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Roles of Murine Macrophages in the Maintenance of Natural Killer (NK) Cell Activity

I. Ontogeny of Macrophage Function for Maintaining NK Cell Activity and an Intrinsic Macrophage Defect in C3H/HeJ Mice*

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Abstract The possibility that macrophages of newborn mice regulate the expression of NK cell activity in a manner different from macrophages of adult mice was investigated in the modulation of NK activity by peritoneal macrophages (PM). PM of newborns, but not those of adults, augmented the NK activity of freshly prepared spleen lymphoid cells. After a 20-hr culture, the NK activity of spleen lymphoid cell preparation was abrogated. The addition of PM of newborns was effective in maintaining NK cell activity during the culture period, but PM from adults were ineffective unless stimulated by lipopolysaccharides (LPS). Unexpectedly PM from newborn C3H/HeJ mice, histocompatible to regular C3H/He mice and genetically defective in the responsiveness to LPS, were unable to maintain the NK cell activity. Since the NK activity of C3H/HeJ spleen lymphoid cells was maintained normally after coculture with PM from newborn C3H/He mice, there seemed to be an intrinsic defect in C3H/HeJ PM. NK activity of spleen cells of two-week-old mice was very low. No effective suppressor cells were detectable in the spleen cell population. These results suggest that any genes other than those involved in the major histocompatibility complex determine the ability to maintain NK activity, and that PM of newborns are in a semi-activated stage, endowed with the potential for recruiting the organization of host defense from other cells at an age still unable to induce the antigen specific immune response.

Introduction

Natural killer (NK) cells are functionally defined as white blood cells with the ability to recognize and lyse certain normal and malignant cell types in the absence of any overt immunization. Morphologically, NK cells contain characteristic large, azurophilic granules and are called large granular lymphocytes. NK cells, although initially recognized for their anticancer activity, appear to be involved in a multitude of biological processes (Lotzová & Herberman, 1986).

Macrophages and NK cells play important roles in the host defense mechanism. Both cell types become immunocompetent without any antigenic stimulation, and act in an antigen nonspecific manner. Macrophages can phagocytize autologous denatured cells and microorganisms invading the host from the environment, without the help of

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T cells or B cells. Virgin NK cells can kill tumor cells in contrast with T cells which differentiate in response to antigenic stimulation.

Among these cell types macrophages seem to play a central role in the host defense mechanism, since they develop ontogenetically earlier than other immunocompetent cells including T cells, B cells (Spear et al., 1973; Inaba et al., 1982), and NK cells. Macrophages from newborn mice have been shown to have higher phagocytic activity (Nakano et al., 1978) and higher antitumor activity than those from adult mice in their lower dependency on LPS for being made cytolytic against tumor cells and in their inhibitory effect on tumor cell growth (Ido et al., 1984). On the other hand, cells expressing NK–1 antigen, a NK-cell surface marker, have been reported to bind but not lyse target tumor cells in newborn mice (Koo et al., 1982; Hackett et al., 1986).

Focussing on the macrophage-NK cell interaction, macrophages have been shown to modulate NK activity positively or negatively. Macrophages have been reported to be required for the augmentation of NK cell activity by a variety of stimulating agents (Djeu et al., 1978), and to support the growth of NK clones with the help of IL–2 and change the target spectrum of NK clones (Minato et al., 1985). On the other hand, several studies have shown that monocytes/macrophages inhibit the development of NK cells and suppress NK activity in the effector phase (Oshimi et al., 1985; Uchida et al., 1984).

The direct interaction of macrophages with NK cells has been investigated in man (Uchida et al., 1982; Munakata et al., 1985; Bloom et al., 1986) and in mice (Suzuki et al., 1984; Minato et al., 1985), but ontogenetic approaches to this interaction have been rarely shown so far. How do macrophages from newborn mice modulate the NK activity of adult spleen lymphoid indicator cells? This study demonstrates that only newborn macrophages were effective in augmenting or maintaining NK cell activity in the absence of stimulation by LPS, although NK activity of spleen cells is low or absent in the early stage of ontogeny.

ABBREVIATIONS

FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; LPS, lipopolysaccharide; NK, natural killer; PEC, peritoneal exudate cells; PGE, prostaglandin E; PM, peritoneal macrophages; TGC, thioglycollate medium.

Materials and Methods

Mice — Inbred female and male mice of C3H/HeSlc (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka) and C3H/HeJ (Institute of Animal Experiments, Kyoto University) were used. Newborn mice were raised in our colony. Peritoneal exudate cells (PEC) were obtained from the newborn mice receiving i.p. injection of a stimulant on the day of birth to two days after birth, and from adult mice at the age of 6 to 12 weeks.

