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Modulation of human natural killer cell activity by pharmacological mediators

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SUMMARY

We have studied the effects of various pharmacological mediators on human NK cell activity. Prostaglandin E_2 (PGE_2) inhibits NK cell activity in a dose-dependent fashion, whereas $PGF_{2\alpha}$ has no significant effect over the same concentration range. Histamine at high doses (10^{-4} M) induced a small but significant inhibition of NK cell activity which was mimicked by both H_1 and H_2 specific histamine receptor agonists. Inhibition of endogenous prostaglandin production by indomethacin did not alter NK cell activity. Inhibition of NK activity by cAMP but not cGMP analogues, together with other data presented suggests that the mechanism of PGE_2-induced inhibition of NK cell activity is not due to impairment of effector cell movement or effector:target cell interaction, but through the adenylate cyclase system which modulates the killing process.

Keywords NK cells pharmacological modulation cyclic AMP

INTRODUCTION

Natural killer (NK) cells are large granular lymphocytes (LGL; Timonen, Ortaldo & Herberman, 1981), which spontaneously kill susceptible target cells and are believed to play a role both in immune surveillance (Jondal, Spina & Targan, 1978; Santoli & Koprowski, 1979) and immune regulation (Cudkowitz & Hochman, 1979; Hansson *et al.*, 1979). There is now compelling evidence that pharmacological mediators modulate immune reactions (Mozes *et al.*, 1974; Melmon, Rocklin & Rosenkranz, 1981) and in particular T cell-mediated responses (Brostoff, Pack & Lydyard, 1980; Boot, Hudspith & Brostoff, 1980). Although the lineage of NK cells in unclear, both T cell markers (West *et al.*, 1977) and monocyte markers (Ortaldo *et al.*, 1981) have been described on human LGL. The presence of both E receptors and Fc receptors for IgG on about 50% of human LGL suggests that an appreciable proportion of these cells can be categorized as T gamma (T_G) cells (Herberman, 1981). Therefore, we have studied the effects of various pharmacological mediators which modulate T cell responses, i.e. histamine and prostaglandins (PG) E_2 and $PGF_{2\alpha}$ on human NK cell activity using K-562 target cells.

Although histamine had a small but variable inhibitory effect on NK activity, PGE_2 was found to be a highly potent inhibitor of NK cell activity and we have studied the mechanism of this inhibition. The results show that like PGE_2, cAMP analogues inhibit NK activity and that the time course of inhibition is identical. Furthermore, PGE_2 does not interfere with effector cell movement or interaction with target cells or the susceptibility of target cells to lysis. The results suggest that PGE_2 inhibition of NK cell activity is via the PGE_2 receptor linked adenylate cyclase system, which modulates the killing process.

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MATERIALS AND METHODS

Preparation of effector and target cells. Peripheral blood mononuclear cells were obtained from normal healthy volunteers by Ficoll-hypaque centrifugation as previously described (Boot *et al.*, 1980). These effector cells (E) were washed twice and resuspended in RPMI 1640 supplemented with 10% fetal calf serum.

K-562, a cell line susceptible to human NK cell activity, was obtained from Dr M. F. Greaves (I.C.R.F., London) and maintained in culture until required. The target cells were washed twice before use in the NK cell assay. Viability of both effector and target cells was routinely >95% as judged by u.v. microscopy using ethidium bromide and acridine orange.

Assay for NK cell activity. NK cell activity was assessed by the method of Jondal & Pross (1975). Briefly, 1×10^4 ^{51}Cr-labelled K-562 target cells were incubated with or without drugs and an appropriate number of effector cells in 0·2 ml medium in round bottomed microtitre plates. The plates were centrifuged for 5 min at 150 *g* and then incubated for 4 h when 0·1 ml of supernatent was removed for counting. Percentage specific cytotoxicity was calculated by the formula: 100 × (supernatant ct/min − spontaneous release ct/min)/(total ct/min − spontaneous release ct/min). The spontaneous chromium release was between 8 and 15% and was not significantly altered by any drugs used in these experiments. The variability among at least triplicate wells was less than 10%. Statistical significance was assessed by Students' paired *t*-test.

Pharmacological mediators. PGE_2 and $PGF_{2\alpha}$ (Sigma Chemicals) were dissolved in ethanol at 10^{-3} M. Further dilutions of this stock were made in RPMI 1640 to give appropriate concentrations.

Indomethacin, a prostaglandin synthetase inhibitor (Sigma Chemicals), was dissolved in ethanol at 4 mg/ml and diluted in medium to give a final concentration of 1 μg/ml in the effector–target cell mixture.

