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The effect of 2′deoxyguanosine on human lymphocyte responses. I. 2′deoxyguanosine enhances T lymphocyte responses

ANGELA C. HANGLOW* & P. M. LYDYARD†* *Division of Immunology, Department of Pathology, University of Cambridge, Addenbrooke's Hospital, Cambridge and †Department of Immunology, Middlesex Hospital Medical School, London, UK*

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SUMMARY

Low concentrations (40 μM) of the purine nucleoside metabolite 2′deoxyguanosine (2′dGuo), were shown to enhance both phytohaemagglutinin- and concanavalin A-induced T cell transformation. Evidence is presented which indicates that this enhancement may be due to the inhibition of suppressor T cell function resulting in the promotion of T cell growth factor production. T cell responses were only inhibited at concentrations of 2′dGuo which were toxic to the lymphocytes.

Keywords 2′deoxyguanosine T cell growth factor

INTRODUCTION

The purine nucleoside metabolite, 2′deoxyguanosine (2′dGuo), has been shown to inhibit proliferation-dependent, antigen specific suppressor T cell (Ts) function in murine (Dosch *et al.*, 1980) and human (Gelfand, Lee & Dosch, 1979) systems. In addition, 2′dGuo has been reported to inhibit mitogen-induced T cell blastogenesis (Gelfand *et al.*, 1979; Cohen *et al.*, 1980). In this paper, the effects of 2′dGuo on mitogen induced human T cell responses have been re-examined in order to evaluate its possible use in understanding immunoregulation in man.

MATERIALS AND METHODS

Preparation of peripheral blood mononuclear cells (PBMC). Defibrinated peripheral blood from normal volunteers was diluted 1 in 2 with sterile phosphate-buffered saline (PBS). PBMC were isolated on Ficoll-Hypaque, density 1·077 g/ml, (Pharmacia) according to the method of Böyum (1968). Mononuclear cells were resuspended in tissue culture medium RPMI 1640 (Flow Labs) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Flow Labs), L-glutamine (40 μM) and gentamycin (40 μg/ml). In some cases, adherent cells were depleted by incubation at 37°C for 45 min in a tissue culture flask.

Cell cultures. One hundred thousand PBMC or adherent cell depleted PBMC were cultured in flat bottomed microtitre plates (Falcon Plastics) in 200 μl tissue culture medium in the presence of either phytohaemagglutinin (PHA, Wellcome Reagents, Beckenham, UK) or concanavalin A (Con A, Sigma). 2′dGuo (Sigma) was added to these cultures at a range of concentrations (10–2,500 μM).

Correspondence: Dr Peter M. Lydyard, Department of Immunology, Middlesex Hospital Medical School, Arthur Stanley House, 40–50 Tottenham Street, London W1P 9PG, UK.

DNA synthesis was assessed during the last 6 h of a 72 h culture period by the addition of 0·1 μCi of ^{125}I-iodo-2′deoxyuridine (IUdR) (Amersham, UK). Cells were harvested onto glass fibre filters using a Titertek Cell Harvester (Flow Labs). Radioactivity incorporated into the DNA was determined by counting on a gamma counter (L.K.B.). Cell recovery and viability were determined by staining with a mixture of acridine orange and ethidium bromide (Lee, Singh & Taylor, 1975).

Assay of human T cell growth factor (TCGF). TCGF production was assessed by the ability of culture supernatants to maintain the proliferation of Con A stimulated murine spleen cells. Briefly, spleen cells from (CBA × BALB/c) F_1 mice (10–12 weeks of age) were cultured at 1×10^7 cells/ml in a 5 mm diameter Petri dish (Falcon Plastics) in 5 ml of tissue culture medium supplemented with 5% FCS and 4 μg/ml Con A. Cells were harvested after 48 h culture at 37°C, washed, counted and resuspended at $2{\cdot}5 \times 10^5$ cells/ml in tissue culture medium supplemented with 20% FCS. One hundred microlitres of doubling dilutions of the test supernatant were added to 100 μl of the suspension of Con A activated spleen cells aliquoted into each well of a flat bottomed microtitre plate. The cultures were incubated for 48 h at 37°C. Proliferation was assessed by the incorporation of IUdR during the last 6 h of culture.

