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Comparison of the cellular cytotoxic activities of colostral lymphocytes and maternal peripheral blood lymphocytes

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Colostral lymphocytes (CL) from mothers 2 to 4 days post-partum and autologous maternal peripheral blood lymphocytes (PBL) were investigated for (1) natural killer (NK) and antibody-dependent cellular cytotoxic (ADCC) activities, (2) target binding ability, (3) interferon (IFN)- and interleukin 2 (IL2)-induced augmentation of NK activity, (4) lectin-dependent cellular cytotoxicity (LDCC), and (5) the ability of culture-derived soluble suppressor factor(s) to inhibit the NK activity of normal allogeneic lymphocytes. CL depleted of adherent cells and Percoll-separated NK-enriched subpopulations of CL demonstrated significantly lower NK and ADCC activities compared to autologous PBL. However, the target binding ability of CL was comparable to autologous PBL. Although the residual NK activity of CL was augmented by IFN and IL2, the activity was not enhanced to the same level shown by autologous PBL. CL also demonstrated a significant enhancement of LDCC activity, although the activity was not stimulated to the levels shown by PBL. Culture supernates of CL manifested greater suppression of the NK ability of allogeneic PBL than culture supernates produced by autologous PBL. These results are consistent with a model that suggests differential partitioning of lymphocyte subpopulations between colostrum and peripheral blood.

Key words: *colostrum, natural killer, interferon, interleukin 2*

Introduction

Numerous studies have demonstrated that breast fed infants are less susceptible than formula fed infants to various infectious agents (Grulee et al., 1934; Windberg and Wessner, 1971; Downham et al., 1976; Cunningham, 1977; Pitt et al., 1977; Larsen and Homer, 1978). Earlier investigations have shown passive transfer of cell mediated immunity from mother to infant through ingestion of breast milk (Mohr, 1973; Ogra et al., 1977). Natural killer (NK) cells and cells mediating antibody-dependent cellular cytotoxic (ADCC) activities have been gaining attention due to their proposed role in immune surveillance against neoplasms and protection against viral infections (O'Toole et al., 1974; Herberman and Holden, 1978; Ching and Lopez, 1979; Nair et al., 1980; Welsh, 1981; Djeu et al., 1982; Engler et al., 1982;

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Bishop et al., 1983). Studies have demonstrated differential surface characteristics and functional activities between colostral and peripheral blood leukocytes (Parmely et al., 1976; Mohr et al., 1980; Pickering et al., 1980; Richie et al., 1980; Kohl et al., 1980; Richie et al., 1982). The current investigations were undertaken to examine the natural and antibody-dependent cellular cytotoxic activities of colostral lymphocytes (CL) against tumor target cells in comparison with peripheral blood lymphocytes (PBL). The results demonstrate a profound deficiency in the NK and ADCC functions of CL against tumor targets in comparison with PBL. Furthermore, these studies support the premise that the cellular subpopulations in CL are different from those in PBL.

Materials and Methods

Isolation of colostral lymphocytes

After obtaining informed consent, colostrum from 17 mothers 2 to 4 days post-partum was collected using a breast pump (type SMB, Egnell, Carry, IL). None of the women had fever or mastitis and received on medication to suppress milk production. Colostrum was diluted 1 : 3 with Ca^{2+}- Mg^{2+}-free Hank's balanced salt solution (HBSS) and centrifuged at $400 \times g$ for 20 min. The fatty top layer and supernates were discarded and the pellet was resuspended in HBSS and centrifuged at $400 \times g$ for 10 min to remove additional fat. The cell pellet was layered on a Ficoll–Hypaque cushion employing a modified method of Boyum (1968). The interface containing mononuclear cells was collected and washed 3 times in HBSS. Slides for differential cell counts were prepared using a cytocentrifuge. The percentage of lymphocytes ranged from 34 to 74% as determined by Wright stain with 82 to 97% viability as evaluated by trypan blue dye exclusion. The percentage of monocytes ranged from 13 to 17% as identified by alpha-naphthyl-acetate esterase staining. To remove adherent cells, washed colostral cells were resuspended in HBSS and allowed to adhere to plastic Petri dishes for 2 h at 37°C in a 5% CO_2 in air atmosphere.