Histamine dihydrochloride (Sigma Chemicals) and the H_1 and H_2 specific histamine receptor agonists (2- and 4-methylhistamine respectively, kindly provided by Smith, Kline & French, Ltd.) were dissolved in RPMI 1640.

N^6,O^2-dibutyryl adenosine 3,5 cyclic monophosphate (DiBcAMP) and N^2,O^2-dibutyryl guanosine 3,5 cyclic monophosphate (DiBcGMP, Sigma Chemicals) were dissolved in RPMI 1640 at 2×10^{-3} M and kept frozen until use.

Mediators were either added at the start of the 4 h assay (direct addition) or to the effector cells for 4 h pre-incubation and included for a further 4 h following addition of ^{51}Cr-labelled target cells. None of the drugs at the highest concentrations used significantly altered the viability of effector or target cells as judged by ethidium bromide/acridine orange staining.

Measurement of effector–target cell conjugation. The method used was a modification of the assay developed by Grimm & Bonavida (1979). Briefly, effector and target cells with and without PGE_2 (10^{-6} M) were centrifuged together as in the ^{51}Cr release assay, but only left in contact for 15 min. One hundred and fifty microlitres of medium was removed, the cells gently resuspended in the remaining medium and diluted in 1·5 ml of 0·7% agarose (Indobiose A37 1BF, lot No. 834) at room temperature. The lymphoid cells binding to target cells were counted using an inverted microscope and expressed as a percentage of bound and unbound lymphoid cells. One hundred mononuclear cells were counted in three different cultures. The viability of both effector and target cells was >97%.

RESULTS

The effect of histamine, H_1 and H_2 agonists on NK cell activity

As shown in Fig. 1, histamine had little effect on NK cell activity over a large concentration range, whether added directly to the 4 h NK assay or after a 4 h pre-incubation period with effector cells prior to addition of target cells. However, when more subjects were studied using the highest concentration of histamine, H_1 and H_2 specific agonists (Fig. 2), a small but significant inhibition of NK cell activity was produced by all three drugs, although individual variation was considerable. Inhibition was greater when the drugs were added directly to the assay than after pre-incubation of effector cells with drugs prior to the addition of target cells.

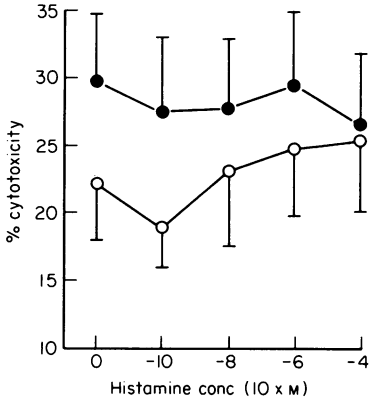

Fig. 1.

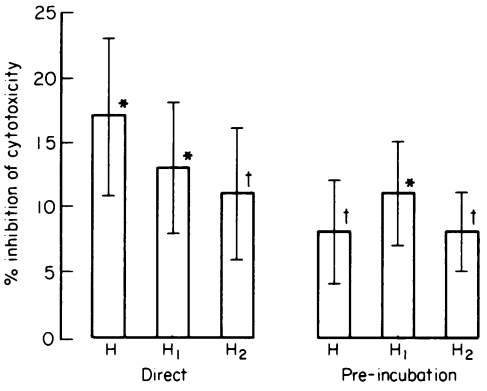

Fig. 2.

Fig. 1. Histamine was added directly to the NK assay (●) or pre-incubated for 4 h with effector cells before addition of target cells (○), at concentrations from 1×10^{-10} to 1×10^{-4} M. Each point represents the mean ±s.e. of experiments on four different subjects. E:T = 20:1.

Fig. 2. Histamine, 2-methyl and 4-methyl histamine (H_1 and H_2 receptor agonists, respectively) were added directly to the NK assay or pre-incubated with effector cells at 1×10^{-4} M final concentration. Each bar represents the mean ±s.e. of 12 different donors studied. Mean control cytotoxicity = 33·2±4·9%. E:T = 20:1. paired Student's *t*-test: * $P < 0.02$; † $P < 0.05$.

Inhibition of NK cell activity by PGE_2 but not $PGF_{2\alpha}$

As shown in Fig. 3, both direct addition and pre-incubation of effector cells with PGE_2 produced a dose-dependent inhibition of NK cell activity (E:T = 20:1). Similar inhibition was observed using E:T ratios of 5:1 and 10:1 (data not shown).