Tumor cells — YAC–1 lymphoma cells of A/Sn-mice origin were used throughout as targets in NK cell assays.

Culture medium — RPMI 1640 (Nissui Seiyaku Co., Tokyo) supplemented with

penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM glutamine and 6% fetal bovine serum (FBS; Hyclone, Logan, UT) were used for the culture of macrophages, spleen cells and YAC–1 cells. Hanks' balanced salt solution (HBSS; Nissui) neutralized with NaOH was used for cell preparation.

Preparation of cells — Single cells of thymus and spleen were obtained by pressing minced tissue through stainless steel meshes, and cell clumps had depleted by passage through a cotton mat. Spleen cells were treated with cold hemolytic Gey's solution for 2 min to eliminate erythrocytes, then washed 3 times with HBSS. Spleen lymphoid cells devoid of adherent cells were prepared by passing spleen cells from male mice through a Sephadex G–10 column. Thymocytes were prepared from mice at the age of one month. PEC were harvested 4 days after i.p. injection of thioglycollate medium (TGC; Brewer's medium, Difco Laboratories, Detroit, MI) by peritoneal lavage with HBSS. Volume of TGC injected was 2 ml for adult mice and body weight-matched volumes for younger mice. The cells harvested were washed twice, resuspended in the culture medium, and incubated for 2 hr in 96-well flat-bottom microplates (No 25860 MP, Corning Glass Works, NY) or in 24-well plates (No 25820 MP, Corning) at 37°C in 5% CO_2–95% air atmosphere. Nonadherent cells were then removed by washing each well 3 times with HBSS. About 75–80% of PEC were adherent. Adherent PEC are interchangeably called peritoneal macrophages (PM) in this paper. The dose of PM described in the text denotes the original dose of PEC. PM were employed as modulator cells for NK cell activity immediately after preparation.

NK cytotoxicity assay — The 4 hr chromium release assay was routinely performed in 96-well U bottom microplates (No 25850 MP, Corning) by using 5,000 ^{51}Cr-labelled YAC–1 cells as targets unless mentioned. Effector cells were tested at effector-to-target cell (E/T) ration of 25 to 160. The percent cytotoxicity was calculated by the following formula.

$$\% \text{ cytotoxicity} = \frac{\text{test cpm} - \text{spontaneous cpm}}{\text{maximum cpm} - \text{spontaneous cpm}} \times 100$$

in which the maximum cpm, the spontaneous cpm and the test cpm indicate, respectively, the radioactivity of the supernatant of 0.1% Triton X–100 treated labelled target cell culture, that of the target cell culture, and that of the culture consisting of target cells and spleen cells.

Assay for NK lysis in the mixture of spleen lymphoid cells and PM — 5×10^5 (E/T=25) spleen lymphoid cells and 2×10^4 ^{51}Cr-labelled YAC–1 cells in 0.2 ml of culture medium with or without 1 μg/ml indomethacin (Sigma, St. Louis, MO), an inhibitor of prostaglandin synthesis, were put into each well of a 96-well flat-bottom microplate where various doses of PM had already been adhered. The plate was then incubated at 37°C for 4 hr to test the cytotoxicity.

Spleen cell culture — Adult spleen lymphoid cells (7.5×10^6) were inoculated into 24-well plates where 4×10^5 PM had already adhered, in 0.5 ml culture medium with 1 μg/ml indomethacin unless otherwise indicated. Lipopolysaccharide B (LPS; *Escherichia coli* 055: B5, Difco) and/or polymyxin B (Sigma) were added to the cultures in some experiments. Indomethacin was routinely added to the cultures, because NK activity of cells after culture with indomethacin was somewhat higher than that without indomethacin, and the results were essentially similar irrespective of the addition of

indomethacin to the culture (data not shown). After a 20-hr incubation, nonadherent cells were recovered by washing each well 3 times with HBSS. To remove detached PM, the recovered cells were passed through Sephadex G–10 columns, resuspended in culture medium and tested for NK activity.