Removal of residual adherent cells

The nonadherent cells were applied to a G-10 column to remove any residual adherent cells as described previously (Nair and Schwartz, 1982). Colostral cells were resuspended in RPMI 1640 medium containing 25 mM HEPES buffer supplemented with 80 μg/ml gentamicin and 300 μg fresh glutamine/ml plus 10% FCS (complete medium) and passed over a 7 ml column of Sephadex G-10 beads (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in the same medium. After 45 min of incubation at 37°C, nonadherent cells were eluted with one bed volume of warm (37°C) medium. The viability of cells was greater than 95% as assessed by trypan blue dye exclusion. Cell recovery was $> 65\%$ of the total input and monocyte contamination as indicated by non-specific esterase staining was $< 3\%$.

Isolation of peripheral blood lymphocytes

Heparinized (20 U/ml) maternal peripheral blood was obtained simultaneously and mononuclear cells were also isolated as described above. Blood was diluted with an equal volume of normal saline and centrifuged at $400 \times g$ for 30 min at 18°C. The mononuclear cell band was harvested, washed 3 times with saline and resuspended in complete medium. Mononuclear cells were depleted of adherent cells by passing through a G-10 column as above. The cell recovery of the G-10 passed population was > 70% of the total input, with 95% viable lymphocytes and < 2% monocytes.

Percoll fractionation of lymphocytes

The enrichment of NK and ADCC effector cells using a discontinuous density gradient of Percoll (Pharmacia) was carried out as described (Timonen and Saksela, 1980). To prepare the density gradient, Percoll solution was mixed at various concentrations with RPMI 1640 medium and 2 ml aliquots (ranging from 47.5 to 37.5% in 2.5% increments) were gently layered into 15 × 130 mm round-bottom glass test tubes. Adherent cell-depleted lymphocytes were layered on top of the gradient and centrifuged at $300 \times g$ for 45 min at 20°C. Five fractions, with the uppermost (37.5%) designated '0' and the lowermost (47.5%) designated 'IV', were collected from the top with a Pasteur pipet and washed twice in RPMI 1640 medium. Recovery of cells was ~ 85% of the input, and viability always exceeded 95% by trypan blue dye exclusion.

Preparation of tumor target cells

The human erythroleukemia cell line, K-562, was used as targets for NK cells. For the ADCC assay, we chose as targets an antibody-coated human B cell leukemia line (SB) because of its resistance to NK activity in a 4 h ^{51}Cr release assay. Tumor target cells were serially passaged in complete medium and used in cytotoxicity assays no more than 48 h after the last passage. To 0.8 ml aliquots of complete medium containing 5×10^6 washed tumor cells, 200 μCi of ^{51}Cr as sodium chromate (New England Nuclear, Boston, MA) were added. The cells were incubated at 37°C for 1 h in a humidified atmosphere of 5% CO_2 in air, with intermittent shaking. After incubation, the cells were washed 3 times with complete medium and resuspended to a concentration of 2×10^5 cells/ml.

Assay for NK activity

NK activity was measured in a direct ^{51}Cr release assay as described previously (Nair and Schwartz, 1981, 1982). A fixed number of viable effector cells in complete medium was added to triplicate cultures of ^{51}Cr-labeled targets in microtitration plates (CoStar, Cambridge, MA). After centrifugation at $40 \times g$ for 2 min, they were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 4 h. At the end of incubation, 100 μl aliquots were removed from each well and transferred to glass tubes and read in a Packard Model 593 gamma counter. Percent cytotoxicity was calculated as follows:

$$\% \text{ cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100 \qquad (1)$$

where spontaneous release represents counts released from control wells containing only 2×10^4 target cells, and total release represents counts obtained from an aliquot of 2×10^4 target cells.

Assay for ADCC

The ADCC activity of effector cells was determined as described previously (Nair and Schwartz, 1981, 1982, 1984). Briefly, 50 μl of varying concentrations of effector cells were added to 50 μl of complete medium containing ^{51}Cr-labeled SB target cells and 100 μl of a 2×10^{-4} dilution of rabbit anti-SB antisera previously found to yield maximum ADCC. Percent cytotoxicity was calculated as described above for NK activity with the following exceptions. Spontaneous release represents counts released in control wells containing effector cells, labeled SB target cells and media instead of anti-SB antibodies, and total release represents counts obtained in an aliquot of 1×10^4 ^{51}Cr-labeled SB target cells.

Interferon

Recombinant IFN α (sp. act., 1×10^8 U/mg protein) has been provided as a gift from Schering Corporation, Bloomfield, NJ. IFN was diluted in RPMI 1640 and stored at $-70°$C before use. The maximum NK augmenting dose was 250 U/ml as determined previously. IFN was diluted in RPMI 1640 medium and stored at $-70°$C before use.

