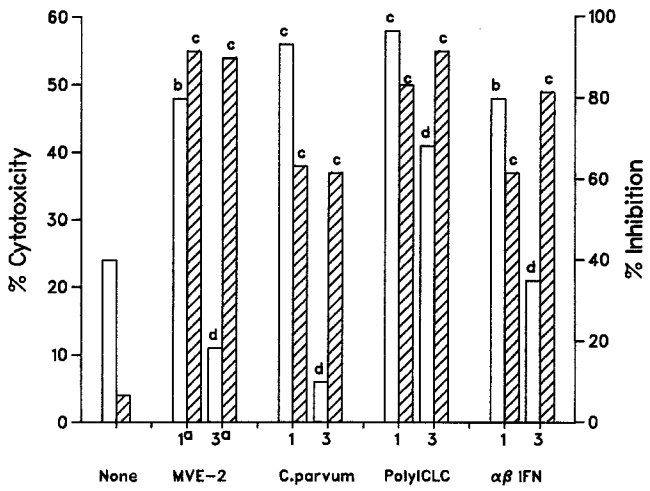

Fig. 4. Sustained augmentation of M0 activity after multiple treatment with BRMs. $B_6C_3F_1$ mice were injected IP with 25 mg/kg MVE-2, 28 mg/kg *C. parvum*, 1 mg/kg PolyICLC, or 10,000 U/mouse $\alpha\beta$-IFN once (on day −3), or three times (on days −9, −6, −3). Spleen cells (□) and peritoneal M0 (▨) of normal and BRM-treated mice were harvested on day 0 and tested for splenic NK-cell cytotoxicity (□, E : T = 50 : 1) and peritoneal M0-mediated tumor cell inhibitory activity (▨, E : T = 10 : 1). *a* number of injection; *b* $P < 0.01$; *c* $P < 0.001$ compared with normal nontreated control; *d* $P < 0.001$ compared with one injection of each BRM. Representative results obtained from three separate experiments

Table 2. Distribution of NK activity and percentage of LGLs in Percoll density fraction of spleen cells from normal mice or mice treated once or three times with BRMs

Treatment[a]		% Cytotoxicity[b]/% LGLs in fraction					
		Unfractionated[c] spleen cells	Percoll density fraction				
			1	2	3	4	5 and 6
None		9.2/2.0	18.9/ 5.0	29.1/11.2	19.8/4.8	5.3/1.3	3.9/0.0
C. parvum	1×	34.6/7.9	65.0/32.3	66.0/31.7	10.3/2.2	1.4/0.0	0.4/1.0
(28 mg/kg)	3×	1.5/0.8	12.1/ 7.3	7.8/ 3.7	0.7/0.0	0.2/0.0	0.6/0.9
None		8.9/4.6	18.8/ 6.3	22.9/ 8.5	15.3/3.1	2.5/0.0	3.0/1.0
PolyICLC	1×	31.0/8.1	55.0/33.0	53.1/27.9	16.2/6.3	0.1/3.0	0.7/0.0
(1 mg/kg)	3×	20.8/6.2	41.1/23.6	44.3/21.1	22.6/6.2	2.1/0.7	0.1/0.0
None		7.4/2.5	17.3/ 4.5	25.4/ 9.8	12.8/4.3	5.2/1.1	4.2/1.5
$\alpha\beta$-IFN	1×	19.1/6.9	52.2/20.2	42.3/15.3	15.2/7.3	2.3/0.5	0.5/0.0
(10^4 units/mouse)		7.1/1.9	25.3/ 8.4	20.3/ 8.5	9.3/3.4	0.5/0.0	0.2/0.7
	3×						

[a] $B_6C_3F_1$ mice were injected IP with BRM once (1×, on day −3) or three times (3×, on days −9, −6, −3), and spleen cells were collected on day 0 to assess % cytotoxicity and % LGL

[b] Cytotoxicity was assessed in a 4-h ^{51}Cr-release assay against YAC-1 target cells (E : T ratio = 10 : 1)

[c] Nylon wool-non-adherent spleen cells prior to gradient separation

After treatment of mice with MVE-2 NK activity was augmented, and upon Percoll density gradient separation the activity was observed in fractions with somewhat lower density than that seen with normal spleen cells, with peak reactivity in fraction 1 (F. 1) (Fig. 3). The marked increase in NK activity in F. 1 was accompanied by a considerable increase in the percentage of LGLs (from 5.5% in normal mice to 35.1% in MVE-2 single-treated mice (Fig. 3), without a major shift in the total distribution of cells (% cell recovery) among the various fractions (Fig. 3). In contrast, three treatments with MVE-2 led to a decrease of NK activity in F. 1 and F. 2 (Fig. 3), associated with a decrease in the percentage of LGLs

(from 35.1% and 11.9% in MVE-2 once-treated mice to 16.3% and 4.1% in three-times-treated mice in F. 1 and F. 2, respectively) (Fig. 3).

Similar results were also obtained after single and multiple treatments with *C. parvum* and $\alpha\beta$-IFN. Single treatments resulted in an increase in NK activity accompanied by an increase in the percentage of LGLs in F. 1 and F. 2 (Table 2), without a major shift in the percent cell recovery among the various fractions (data not shown). In contrast, multiple treatments with MVE-2, *C. parvum,* or $\alpha\beta$-IFN resulted in a decrease in both NK activity and the percentage of LGLs (Table 2). However, multiple treatment with PolyICLC led to high NK activity in F. 1 and F. 2, just slightly below the level produced by one treatment in each case, and the percentage of LGLs remained considerably higher than in the normal controls.