Results

PM from newborns augment NK activity in a few hours

As shown in Figure 1, only newborn mouse PM augmented NK cell activity of adult spleen lymphoid cells by 52 to 62% in the presence of 1 μg/ml indomethacin in only 4 hr, but neither adult mouse nor newborn mouse PM could augment NK activity without indomethacin. Prostaglandin E_2 (PGE_2) has an inhibitory effect on the cytolytic activity of NK cells (Kendall & Targan, 1980) and PM of newborns produce PGE_2 in considerable amounts (Snyder et al., 1982a, 1982b). This may explain why PM-mediated augmentation was not observed with the PM of newborn mice in the absence of indomethacin. The augmentation of cytotoxicity was probably not caused by the cytotoxicity of the PM themselves, since PM were not cytotoxic against YAC–1 in a 4-hr culture (closed symbols).

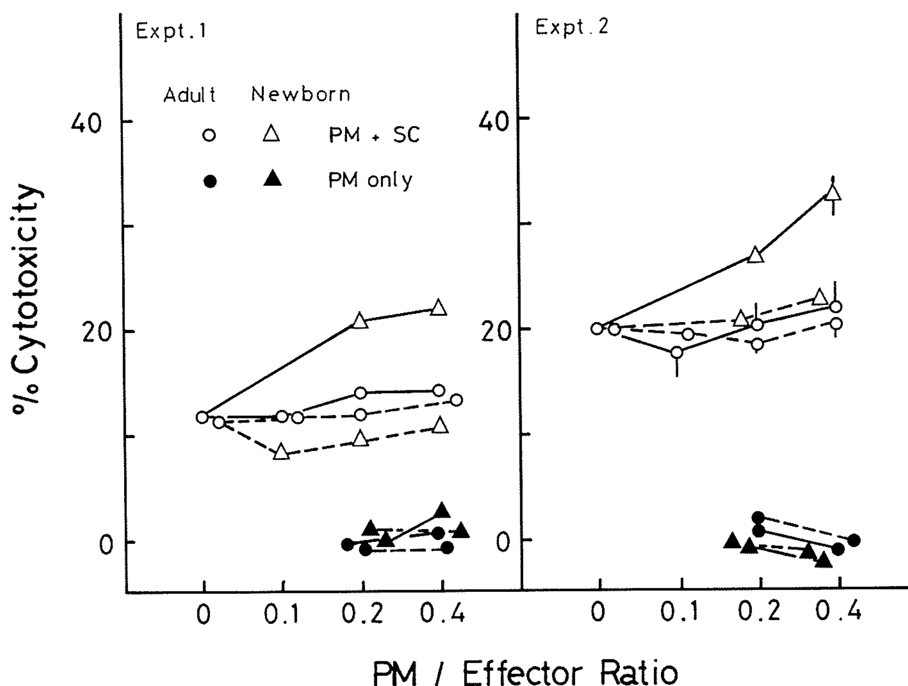

Fig. 1. Newborn mouse PM augment NK cell activity in a few hours. Various numbers of PM were cultured in 0.2 ml medium for two hr in 96-well flat-bottom plates. Medium was then drained, and 5×10^5 spleen lymphoid cells (SC) and 2×10^4 ^{51}Cr-labelled YAC–1 cells in 0.2 ml of culture medium with (——) or without 1 μg/ml indomethacin (– – –) were added to the layers of PM. The cells were tested for cytotoxic activity. Each symbol and vertical bar represent the arithmetic mean of triplicate cultures and SEM.

Table 1. PM of newborn, but not adult mice are effective in maintaining NK activity of spleen lymphoid cells of adult mice.[a]

PM		% Cytotoxicity				
		expt. 1	expt. 2		expt. 3	
		80 : 1[b]	40 : 1	80 : 1	40 : 1	80 : 1
Newborns	8×10^5	32.1±0.7[c]	7.9±0.7	15.4±1.7	17.5±0.4	32.4±0.8
	4×10^5	N.T.	3.8±0.6	5.4±1.1	15.3±0.1	30.2±2.3
	2×10^5	N.T.	1.3±0.1	1.2±1.0	N.T.	N.T.
Adults	8×10^5	6.2±0.4	1.6±0.1	1.5±0.6	3.5±0.2	5.0±0.2
	4×10^5	N.T.	<1.0	1.0±0.9	2.7±0.2	4.0±0.3
–	–	<1.0	<1.0	<1.0	2.0±0.1	2.4±0.0

[a] Spleen lymphoid cells of adult mice (7.5×10^6 G-10-passed cells) were cultured for 20 hr with PM in the presence of 1 μg/ml indomethacin. Recovered nonadherent cells were passed through Sephadex G-10 columns to remove detached PM, and G10-passed lymphoid cells were tested for NK activity.
[b] Effector-to-target cell ratio; target cells, 5,000 per well
[c] Mean±SEM of triplicate cultures

PM of newborns are effective in maintaining NK cell activity, but those of adults are not

As reported before (Reynolds et al., 1981; Cohen et al., 1986), NK activity of spleen lymphoid cells cultured alone decreased or was almost abolished after 20 hr. NK activity of fresh spleen lymphoid cells was 26.5% (n=11), and that of the cells after a 20-hr culture was 1.7% (n=8) at the E/T ratio of 80 (Table 1). However, NK activity of spleen lymphoid cells was fully maintained by coexistence of PM from newborns depending on the dose of the PM.