Interleukin-2 (IL-2)

Commercially available IL-2 (Electro-Nucleonics Labs, Inc., Silver Spring, MD) was diluted in RPMI 1640 and stored at $-70°$C before use. A 10% final concentration, the maximum NK augmenting dose, as determined previously, was used.

Treatment of effector cells with IFN or IL-2

Effector cells (2×10^6/ml) were washed and suspended in 1 ml of RPMI 1640 plus 5% FCS to which IFN or IL2 was added as above. The cultures were incubated for 24 h at 37°C in a humidified atmosphere of 5% CO_2 in air, washed twice and resuspended in medium. Control cultures were treated identically with the exception that either IFN or IL2 was not added. Viability of treated lymphocytes was unaffected as assessed by trypan blue dye exlusion. Both treated and control cultures were assayed for NK activity.

Target binding assay

A target binding assay was used as described by Haliotis et al. (1980). In brief, lymphocytes were washed twice and mixed with a 5-fold excess of tumor cells, centrifuged at $150 \times g$ for 5 min and incubated at 37°C for 5 min. Following incubation, lymphocyte target mixtures were transferred to ice, the pellets were gently resuspended and the number of lymphocytes binding to tumor targets was determined using a hemocytometer.

Lectin dependent cell mediated cytotoxicity (LDCC) assay

The LDCC assay was carried out similar to the NK assay except that NK-resistant SB cells were used as targets. Briefly, appropriate numbers of target and effector cells were added to V-bottom microtitration plates and various concentrations of phytohemagglutinin-P (PHA, Difco Laboratories, Detroit, MI), 1, 2.5 and 5 μg/ml, were added to a final volume of 0.2 ml and incubated for 4 h at 37°C in a humidified 5% CO_2 in air incubator. Percent LDCC activity was calculated as described for NK activity and was compared with cytotoxicity observed with control cultures containing effector and target cells in the absence of PHA. Percent cytotoxicity was calculated as in eqn. 1, where spontaneous release represents counts released from control wells containing 2×10^4 target cells plus lectin and total release represents counts obtained from an aliquot of 2×10^4 target cells. Statistical significance of all the results was determined using a single tail Student's '*t*'-test.

Preparation of soluble suppressor factors (SSF)

SSF was prepared as described previously (Shou et al., 1980; Nair and Schwartz, 1982). Briefly, CL or PBL were washed three times with normal saline and resuspended in RPMI 1640 medium containing only gentamicin and glutamine. Suspensions of 5×10^6 cells/ml were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 5 to 7 days. After incubation, supernates were separated by centrifugation at $500 \times g$ for 30 min and stored at -20°C. Control supernates prepared in the absence of either CL or PBL were treated similarly and termed as mock SSF.

Incubation of effector cells with SSF

Duplicate sets of cultures containing 2×10^6 lymphocytes from healthy unrelated donors in 0.8 ml of RPMI 1640 plus glutamine, gentamicin and 5% FCS were added to tubes containing 200 μl of SSF or mock SSF (20% v/v) and incubated in a humidified environment of 5% CO_2 in air, at 37°C for 72 h. Cells were then washed twice and resuspended in complete medium. Viability of SSF treated cultures was comparable with that of mock SSF treated cultures as determined by trypan blue dye exclusion and was 80 to 90%. Treated and control cultures were tested for their ability to mediate NK activity.

Results

NK and ADCC activities of colostral lymphocytes

CL have been shown to be poor effectors of cellular cytotoxicity against virus infected targets as well as in bactericidal assays (Kohl et al., 1980; Pickering et al., 1980). Mononuclear cells depleted of adherent cells and NK-enriched Percoll separated colostral cells were tested for their NK and ADCC activity against tumor target cells. Data presented in Table 1, show that CL depleted of adherent cells demonstrated significantly lower NK and ADCC activities, respectively, against K562 and antibody-coated SB target cells compared to maternal PBL at all E : T cell

TABLE 1

NK and ADCC activities of colostral and peripheral blood lymphocytes [a]