Generation of TCGF by human PBMC. PBMC were cultured at 2×10^6/ml in tissue culture medium supplemented with 2% heat-inactivated normal human serum (NHS) in the presence of 20 μg/ml Con A and in the presence or absence of 40 μM 2′dGuo. Supernatants from these cultures were collected after 24 h and incubated on a roller with Sephadex G-100 for 3 1 h periods (2 ml of serum to 1 ml of packed Sephadex) to remove contaminating Con A (Mochizuki, Watson & Gillis 1980). The supernatants were stored at −20°C until tested.

Expression of TCGF receptors on human PBMC. PBMC were cultured at 2×10^6 cells/ml in RPMI supplemented with 10% heat-inactivated FCS and 20 μg/ml Con A and in the presence or absence of 40 μM 2′dGuo. Cells were harvested after 48 h, washed and resuspended at 4×10^6/ml in a Con A depleted tonsil supernatant containing TCGF which was used as a standard preparation. It was prepared by culturing tonsil cells from two donors in a 2 way mixed lymphocyte culture (MLC) in the presence of 20 μg/ml Con A and 2% heat-inactivated NHS at 4×10^6 cells/ml for a 24 h period. Con A stimulated PBMC were incubated in the standard TCGF preparation for 4 h at 37°C with continuous rotation. After this time the supernatants were recovered and tested for TCGF activity (as previously described).

Statistical analysis. This was performed using the paired Student's *t*-test.

RESULTS

Enhancement of PHA- and Con A-induced cell transformation

The addition of 2′dGuo to cultures of human PBMC resulted in enhancement of cell transformation induced by both PHA and Con A (Fig. 1). Generally, an enhancement was only observed at optimal mitogen concentrations (12·5 μg/ml PHA and 20 μg/ml Con A) and at 40 μM 2′dGuo. Inhibition of transformation was observed at higher concentrations of the metabolite (150 μM). This inhibition was due to cytotoxicity as shown by a decrease in cell numbers and viability (Fig. 2). At suboptimal concentrations of both mitogens, the effect of 40 μM 2′dGuo was inconsistent between different individuals. In some individuals, lymphocyte transformation was enhanced whilst in others it was suppressed. It was unlikely that this inconsistency was due to different numbers of monocytes contaminating the lymphocyte preparations since similar data was obtained with PBMC depleted of adherent cells (data not shown).

Enhancement of TCGF production

The enhancement of mitogen-induced cell transformation by 2′dGuo might be explained by increased production of TCGF and/or enhanced expression of receptors for this lymphokine. Experiments designed to investigate this possibility showed that 2′dGuo significantly enhanced the production of TCGF by normal human PBMC stimulated with optimal concentrations of Con A (Table 1). The enhancement of murine blast cell proliferation was not due to the direct effects of 2′dGuo present in the supernatants. Control experiments showed that 40 μM 2′dGuo did not