Sustained augmentation of M0 activity after multiple treatments with BRMs

Splenic NK cell and peritoneal M0 activity were examined from the same mice that received one or three treatments with each of the four BRMs (Fig. 4), to assess whether M0 activity would also be altered. As previously shown (Fig. 2), multiple treatments resulted in depressed NK activity, in contrast to the significant increases achieved with one treatment. In contrast, augmented M0 activity was seen even after multiple treatments with all of the BRMs. Multiple treatments did not lead to any reduction in the levels of augmentation of activity, as was seen with NK-cell activity; rather, the level of M0 boosting remained at or near the levels attained with a single treatment.

Discussion

The present result indicate that multiple treatments with a variety of BRMs induced little or no augmentation of NK activity, even though each agent was quite effective in boosting NK activity after a single treatment. Of the four agents tested here, three (MVE-2, *C. parvum,* and $\alpha\beta$-IFN) clearly showed such divergent results, and the fourth, PolyICLC, showed a trend in the same direction, although significant levels of NK boosting continued to be observed even after three treatments. These results extend our previous observations with MVE-2 [22] and indicate that such hyporesponsiveness to sustained boosting of splenic NK activity in mice after multiple treatments may be a general phenomenon with BRMs. These observations with NK activity were in striking contrast to those obtained with the M0-mediated tumor growth inhibition and suggest that the mechanisms for regulation of these effector cell activities are quite different.

One might consider several possible mechanisms for the hyporesponsiveness to NK boosting after multiple treatments with BRMs. We examined the possiblities that repeated, frequent exposure to BRMs might be toxic to spleen cells or, alternatively, might stimulate the expansion of noneffector cells and thereby dilute out the NK-cell pools. However, our results failed to support either possibility: decreases in spleen cell numbers were not seen; rather there was a modest increase in the overall population, albeit not of a magnitude sufficient to account for the reduced NK activity. Using MVE-2 as a representative BRM to explore this phenomenon in more depth, we have examined the possible role of prostaglandin E [4, 16] or the generation of cells capable of suppressing NK-effector function [24, 25]. In a report to be published elsewhere (Saito T, Welker RD, Fukui H, Herberman RB, and Chirigos MA, *Cellular Immunology,* 1984, in press), we will present evidence against either of these potential mechanisms contributing to the development of hyporesponsiveness. Rather, we obtained evidence for a reduction in the size of the LGL population in the spleen and we have therefore focused on this aspect in the present study with the various BRMs. Similar observations were obtained with each of the three BRMs that induced hyporesponsiveness upon multiple treatments. As previously reported (Santoni A, submitted for publication) single treatments with each of these agents caused a considerable increase in the percentage of splenic LGLs, which showed a lower density distribution in Percoll gradients. In contrast, our present studies show that multiple treatments induced a less pronounced (MVE-2 and *C. parvum*) or no ($\alpha\beta$-IFN) increase in the percentage of LGLs in the low-density Percoll fractions. From the studies of Santoni et al. (submitted for publication) and Biron et al. [2], the increase in splenic NK cells after one treatment with BRMs or viruses appeared to be attributable to a stimulation of the proliferation of NK cells, with an expansion of the effector cell populations, rather than to a redistribution of these cells from other sites to the spleen. The present results indicate that repeated, frequent exposure to the same agents results in some block in the maintenance or reappearance of this expansion of the effector cell pool. Thus, the substantially lower levels of NK activity after multiple versus one treatment with a BRM appear to be attributable, at least to a large extent, to the smaller number of LGLs.

The lack of substantial or cyclic augmentation of NK activity that we have observed upon multiple treatments with $\alpha\beta$-IFN, in contrast with the sustained M0 cytostatic activity, is directly analogous to the pattern of results obtained in clinical trials in cancer patients with preparations of natural or recombinant αIFN [19]. Such results with IFN suggest that the hyporesponsiveness to NK boosting by other BRMs cannot be simply attributed to decreased induction of IFN after multiple treatments. If that were the main explanation for the hyporesponsiveness to NK boosting, the administration of IFN itself could be expected to be effective in overcoming the block.

The present results have some obvious implications for attempts at therapy of cancer by augmentation of NK activity with BRMs. It appears that frequent, repeated treatments with several different BRMs would not only fail to be optimal for such a therapeutic approach but would actually be counterproductive, decreasing or eliminating the possible benefits from single treatments. It therefore seems necessary to develop alternative treatment protocols, or devise procedures to reverse or overcome the hyporesponsiveness, to achieve maintenance, or repeated strong stimulation, of augmented NK activity. The present results suggest that monitoring of the effects of various treatments on the number of LGLs, as well as on the levels of NK activity, may help to select improved approaches. An emphasis on such issues seems warranted, since current treatment regimens with BRMs are often unsuccesful in the eradication of tumors, and recent studies with some BRMs, e.g., MVE-2, indicate that boosting of NK activity may be an important or even essential aspect of the antitumor effects [6].

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