Targets	Effector source	Effector : target cell ratio % cytotoxicity [b]		
		100:1	50:1	20:1
	PBL	52.8 ± 8.3	33.3 ± 6.3	21.0 ± 3.6
K562 (NK)		($P < 0.0005$)	($P < 0.0005$)	($P < 0.0005$)
	CL	4.5 ± 1.2	3.1 ± 1.2	1.5 ± 0.5
	PBL	33.0 ± 4.9	22.1 ± 2.8	9.5 ± 1.5
SB (ADCC)		($P < 0.0005$)	($P < 0.0005$)	($P < 0.0005$)
	CL	7.3 ± 2.1	0.8 ± 0.1	0.8 ± 0.2

[a] Effector cells were mixed with target cells as described in Materials and Methods in a 4 h ^{51}Cr release assay. The results are the mean of 14 different colostral lymphocyte samples and 7 different maternal peripheral blood lymphocytes samples ± SD performed in triplicates.

[b] Calculated as in Materials and Methods.

ratios. The possibility that dilution of NK cells with other lymphocytes in the colostral lymphocyte pool may be responsible for the diminution of NK and ADCC activities was examined by investigating the cytotoxic activities of Percoll-enriched NK and ADCC effector cells. The data presented in Fig. 1 reveal that NK- and ADCC-enriched CL from the 37.5% Percoll fraction 0 also showed significantly diminished NK (8%; $P < 0.005$) and ADCC (5%; $P < 0.01$) activities compared to higher NK (29%) and ADCC (24%) activities displayed by a similar Percoll fraction of maternal PBL. CL from fraction II also manifested lower levels of NK (1.2%) and ADCC activities (0.5%) as contrasted with higher levels of NK (16%) and ADCC

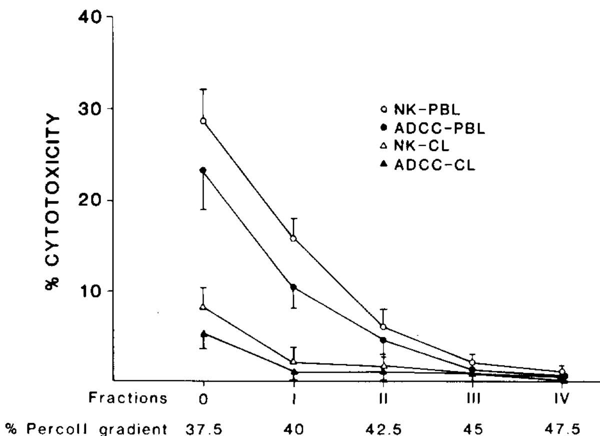

Fig. 1. NK and ADCC activities of Percoll-enriched colostral lymphocytes. Lymphocytes depleted of adherent cells were separated on a discontinuous Percoll gradient as in Materials and Methods. Values represent mean percent cytotoxicity ± SD of three separate experiments with triplicate determinations for each experiment at a 10:1 E:T cell ratio.

TABLE 2

Target binding ability of colostral lymphocytes

Effector cells	Target binding lymphocytes (%)
PBL	12.6 ± 4.1
CL	13.6 ± 3.5

Effector and target (K562) cells at an effector: target ratio of 5:1 and incubated at 37°C for 30 min, followed by 0°C for 1 h. Aliquots were examined in a hemocytometer and a positive target binding cell was scored for each lymphocyte binding 2 or more target cells. The results are expressed as mean ± SD of 4 experiments performed in duplicate.

(11%) activities manifested by the same fraction of PBL. Lymphocytes from nonlytic fractions, II to IV of both CL and PBL did not manifest significant differences in NK or ADCC activities.

To examine whether the lack of cytotoxicity observed with colostral cells is due to interference with a receptor-associated target recognition structure rather than inhibition of the lytic mechanism, target binding assays were performed. The results presented in Table 2 show that 13% of CL formed conjugates with K562 target cells compared to 12% formed by PBL. This suggests that the NK deficiency of CL may be associated with post binding events such as the production or release of soluble NK cytotoxic factor(s) (NKCF), rather than their target binding capacity.