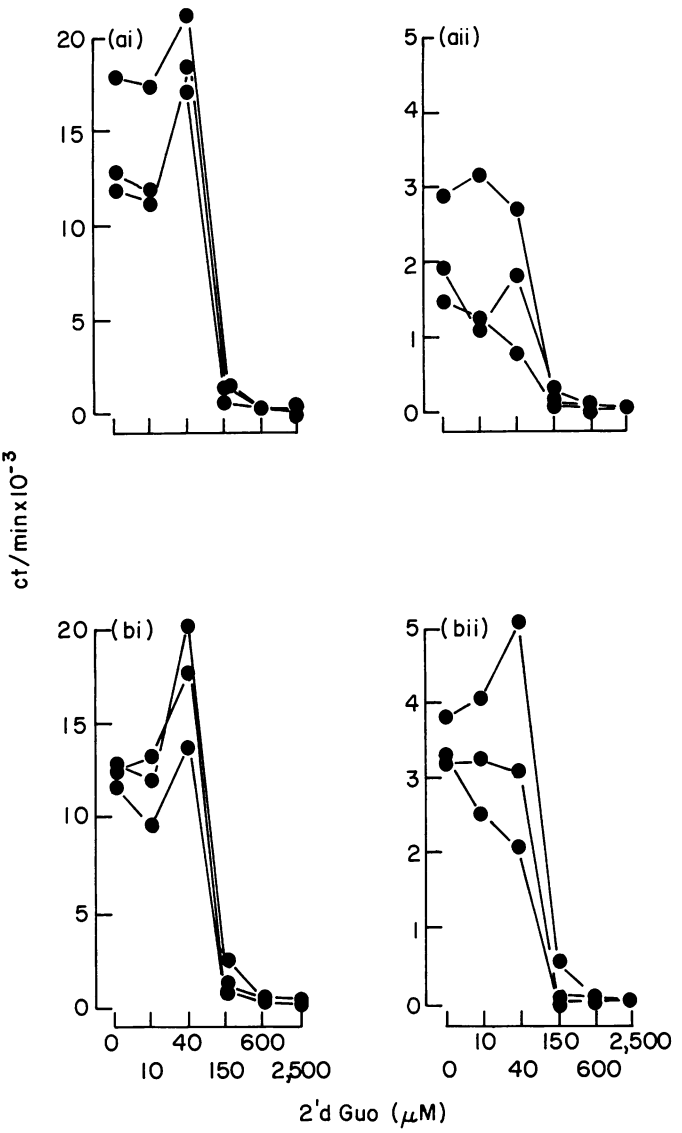

Fig. 1. Enhancement of (a) PHA- and (b) Con A-induced cell transformation by 2'dGuo. 2'dGuo (10–2,500 μM) was added to PBL cultures in the presence of optimal (i, 12·5 & 20 μg/ml) and suboptimal (ii, 1·25 & 5 μg/ml) concentrations of PHA and Con A, respectively. IUdR incorporation was assessed as described in Materials and Methods. Representative data is shown on three individuals. Standard errors did not exceed 10% mean. $P < 0{\cdot}05$ at optimal concentrations of both PHA (12·5 μg/ml) and Con A (20 μg/ml) in the presence of 40 μM 2'dGuo.

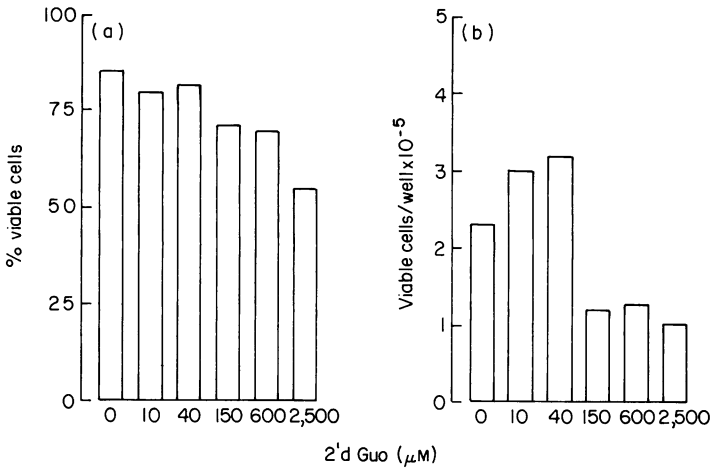

Fig. 2. The effect of 2'dGuo on lymphocyte viability in Con A cultures. The viability of PBL cultured with Con A (20 μg/ml) for 72 h was determined both by (a) assessing the percentage of viable cells and (b) total number of viable cells per culture.

Table 1. Effect of 2'dGuo on the generation of TCGF

		Stimulation index		
		Reciprocal of supernatant dilution		
Expt.	2'dGuo	2	4	8
1	–	4·1±0·2	1·4±0·05	1·4±0·04
	+	7·1±0·4	4·0±0·1	4·2±0·3
2	–	3·8±0·2	2·3±0·02	1·8±0·06
	+	3·3±0·3	1·4±0·07	1·5±0·04
3	–	8·2±0·2	2·7±0·1	1·0±0·03
	+	10·0±0·1	2·5±0·1	2·6±0·08
4	–	5·6±0·2	2·5±0·3	0·9±0·02
	+	9·6±0·4	3·6±0·1	1·0±0·1
Significance level		$P=0·05$	$P=$N.S.	$P=$N.S.

PBMC were cultured with 20 μg/ml Con A and 2% heat-inactivated NHS in the presence or absence of 40 μM 2'dGuo. Supernatants were harvested after 24 h, and depleted of Con A and tested for TCGF activity (as described in Materials and Methods).

$$\text{Stimulation index} = \frac{\text{ct/min supernatant from PBL + Con A + 2'dGuo}}{\text{ct/min control supernatant}}$$

Control cultures were supplemented with 20 μg/ml Con A and 40 μM 2'dGuo 1 h before harvesting.

significantly affect IUdR incorporation by these cells (Table 2). There was no corresponding increase in the absorption of TCGF by Con A stimulated blast cells in the presence of 2'dGuo (Table 3).