$PGF_{2\alpha}$ was also tested for its effect on NK cell activity and over the same concentration range as PGE_2, direct addition of $PGF_{2\alpha}$ had no significant effect on the NK cell assay (Fig. 3).Pre-incubation of effector cells with 10^{-6} M $PGF_{2\alpha}$ prior to addition of target cells also failed to significantly affect cytotoxicity (data not shown).

Since ethanol was used as carrier for solubilizing the prostaglandins, it seemed possible that the inhibiting effects of PGE_2 might, in part, be due to the effects of ethanol *per se*. We therefore investigated the effect of ethanol under conditions in which 10^{-6} M PGE_2 inhibited NK cell activity (i.e. 0·03%). No significant difference was seen between control cytotoxicity and cytotoxicity in the presence of ethanol was observed (18·9±2·6% *vs* 19·8±3·1% respectively, mean±s.e., $n = 5$,

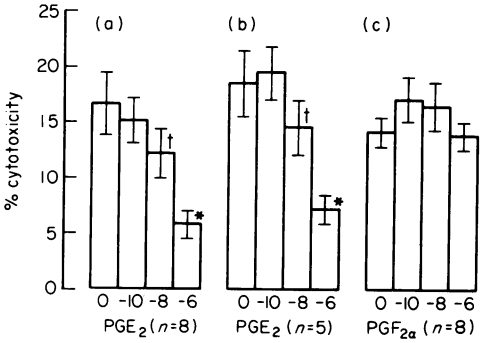

Fig. 3. The effect of PGE_2 and $PGF_{2\alpha}$ on NK cell activity. PGE_2 produced a dose-dependent inhibition of cytotoxicity whether added (a) directly to the assay or (b) after a 4 h pre-incubation period with effector cells. In contrast, (c) $PGF_{2\alpha}$ has no effect on NK cell activity (direct addition) over the same dose range (1×10^{-10} to 1×10^{-6} M). □ E:T = 20:1. Student's paired *t*-test: * $P < 0.001$; † $P < 0.02$.

$P > 0{\cdot}2$). Neither PGE_2 nor ethanol under the conditions used in these experiments were toxic to the effector cells as shown by AO/EB staining.

Lack of effect of indomethacin on NK cell activity

Since peripheral blood monocytes are capable of producing and releasing prostaglandins (Bray, Powell & Lydyard, 1981), we considered the possibility that small amounts of endogenous PGE_2 produced by adherent monocytes present during the assay might inhibit cytotoxicity. However, indomethacin (1 μg/ml) added directly to the 4 h assay failed to significantly enhance NK cell activity ($28{\cdot}2 \pm 3{\cdot}7\%$ specific cytotoxicity with indomethacin compared with $28{\cdot}8 \pm 2.0\%$ in control cultures, $P > 0{\cdot}3$, $n = 9$). In addition, pre-incubation of indomethacin with effector cells prior to addition to target cells failed to affect NK cell activity (data not shown).

Lack of inhibition of effector–target cell conjugation by PGE_2

Since the inhibition of NK cell activity obtained with PGE_2 was greater and more consistent than the histamine-induced inhibition, we chose PGE_2 to examine the mechanism(s) by which inhibition may be achieved.

One possible mechanism for the inhibition of NK cell activity by PGE_2 is the prevention of close contact between effector and target cells. In these experiments, the NK cell assay was set up with and without 10^{-6} M PGE_2 and the cell mixtures made up in an agar support medium to prevent dissociation of effector target cells. An E/T of 1:1 was used to facilitate enumeration of the target bound effector cells. As shown in Table 1, the percentage of effector cells bound to target cells were not significantly altered by the presence of PGE_2 during the assay. However, the NK activity of the same effector cells (E/T = 20:1) was significantly inhibited by PGE_2 (10^{-6} M) in the ^{51}Cr release assay (Table 1).

Evidence to suggest lack of effect of PGE_2 on NK cell movement and target cell lysability

To determine whether or not PGE_2 inhibits NK cell movement between target cells, effector–target mixtures were kept in suspension by gentle rocking during the 4 h killing assay thus allowing random movement of cells in suspension. As shown in Table 2, although cytotoxicity is considerably lower when cells were maintained in suspension (compared to cells spun down), the inhibition of cytotoxicity by PGE_2 was very similar in both cases.