Effect of IFN and IL2 on the NK activity of colostral lymphocytes

We investigated whether IFN and IL2, agents known to augment the NK activity of lymphocytes, could enhance the cytotoxicity of CL to similar levels shown by PBL. Data presented in Table 3 demonstrate that PBL pretreated with IFN for 24 h produce 49% NK activity compared to 21% cytotoxicity displayed by untreated lymphocytes ($P < 0.0025$). PBL precultured with IL2 also demonstrated a signifi-

TABLE 3

Effect of interferon (IFN) and interleukin 2 (IL2) on NK activity of colostral lymphocytes [a]

Effector cells	Treatment	% Cytotoxicity [b]	% Enhancement [c]
PBL	none	21.2 ± 3.8 [d]	
PBL	IFN	49.2 ± 6.8 ($P < 0.0025$)	132.1
PBL	IL2	61.2 ± 8.2 ($P < 0.0005$)	188.6
CL	none	1.5 ± 0.2	
CL	IFN	2.8 ± 0.2 ($P < 0.05$)	86.6
CL	IL2	3.1 ± 0.7 ($P < 0.05$)	106.6

[a] Effector cells were cultured alone or with IFN (250 U/ml) or IL2 (10% v/v), for 24 h at 37°C, washed and tested for NK activity against K562 target cells at a 20:1 E:T cell ratio.

[b] Calculated as in Materials and Methods.

[c] % Enhancement was calculated in comparison to the cytotoxicity obtained when lymphocytes were precultured in media alone.

[d] Values represent mean % cytotoxicity ± SD of 4 separate experiments done in triplicate.

cantly higher cytotoxicity of 61% compared to 21% cytotoxicity shown by untreated lymphocytes ($P < 0.0005$). CL pretreated with IFN and IL2 showed 2.8% ($P < 0.05$) and 3.1% ($P < 0.05$) cytotoxicity, respectively, compared to 1.5% cytotoxicity demonstrated by untreated colostral cells. This suggests that although the residual cytotoxicity of CL could be enhanced by either IFN or IL2, the cytotoxicity could not be augmented to the extent shown by PBL.

Effect of lectin on cellular cytotoxicity of colostral lymphocytes

Since CL demonstrated diminished NK and ADCC activities and IFN- and IL2-induced NK activities compared to PBL, we examined whether the cytotoxicity of CL could be activated with lectin. Data presented in Table 4 demonstrate that PBL at a 50:1 E:T cell ratio produced 44 ($P < 0.005$), 47 ($P < 0.0005$) and 40% ($P < 0.005$) LDCC activity against SB targets at 1, 2.5 and 5 μg/ml of PHA, respectively, compared to 6.9% cytotoxicity displayed in absence of PHA. CL exhibited 0.6% cytotoxicity without PHA, which was significantly enhanced to 12 ($P < 0.0025$), 15 ($P < 0.0005$) and 11% ($P < 0.005$) by PHA at 1, 2.5 and 5 μg/ml concentrations, respectively. Although PHA could significantly enhance the low cytotoxic activity of CL it was not stimulated to the levels shown by PBL.

Effect of colostral lymphocyte culture supernates on the NK activity of normal lymphocytes

Since CL demonstrated reduced NK activity compared to PBL, we examined whether abnormal synthesis and secretion of culture-induced soluble suppressor factor(s) (SSF) by CL might be responsible for such reduced NK activity. Data presented in Table 5 demonstrate that lymphocytes from healthy unrelated donors precultured separately with different culture supernates prepared from other unrelated normal donor PBL manifested 40, 42, 40 and 34% cytotoxicity compared to a mean of 54% cytotoxicity shown by lymphocytes precultured in medium alone (26, 22, 26 and 36% suppression of cytotoxicity, respectively). Lymphocytes precultured separately with different CL culture supernates showed a variable suppression of cytotoxicity ranging from 12.5 to 79.7%. CL culture supernates from samples 2, 3, 5

TABLE 4

Effect of lectin on cellular cytotoxicity of colostral lymphocytes [a]

Lymphocyte source	PHA concentrations (μg/ml) % cytotoxicity			
	0	1	2.5	5
PBL	6.9 ± 2.2 [b]	44.4 ± 9.1 ($P < 0.005$)	47.5 ± 6.2 ($P < 0.0005$)	40.6 ± 7.2 ($P < 0.005$)
CL	0.65 ± 0.07	12.3 ± 2.5 ($P < 0.0025$)	15.3 ± 2.7 ($P < 0.0005$)	11.6 ± 2.8 ($P < 0.005$)

[a] Effector cells were mixed with SB targets and % cytotoxicity was calculated as in Materials and Methods.

[b] Values are mean ± cytotoxicity ± SD of 4 separate experiments employing 5 CL and 4 PBL samples. Experiments were performed in triplicate at a 50:1 E:T cell ratio.

and 7 showed highly increased inhibition of NK activity of allogeneic lymphocytes compared to inhibition shown by culture supernates from normal PBL. This suggests that CL can secrete a soluble suppressor factor(s) into the culture supernates and such factors may be partly responsible for their decreased NK activity.