Why were the PM from adults ineffective in maintaining NK activity? It is unlikely that the PM from adults bound NK cells more tightly, so that recovered cell preparations contained few NK cells. The number of recovered cells from the coculture with the PM of adults was not smaller than that from the coculture with the PM of newborns. Percent recovery of cells cultured with the PM of adults, with PM of newborns, and without PM was, respectively, 41.7, 41.6 and 45.0% (n=14). After washing out nonadherent cells from the culture even weakly, the adherent preparation, which contained adult mouse PM and many lymphocytes, showed low cytotoxicity against YAC–1 (data not shown).

Effect of LPS dose on PM-mediated maintenance of NK activity of spleen lymphoid cells

The culture condition seemed to affect the ability of the PM from the adults in maintaining NK activity. In fact, in culture medium supplemented with certain lots of FBS, the PM from the adults were also effective in maintaining NK activity. To investigate the involvement of LPS in the accidentally observed maintenance of NK activity by the PM of adults, various doses of LPS were added to each culture. Figure 2 shows the enhancement of maintenance of NK activity mediated by the PM of adults. The cytolytic activity of lymphoid cells after culture alone was subtracted from the cytolytic activity after coculture with PM. Depending on LPS dose, the NK activity of

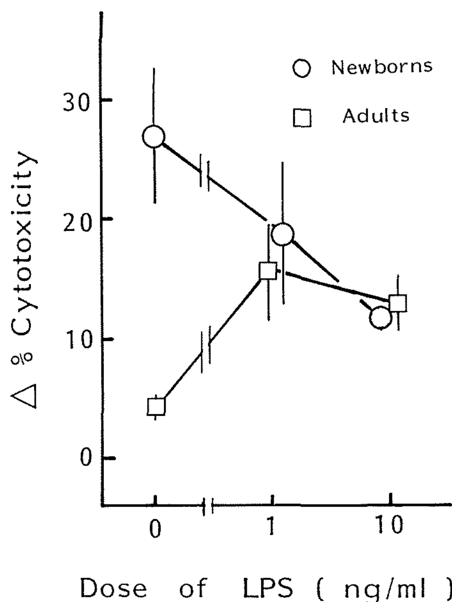

Fig. 2. Effect of LPS dose on PM-mediated maintenance of NK activity of spleen lymphoid cells. G-10-passed cells from adults (7.5×10^6) and PM (4×10^5) were cocultured for 20 hr with the indicated concentration of LPS. Macrophages were then removed by passing recovered cells through Sephadex G–10 columns, and the lymphoid cells were tested for NK activity. The cytolytic activities of lymphoid cells only compared to the cytolytic activities of lymphoid cells cocultured with macrophages were subtracted from each activity respectively (LPS(–), 3.8%; 1 ng/ml, 7.5%; 10 ng/ml, 9.7%). Each point represent the mean±SEM of three independent experiments.

spleen lymphoid cell preparation seemed to be maintained, but contamination by spleen macrophages may maintain NK activity. In contrast to the PM of adults, the PM of newborns were less effective in maintaining NK activity. Because the PM of newborns were greatly effective in maintaining NK activity in the absence of stimulation by LPS, some negative feedback regulation likely affects the PM of newborns.

No effect of polymyxin B on maintenance of NK activity mediated by PM of newborns

PM of newborns are more sensitive to stimulation by LPS and readily acquire cytolytic activity than those of adults (Ido et al., 1984). A small quantity of LPS might contaminate the culture and activate newborn mouse PM to maintain the NK activity of spleen cells. However this appeared unlikely. Polymyxin B is a reagent that binds at the ratio of 1 to 1 to lipid A, which is an active component of LPS, and blocks the action of LPS. As shown in Table 2, PM of newborns were also effective in maintaining the NK activity of spleen lymphoid cells, in the presence of 0.5 μg/ml polymyxin B through the preculture to NK assay. On the other hand, the presence of 5 μg/ml polymyxin B really inhibited the action of LPS on the maintenance mediated by PM of adults. The PM of newborns seem to have the ability to maintain NK activity in the absence of stimulation by LPS.