To ensure that PGE_2 was not affecting the lysability of K-562 cells, ^{51}Cr-labelled target cells were

Table 1. Lack of effect of PGE_2 on effector–target cell conjugation

	% effector cells binding to target cells*		% cytotoxicity in ^{51}Cr release assay†	
Expt.	Control	PGE_2	Control	PGE_2
1	9·0	10·5	18·7	10·7
2	8·0	8·7	25·0	12·7
3	7·8	8·3	11·3	5·3
4	12·0	11·7	22·3	13·5
5	10·0	9·5	17·1	12·0

* Effector cells were mixed with an equal number of K-562 target cells in the presence or absence of PGE_2 (1×10^{-6} M) and the number of effector cells binding to target cells evaluated (E:T = 1:1; $P > 0{\cdot}2$).

† Effector cells were mixed with ^{51}Cr-labelled K-562 target cells (E:T = 20:1) for 4 h in the presence or absence of PGE_2 (1×10^{-6} M) and the % cytotoxicity determined (PGE_2 *vs* control; $P < 0{\cdot}005$).

Table 2. The effect of PGE_2 on effector cell movement

	Cells spun down*		Cells not spun down†	
Expt.	% cytotoxicity	% inhibition by PGE_2	% cytotoxicity	% inhibition by PGE_2
1	31·8	41	14·1	56
2	44·5	58	18·1	59
3	10·4	90	4·6	56
4	33·9	68	13·5	60
5	25·6	84	15·6	82
Mean ± s.e.	29·2 ± 6·3	68 ± 10	13·2 ± 2·6	63 ± 5

Effector and target cells were mixed in the presence or absence of PGE_2 (1×10^{-6} M) and either *spun down at the start of the 4 h killing assay or †maintained is suspension by gentle rocking during the 4 h killing assay.

Control cytotoxicity; *vs* †, $P < 0{\cdot}02$; % inhibition by PGE_2 * *vs* †, $P > 0{\cdot}5$.

preincubated with PGE_2 for 30 min, washed three times and assayed with untreated effector cells (E:T = 20:1). Pre-treatment of target cells with PGE_2 had no effect on their susceptibility to lysis by normal effector cells (data not shown).

The effect of dibutyryl (DiB) cAMP and cGMP on NK cell activity

As shown in Fig. 4, DiBcAMP inhibits NK cell activity in a dose-dependent fashion, producing almost total inhibition at the highest, non-toxic concentration used (5×10^{-4} M). In contrast, DiBcGMP had no significant effect at any dose over the same concentration range.

If PGE_2 inhibits NK activity via cAMP then it would be expected that the kinetics of inhibition by PGE_2 and DiBcAMP would be similar. Therefore, the drugs were added at 0, 0·5, 1, 2 and 3 h after the initiation of the 4 h killing assay. As shown in Fig. 5, the time course of inhibition was very similar for both drugs in parallel experiments, indicating a common inhibitory pathway.

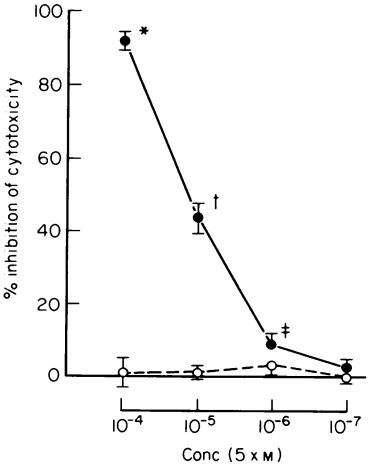

Fig. 4. The effect of cyclic nucleotide analogues on NK cell activity. DiBcAMP (●) produces a dose-dependent inhibition of cytotoxicity, whilst DiBcGMP (○) has no significant effect over the same dose range (5×10^{-4} to 5×10^{-7} M). Each point represents the mean ± s.e. of six different donors studied. Mean control cytotoxicity = 27·9 ± 2·8. E:T = 20:1. Student's paired *t*-test: * $P < 0{\cdot}001$; † $P < 0{\cdot}005$; ‡ $P < 0{\cdot}02$.

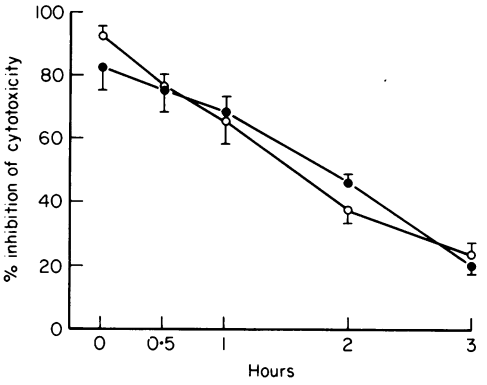

Fig. 5. PGE_2 (●; 1×10^{-6} M) and DiBcAMP (○; 5×10^{-4} M) were added in parallel at various times after initiation of the 4 h NK assay. Each point represents the mean ± s.e. for four subjects studied. E:T = 20·1. Mean control cytotoxicity = 26·7 ± 5.2%.