Table 2. Effect of 40 μM d'Guo on the proliferation of murine spleen blast cells maintained in TCGF

		Stimulation index		
		Reciprocal of supernatant dilution		
Expt.	2'dGuo	2	4	8
1	–	13·0±1·0	5·3±0·6	2·9±0·1
	+	11·4±0·5	6·7±0·7	4·0±0·2
2	–	6·7±0·2	2·0±0·1	1·2±0·1
	+	5·5±0·3	2·4±0·2	1·8±0·3
3	–	12·5±0·4	6·7±0·3	3·0±0·2
	+	7·1±0·3	5·1±0·2	2·7±0·2
Significance level		P=N.S.	P=N.S.	P=N.S.

F_1 CBA × BALB/c spleen cells were cultured for 48 h in the presence of 2 μg/ml Con A and 5% heat-inactivated FCS. The blast cells were recovered, washed and recultured in TCGF supplemented medium in the presence or absence of 40 μM 2'dGuo.

$$\text{Stimulation index} = \frac{\text{ct/min TCGF cultures } (\pm 2'\text{dGuo})}{\text{ct/min control cultures } (\pm 2'\text{dGuo})}.$$

Table 3. 2'dGuo does not affect the absorption of TCGF by Con A stimulated PBMC

	% absorption of TCGF by Con A stimulated PBL generated	
Expt.	Without 2'dGuo	With 2'dGuo
1	63±7	66±6
2	84±9	79±8
3	81±10	86±7
4	84±8	78±9
5	91±8	92±7
Significance level		P=N.S.

PBMC were cultured with 10% heat-inactivated FCS and 20 μg/ml Con A in the presence or absence of 40 μM 2'dGuo. These cells were recovered after 48 h and their capacity to absorb out TCGF determined (see Materials and Methods). Control cultures received 2'dGuo but not Con A.

DISCUSSION

In this paper evidence is presented which shows that low concentrations of 2'dGuo can enhance mitogen-induced T cell responses. A number of papers have reported that 2'dGuo inhibits mitogen-induced T cell blastogenesis (Gelfand *et al.*, 1979; Cohen *et al.*, 1980). However, data in this

paper suggest that T cell proliferative responses are in fact enhanced by 40 μM 2′dGuo and only inhibited at toxic concentrations of the deoxynucleoside.

Athough it is possible that 2′dGuo has a direct stimulatory effect on T cells it is unlikely, since 40 μM 2′dGuo actually inhibited proliferation of two human T cell lines (data not shown). However, malignant T and B cell lines have previously been shown to be more susceptible to inhibition by 2′dGuo than mitogen-induced PBL (Gelfand *et al.*, 1979), indicating that malignant cell lines may not be representative of lectin activated T cells.

One mechanism by which 2′dGuo could enhance mitogen-induced T cell transformation is by increasing TCGF production. A direct stimulatory effect on TCGF producing cells seems unlikely. The more plausible explanation is that 2′dGuo inhibits Ts cells which normally regulate TCGF production (Gullberg *et al.*, 1981; Gullberg & Larsson, 1982). In addition, Palacios & Moller (1981) have proposed that Con A-induced suppression may be mediated via removal of TCGF by blast cells thereby limiting the concentration of available lymphokine. It is conceivable that 2′dGuo enhances mitogen-induced T cell transformation by inhibiting such non-specific Ts cell function. As a result, more TCGF would be available in the culture supernatants.

The possibility that 2′dGuo operates via monocytes has not been formally excluded. However, enhancement of mitogen-induced cell transformation was observed with populations of both adherent cell depleted and total PBMC suggesting that monocytes (which are part of the adherent cell population) are not essential. The enhancement of TCGF production by 2′dGuo demonstrates that the nucleoside does not inhibit monocyte production of interleukin-1 which is required for the production of TCGF (Smith *et al.*, 1980). In fact, it cannot be excluded that interleukin-1 production is enhanced by 2′dGuo and this is currently under investigation. Thus, low concentrations of 2′dGuo have been shown to enhance mitogen-induced T cell proliferation. This enhancement is best explained by the inhibition of Ts cells by 2′dGuo resulting in the increased production of the lymphokine TCGF, which is required for T cell proliferation.

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