Table 2. Effect of polymyxin B on PM-mediated maintenance of NK activity.

PM 8×10^5	Polymyxin B[a] μg/ml	% Cytotoxicity LPS (−)[b]	LPS (+)[c]
Newborns	0	33.3±1.1	12.7±0.1
	0.5	32.1±0.7	N.T.
	5	N.T.	16.4±0.2
Adults	0	7.9±0.4	18.2±0.6
	0.5	7.3±0.6	N.T.
	5	N.T.	3.8±0.2
-	0	3.3±0.7	<1.0
	0.5	4.1±0.6	N.T.
	5	N.T.	<1.0

[a] Polymyxin B was supplemented throughout the preculture.
[b] E/T=80
[c] E/T=40, LPS 1 ng/ml
Other indications are the same as in the notes of Table 1.

Deficiency in the ability of C3H/HeJ PM to maintain NK activity

Because both newborn and adult PM were affected by LPS, it was of interest how PM from C3H/HeJ mice, genetically defective in their responsiveness to LPS, act on spleen lymphoid cells and affect the maintenance of the NK activity. We expected that, irrespective of the dose of LPS, the PM from newborn mice were effective in maintaining NK activity, but those from adult mice were not. However, unexpectedly, as shown in Table 3, not only the PM from adult mice but also those from newborn mice from C3H/HeJ strain were ineffective in maintaining the NK activity of spleen lymphoid cells from C3H/HeJ, though PM of newborn C3H/HeSlc mice were capable of maintaining the NK activity of spleen lymphoid cells from C3H/HeSlc.

Normal ability of C3H/HeJ spleen lymphoid cells to respond to PM and mainfest NK activity

There may be an intrinsic defect in PM and/or in spleen lymphoid cells of C3H/HeJ mice with respect to expression of NK activity. To discriminate these possibilities,

Table 3. Deficiency in the ability of C3H/HeJ PM to maintain NK activity.

Strain	PM 8×10^5	% Cytotoxicity[a] expt. 1	expt. 2
C3H/HeSlc	Newborns	14.0±0.3	18.2±0.4
	-	<1.0	<1.0
C3H/HeJ	Newborns	2.1±0.3	<1.0
	Adults	<1.0	<1.0
	-	<1.0	<1.0

[a] E/T=40
Other indications are the same as in the notes of Table 1.

Table 4. Normal ability of C3H/HeJ spleen lymphoid cells to respond to PM and manifest NK activity.

4×10^5 PM		% Cytotoxicity of spleen lymphoid cells[a)]			
		expt. 1		expt. 2	
		C3H/HeSlc	C3H/HeJ	C3H/HeSlc	C3H/HeJ
C3H/HeSlc	Newborns	10.6±1.1	7.6±0.2	26.7±0.4	22.7±0.8
	Adults	5.4±0.4	3.0±0.6	3.2±0.3	3.2±0.4
	–	1.4±0.4	<1.0	1.3±0.7	1.6±0.3
C3H/HeJ	Newborns	<1.0	1.0		
	Adults	2.5±0.2	<1.0	N.T.	N.T.
	–	1.4±0.4	<1.0		

[a)] Expt. 1, E/T=40; Expt. 2, E/T=80
Other indications are the same as in the notes of Table 1.

cross-combination experiments were executed, as shown in Table 4. When cocultured with PM preparations from C3H/HeJ, spleen lymphoid cells from both strains were unable to preserve their NK activity. Conversely, when cocultured with PM preparations from C3H/HeSlc, spleen lymphoid cells from both strains preserved their NK activity. There was probably an intrinsic defect clearly in C3H/HeJ PM, not in the C3H/HeJ spleen lymphoid cells.