DISCUSSION

Histamine at 10^{-4} M produced a small but significant inhibition of NK cell activity, which was more obvious with direct addition to the NK assay than after pre-incubation with effector cells. Similarly, both H_1 and H_2 agonists produced inhibition, the H_1 agonist being the more active of the two. In a limited study by others using different target cells (Strannegard & Strannegard, 1980), NK cell activity by lymphoid cells from atopic subjects was more inhibited by 3×10^{-6} M histamine than non-atopic controls. Although our data was obtained from both normal ($n = 6$) and atopic ($n = 6$) laboratory personnel, there was no significant difference to histamine between the two groups. In contrast to the modest effect of histamine, a dose-dependent inhibition of NK cell activity was shown by PGE_2 when added directly to the NK assay or after pre-incubation with effector cells alone. This effect was not due to the ethanol in which the PGE_2 was dissolved, since ethanol alone at the highest concentration used had no effect on NK cell activity. In addition, $PGF_{2\alpha}$ had no significant effect on NK cell activity although dissolved in the same concentration of ethanol and used over the same dose range as PGE_2.

There appears to be two main mechanisms which might explain the inhibition of NK cell activity by PGE_2: (i) physical impairment of effector cell–target cell interaction. It is clear that effector cell movement and interaction with target cells is not influenced by PGE_2 and that target cell susceptability to lysis is similarly unaffected. (ii) A direct effect of PGE_2 on the NK effector cells. It has been suggested by Katz, Zaytoun & Fauci (1982), that drugs elevating cAMP, e.g. isoproterenol, theophylline and DiBcAMP inhibit NK activity, whilst those increasing cGMP levels, e.g. acetylcholine, carbachol and DiBcGMP, enhance cytotoxicity. Although DiBcGMP had no effect on NK activity in our hands (correlating with the lack of effect of $PGF_{2\alpha}$, which has been associated with elevation of cGMP; Kaliner, 1978), DiBcAMP was found to be highly inhibitory and the time course of inhibition by PGE_2 and DiBcAMP in parallel experiments was very similar (Fig. 5), suggesting that PGE_2 also inhibits NK cell activity via cAMP. It seems unlikely that the inhibition of NK cell activity by PGE_2 in the experiments reported here using unseparated cells is via another cell bearing PGE_2 receptors, since Katz *et al.* (1982) were able to show the inhibitory effects of DiBcAMP on highly purified NK cells (> 70% LGL). Furthermore, Goodwin, Kaszubowski & Williams (1979) have shown that the PGE_2 induced stimulation of cAMP by purified T cells resides largely in the T_G population, which also includes an appreciable proportion (~50%) of NK cell activity (Herberman, 1981). Since the inhibition of NK activity by PGE_2 is ~70%, this suggests that a substantial proportion of functional, 'mature' NK cells are T_G cells.

That small amounts of PGE_2 released by mononuclear cells containing the NK effector cells (Bray *et al.*, 1981) or by the K-562 target cells could damp down NK cell activity is unlikely since the prostaglandin synthetase inhibitor, indomethacin, did not enhance (augment) NK cell activity

when present in the cultures or when pre-incubated with the effector cells. However, the release of PGE_2 by some tumour target cells inhibits NK cell activity (Droller, Schneider & Perman, 1978) and this could be an important 'adaptive' mechanism for tumour survival. Although normal monocytes would appear to produce insufficient amounts of PGE_2 to modulate NK cell activity, activated monocytes found in patients with Hodgkin's disease (Goodwin *et al.*, 1977) and sarcoidosis (Goodwin *et al.*, 1979) which produce larger amounts of PGE_2 than normal monocytes in culture, could be expected to result in decreased NK cell activity. In fact, it has been shown that NK cell activity is decreased in Hodgkin's patients (Gupta & Fernandez, 1981). In addition, NK cell activity might be expected to be altered in patients with abnormal numbers of circulating T_G cells and in this context, Dobloug *et al.* (1982) have shown that both NK activity and T_G cells are reduced in juvenile rheumatoid arthritis. Since NK cell activity has been shown to play a role in immune regulation (Cudkowitz & Hochman, 1979), then high levels of PGE_2 in patients with chronic inflammatory disease, such as immune complex disease, may compromise the immunoregulatory function of NK cells.

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ADDENDUM

Whilst this paper was under consideration for publication, Goto *et al.* (J. Immunol. **130,** 1350) also reported that PGE_2 inhibits NK cell activity by elevating cyclic AMP.

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