Discussion

Although the protective effects of humoral factors from colostrum have been established, the cellular activities of colostrum remain under active investigation (Beer et al., 1974; Pett et al., 1977; Pittard et al., 1977; Ho and Lawson, 1978; Ogra and Ogra, 1978; Head and Beer, 1979; Meggs and Beer, 1979; Cleary et al., 1980; Kohl et al., 1980; 1982; Keller et al., 1981). Several earlier investigations have shown that CL represent a population of lymphocytes different from those in peripheral blood (Kohl et al., 1978; Richie et al., 1980; 1982; Kohl et al., 1982). Richie et al. (1980) demonstrated that lymphocytes from colostrum form E rosettes at 4 and 37°C whereas autologous peripheral blood lymphocytes form E rosettes only at 4°C. They further observed (Richie et al., 1982) a relatively low ratio of OKT4/OKT8-positive T cell subsets in colostrum compared to PBL. Parmely et al. (1976) showed that CL were hyporesponsive to nonspecific mitogens and alloantigens as contrasted with PBL. Reduced cellular cytotoxicity of colostral leukocytes against virus-infected

TABLE 5

Effect of soluble suppressor factor (SSF) on NK activity of lymphocytes [a]

Source of SSF	SSF sample no.	Cytotoxicity [b] (%)	Suppression (%)
MOCK	1	51.2	–
	2	58.6	–
	3	53.1	–
PBL	1	40.2	25.9
	2	42.4	21.9
	3	39.9	26.5
	4	34.5	36.4
CL	1	44.5	17.4
	2	11.0	79.7
	3	14.7	72.9
	4	47.5	12.5
	5	20.0	63.1
	6	41.3	23.9
	7	18.6	65.7
	8	34.5	36.4

[a] Effector cells depleted of adherent cells were precultured with either SSF or mock SSF, and tested for their NK activity at a 50:1 E:T cell ratio.

[b] Calculated as in Materials and Methods.

[c] % Suppression of cytotoxicity was calculated on the basis of the mean cytotoxicity (54.3 ± SD 3.8) obtained when media alone were used in the culture.

targets and in bactericidal assays has also been reported (Kohl et al., 1980; Pickering et al., 1980). Cytotoxic T cells directed against certain HLA phenotypes were also shown to be absent in colostrum in comparison with blood lymphocytes (Parmely and Williams, 1979). Kohl et al. (1980) demonstrated that colostral cells do not mediate cytotoxicity against Herpes simplex virus-infected target cells and also displayed a lower level of ADCC against the same target cells. The present investigation shows that CL demonstrate reduced NK and ADCC activities against tumor target cells compared to PBL. The reduced cytotoxicity is not due to the dilution of effector cells with neutrophils and adherent cells which are initially present in large numbers in the colostrum leukocyte population. The removal of neutrophils on a Ficoll–Hypaque density gradient, and adherent cells by adherence on Petri dishes and passage through G-10 columns yielded > 87% lymphocytes as measured by Wright and esterase staining. These partially purified colostral cells demonstrated reduced cytotoxicity compared to a total mononuclear cell preparation from peripheral blood which also contained about 80 to 87% lymphocytes (data not presented). Timonen and Saksela (1980) described the sedimentation characteristics of human NK cells effective against K562 targets by using a discontinuous Percoll gradient. In the present study, colostral effector cells enriched on a Percoll gradient (fraction 0 and I) also demonstrated significantly lower NK and ADCC activities compared to PBL (Fig. 1). CL from fractions II to IV did not show any detectable cytotoxicity suggesting that colostral cytotoxic effector cells do not have sedimentation characteristics different from PBL.

Further, we examined whether the lack of cytotoxicity by CL may be due to a decreased target binding ability. It was observed that CL exhibited target binding capacity comparable to PBL (Table 2). Analysis of lymphocyte surface markers as defined by monoclonal antibodies and fluorescence-activated flow cytometry demonstrated that CL contained 3 to 5% $Leu7^+$ cells (data not presented). This suggests that the reduced cytotoxicity displayed by CL may be due to intrinsic defects of the effector cells rather than a defective target binding capacity or lack of adequate number of phenotypically positive NK cells. Alternatively, the Leu 7 + cells in colostrum may not be functional NK cells.