Ontogeny of NK activity of spleen cells

NK activity of spleen cells is not detected in newborn mice, begins to appear at the age of 2 weeks, reaches a maximal response between 5 and 8 weeks old, and

Fig. 3. Ontogeny of NK activity of spleen cells. Spleen cells from male mice of various ages were tested for NK activity at a effector-to-target ratio of 25 (○) or 50 (●). Each symbol and vertical bar represent the mean of triplicate cultures and SEM. The number of target cells per well was 20,000.

decreases thereafer (Kiessling et al., 1975; Herberman et al., 1975). We obtained similar results (Fig. 3). Low NK activity showed by spleen cells from 2-week-old mice seemed unlikely to be due to suppressor cells. NK activity of a graded number of adult spleen cells was not affected by the addition of newborn spleen cells at the indicated

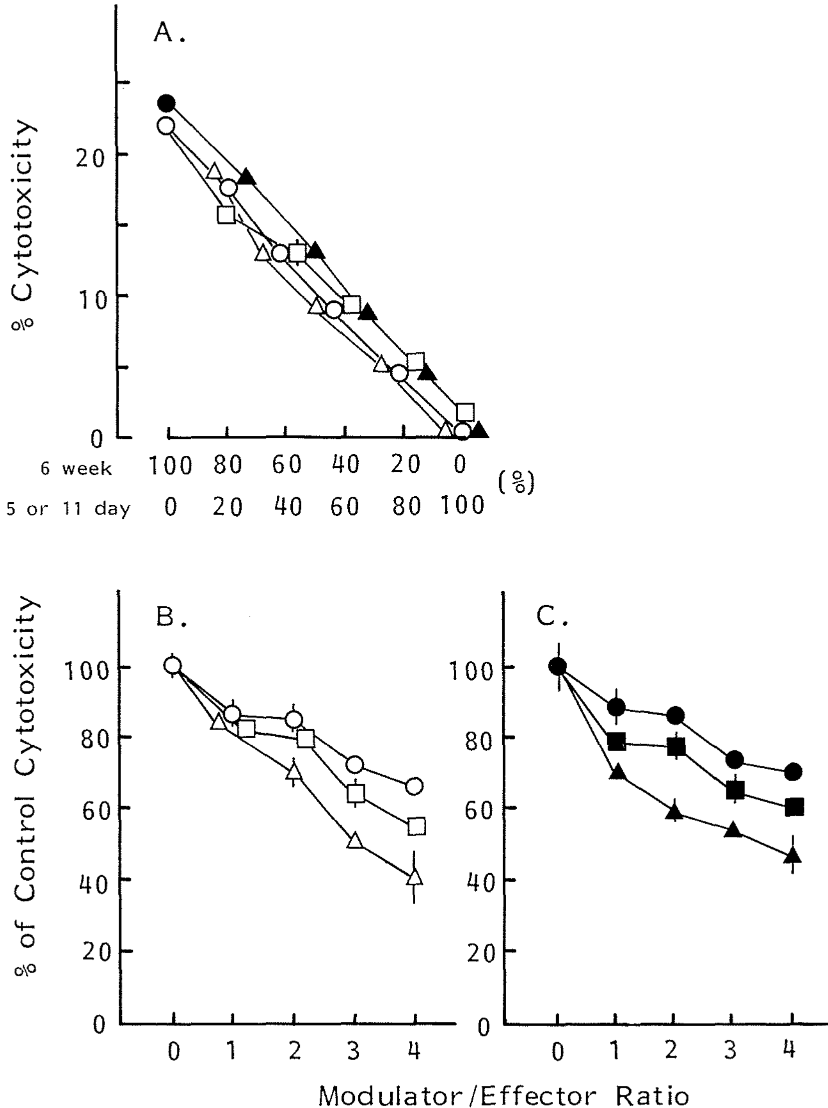

Fig. 4. Spleen cells of newborns were ineffective in suppressing NK activity of spleen cells of adults. In Panel A, mixtures of spleen cells from 6-week-old mice with spleen cells from 11-day-old mice (□) or from 5-day-old mice (△, ▲) were tested for NK activity at E/T=100, using a fixed cell density. Thymocytes served as control cells (○). In some cultures, 1 μg/ml indomethacin were present through NK assay (▲). In Panel B (without indomethacin) and Panel C (with indomethacin), similar mixtures of spleen cells as in Panel A were tested for NK activity at E/T=50, using a fixed number of spleen effector cells from 6-week-old mice. Thymocytes served as filler cells (○).

ratio (Fig. 4A). Thymocytes were used as a control cell preparation containing neither effector nor inhibitory cells (Cudkowicz & Hochman, 1979). Spleen cells from 5-day-old mice were slightly suppressive (Fig. 4B) and this effecet was similar in the presence of 1 μg/ml indomethacin (Fig. 4C). This apparent suppressive effect (Figs. 4B and 4C) may be due to competitive inhibition between mature NK cells and immature NK cells which bind but do not lyse target cells.

Discussion

Some functions of the macrophages have been shown to emerge earlier in the ontogenetic development than the functions of T cells, B cells (Spear et al., 1973; Inaba et al., 1982), and NK cells (Kiessling et al., 1975; Herberman et al., 1975, Hackett et al., 1986). In fact, PM from newborns have shown to have higher phagocytic activity (Nakano et al., 1978) and higher antitumor activity than those from adults in their lower dependency on LPS for being made cytolytic against tumor cells and in their inhibitory effect on tumor cell growth (Ido et al., 1984). How such macrophages affect the maintenance of NK activity of adult spleen lymphoid cells as indicator cells is an interesting problem.

Monocytes/macrophages have been reported to augment NK activity of lymphocytes rapidly during NK assay, and not to require or have low dependency on de novo RNA or protein synthesis (Suzuki el al., 1984; Bloom et al., 1986), but to have to be viable (Bloom et al., 1986). IL 1 and/or IFN are possibly involved in the direct augmentation of NK activity by newborn mouse PM, but, if so, the effect of monokines may be masked by the suppressive effect of prostaglandins.

IL 1 has been shown to affect tumor cells directly and alter them to increase their capacity to bind large granular lymphocytes and to be made vulnerable to the attack of the lymphocytes (Herman et al., 1985). In our preliminary experiments, newborn mouse PM were found to produce and release a higher titer of IL 1 to the supernatant of PM cultures stimulated by phagocytosis of latex particles in comparison with adult mouse PM (data not shown). IFN β has been reported to be spontaneously produced by newborn mouse PM adhering to plastic substrata (Inaba et al., 1986). Therefore, if there are intracellular pools of IL 1 or IFN in the PM of newborns, which did not require protein synthesis for secretion of them, these monokines may be involved in augmentation of NK activity by the PM of newborns.

Inhibition of prostaglandin synthesis by indomethacin was required for rapid augmentation of NK activity by the PM of newborns. This requirement is in accordance with the report that the Ia expression of the PM of newborns is inhibited by the prostaglandins produced (Snyder et al., 1982a; 1982b). The PM of newborns appeared to have both a positive effect (IFN), which appeared first in the presence of indomethacin, and a negative effect (prostaglandins), on rapid augmentation of NK activity.

Although the PM from adults stimulated by TGC *in vivo* did not rapidly augment NK activity in our experiments, another study showed that PM stimulated *in vivo* by BCG, OK–432 or glycogen augment NK activity (Suzuki et al., 1984). Since BCG and OK–432 are strong biological-response-modifiers, PM stimulated by one of them appears to be different from PM stimulated by TGC.

Monocytes/macrophages have been reported to be effective in augmentation of NK activity of lymphocytes during about one day coculture *in vitro* in rats (Reynolds et al., 1981) and in man (Bloom et al., 1986). The following three findings suggest that IFN produced by PM are important in such augmentation or maintenance of NK activity: 1) De novo RNA and protein synthesis by PM are required (Bloom et al., 1986); 2) The addition of indomethacin to the cultures was not necessarily required (data not shown). This was differnt from the case of the rapid augmentation of NK activity. In fact, IFN have been shown to decrease the production of prostaglandin E_2 by PM (Boraschi et al., 1984) and to decrease the sensitivity of NK cells to suppression by prostaglandin E_2 (Leung & Koren, 1982; 1984; Bash & Vogel, 1984); 3) PM-mediated augmentation was abrogated by the addition of anti-IFN α/β to the culture (Kura, 1988). IFN must be an important lymphokine in the maintenance of NK activity.

We showed that macrophages from the peritoneal cavity of adults were ineffective in maintaining NK activity, unless stimulated by LPS, but some investigators have shown that monocytes/macrophages of adults without any overt stimulation are effective in augmenting NK activity. Several factors may explain this discrepancy. First, monocytes/macrophages are derived from different organs and/or strains (Reynolds et al., 1981; Bloom et al., 1986). Second, a small amount of LPS contaminating the cultures might stimulate adult monocytes/macrophages. Third, independent of LPS, monocytes may already be somewhat activated as a consequence of the adherence procedure used to isolate them (Bloom et al., 1986). At least, under our conditions using murine PM, the PM of newborns were effective in maintaining NK activity without any overt stimulation, but those from adults were ineffective unless stimulated by LPS, or other agents.