IFN and IL2 seem to exert a major role in immunoregulation (Gillis and Watson, 1980; Johnson and Blalock, 1980; Kadish et al., 1980; Attallah et al., 1981; Ruscetti and Gallo, 1981; Nair and Schwartz, 1983). IFN and IL2 augment NK activity both in vitro and in vivo (Herberman et al., 1979; Henny et al., 1981; Perussia and Trinchieri, 1981; Nair and Schwartz, 1982). IFN appears to enhance NK by more than one mechanism. IFN may activate non-cytotoxic pre-NK cells to mature cytotoxic effector cells (Saksela et al., 1979) or activate mature NK cells that are transiently inactive (Perussia and Trinchieri, 1981; Bishop and Schwartz, 1982). Alternatively, IFN may potentiate endogenous lytic mechanisms by developing new receptors or modulating preexisting cell surface molecules or their activity (Saksela et al., 1979; Ortaldo et al., 1980; Gustafsson and Lundgren, 1981; Perussia and Trinchieri, 1981). It was reported that milk lymphocytes could produce lymphocyte derived chemotactic factor (LDCF) and IFN (Emodi and Just, 1974; Lawton et al., 1979; Keller et al., 1981). Previously, it was demonstrated that IL2 was involved in

the production of IFN and in the establishment of NK clones (Dennert, 1980; Dennert et al., 1981; Nabel et al., 1981; Handa et al., 1983). The present investigation describes a deficiency in the cytotoxic response of CL to IFN and IL2. Although residual NK activity of CL was augmented by IFN and IL2, the activity was not enhanced to the same level shown by autologous PBL (Table 3).

NK cells appear to have some selectivity since certain targets are very susceptible whereas others are resistant. This may be due to differences in the antigenic structures present on target cells or the receptor sites on NK cells or both (Bishop et al., 1983). It has been demonstrated that lymphocyte cytotoxicity can be activated by lectin (PHA). The exact function of lectin activation is not presently understood, but it has been suggested that it may act directly on cytotoxic cells by rearranging cryptic receptors for recognition sites on target cells providing sufficient signals to trigger subsequent lytic events (Brunda et al., 1982; Spits et al., 1982). In the present report the cytotoxicity of CL could be significantly enhanced by PHA, even though cytotoxicity could not be enhanced to the levels shown by PBL (Table 4), suggesting a deficiency in the LDCC activity of CL.

The role of soluble suppressor factors as mediators of suppressor cell activities has been well documented (Hofman et al., 1980; Sasports et al., 1980; Shou et al., 1980; Broder et al., 1981; Fleisher et al., 1981; Lederman et al., 1981). We have reported that a SSF could be isolated from the culture supernates of PBL from healthy donors which was capable of inhibiting NK and ADCC activities of normal effector cells (Nair and Schwartz, 1982). In the present investigation, 4 of 8 colostral culture supernates showed greater suppression of the NK activity of allogeneic PBL than SSF produced by PBL (Table 5) suggesting that the depressed NK activity of CL may be correlated with the development of highly active SSF. Further, it has been demonstrated that NK cells possess low avidity Fc receptors (Bakacs et al., 1977; Kall and Koren, 1978; Perussia and Trinchieri, 1984) and blocking of such receptors may lower NK activity (Koren and Williams, 1978). The greater suppressor activity of CL supernates may also be attributed to a nonspecific interference or blockage of Fc receptors by IgA which is shed by CL during preculture. This is consistent with the finding of a decrease in Fc receptor activity of blood granulocytes and monocytes after incubation with colostrum (Kohl et al., 1980; Pickering et al., 1980).

The apparent lack of cytotoxicity by CL suggests that the mammary gland may be capable of recruiting certain lymphocyte subsets that are functionally inactive in mediating NK and ADCC activities. It is also possible that immunologic and hormonal events occurring during pregnancy may render potential cytotoxic cells to an inactive phase. This is supported by the evidence of reduced NK cell activity during pregnancy (Barrett et al., 1982). The absence of cytotoxic activities of CL may also be due to a lack of amplifier subpopulations, target binding/post binding phase activities or the ability of effector cells to produce cytotoxic mediators like soluble NKCF. Such studies are presently being undertaken. An interesting hypothesis arising from these studies is that the suppression of potentially cytotoxic cells in breast milk may protect the immunologically immature infant analogous to those mechanisms protecting the fetal allograft.

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