Endogenous factors may affect the maintenance of NK activity other than exogenous factors like LPS-stimulation. In fact, when the PM were derived from C3H/HeJ mice, genetically unresponsive to LPS, even the PM of newborn mice were ineffective in maintaining NK activity. This phenomena may reflect endogenous factors defective in C3H/HeJ mice. Alternatively, there is a possibility that newborn PM were readily stimulated by LPS contaminating the cultures and the difference between the PM from newborns and adults in the maintenance of NK activity reflected the difference in sensitivity to LPS. This possibility is unlikely based on the finding that the PM of newborns were effective in maintaining NK activity even in the presence of polymyxin B. Conversely, C3H/HeJ mice have been reported to be defective in several macrophage functions independent of LPS (Vogel & Rosenstreich, 1979; Morgan & Weigle, 1980; Nowakowski et al., 1980; Vogel et al., 1981; Vetvicka et al., 1986). We would consider that the defect in maintenance of NK activity by C3H/HeJ PM reflect endogenous factors in regulation of NK activity.

Major histocompatibility complex did not seem to be involved in the maintenance of NK activity since C3H/HeSlc and C3H/HeJ have the same H–2 haplotype. On the other hand, we do not know whether the ineffective maintenance of NK activity by C3H/HeJ is attributed to the gene(s) that controls LPS responsiveness, because these mice are not congenic with C3H/HeSlc.

NK activity of newborn mouse spleen cells is low, and suppressor cells appeared not to be involved in the expression of the NK activity since NK–1^+ cells purified by

cell sorting do not express cytolytic activity against YAC–1 (Hackett et al., 1986). By mixing spleen cells of adult and newborn mice, we also confirmed that the low NK activity of newborn mouse spleen cells was not due to suppressor cells in the preparation.

The NK activity of the spleen cells of adults have apparently decreased by the addition of the spleen cells of newborns to the assay culture (Savary & Lotzová, 1978; Cudkowicz & Hochman, 1979). This may be due to the competitive inhibition for binding target cells between adult NK cells and newborn NK–1^+ cells (Koo et al., 1982), although the possibility of newborn suppressor cells cannot be excluded because of the production of prostaglandin or α-fetoprotein in neonates (Snyder et al., 1982a, 1982b; Kendall & Targan, 1980; Cohen et al., 1986).

Most investigators have detected the augmentation of NK activity of cells of newborn animals by IFN to some degree. Since the degree of the augmentation has been low, some investigators have reported that NK activity of cells of newborn animals is augmented by IFN (Flexman & Shellam, 1984; Read & Williams, 1984; Charley et al., 1985; Cohen et al., 1986), but others have reported that it is not (Koo et al., 1982; Hackett et al., 1986). The present finding that the PM of newborns were effective in maintaining NK activity of the spleen lymphoid cells of adults and the accompanying finding that IFN α/β is involved in the maintenance of NK activity by the PM of newborn mice (Kura, 1988) strongly suggest that the cytolytic activity of the spleen cells of newborn mice are regulated physiologically by IFN.

Several findings indicate that the PM of the newborns themselves or other cells affected by these PM are involved in suppression of cell growth, such as allo-reactions and tumor propagation (Argyris, 1979, 1982; Ido et al., 1984; Jadus & Peck, 1986). In addition to macrophages and NK cells, there are NS (natural suppressor) cells in neonate (Argyris, 1978) and NC (natural cytotoxic) cells in neonate and adult as inhibitory cells for cell growth. The cytolytic activity of NC cells have been shown not to fluctuated much during the ontogenetic development (Stutman et al., 1978). We would consider the function of newborn mouse PM for maintaining NK activity effectively *in vitro*, as an inhibitory function of the NC cells, NK cells, NS cells, and macrophages in the early stage of the ontogenetic development.

Suppressor cells in newborns appear to protect the host from the growth of mutated cells in the ontogenetic stage absent from antigen-specific immune responsiveness. Such suppressor cells may also inhibit host-versus-graft-like reactions of leaked maternal lymphocytes to the fetus, and inhibit the reactions of harmful self-reactive T cells in the thymus and insuring self-tolerance (Hansson et al., 1981, Holmberg at al., 1984, Maier et al., 1985, Hooper et al., 1986). In a later stage of life, such cells may regulate the growth and differentiation of bone-marrow cells.

In conclusion, there are significant functional differences between macrophages of newborn and adult mice in the ontogenetic development. PM of newborn mice appear to be in a semi-activated stage, endowed with the potential for recruiting the organization of host defense from other cells when they cannot yet induce an antigen specific immune response. On the other hand, PM of adult mice usually appear to be silent, but to be able to regulate the activity of cells participating in the organization of host defense, which are already constructed, in response to the environmental or endogenous stimulation.

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