Inhibition by gold of human lymphocyte stimulation

An *in vitro* study

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SUMMARY Transformation of lymphocytes stimulated by mitogen, purified protein derivative, and in the mixed lymphocyte culture was significantly inhibited by gold sodium thiomate and gold sodium thiosulphate at concentrations of 5×10^{-4} to 5×10^{-5} mol/l.

The mechanism of the therapeutic effect of gold is not known. Because of the accumulating evidence of lymphocyte abnormalities in rheumatic disease (Yu and Peter, 1974) and the beneficial results of treatment by agents which alter lymphocyte function (Decker *et al.*, 1973; Abdou, *et al.*, 1973; Pearson *et al.*, 1975), the possible effects by gold on human lymphocytes stimulated by various agents were studied. Investigations were carried out on lymphocytes stimulated by (1) the mitogens PHA, Con A, and PWM, (2) the antigen PPD, and (3) other lymphocytes in the mixed lymphocyte culture (MLC).

Methods

Human peripheral blood lymphocytes obtained from healthy donors were isolated by Ficoll-Hypaque density gradient centrifugation. In standard tests 1.5×10^6 lymphocytes in 1 cm^3 of RPMI-1640 with 5% human AB serum were cultured at 37° in plastic tubes for 72 hours in the presence or absence of the stimulating agent or in the presence or absence of the gold compound. The degree of transformation was determined by the uptake of ^3H thymidine in a standard assay during the final 24 hours of the culture (48–72 hours). Because gold was found to be more inhibitory if added before mitogen or antigen (see Table 4), unless otherwise indicated gold was added at the time of the initiation of the culture (time 0) and the stimulating agent added 24 hours after the culture initiation. Mitogen or PPD was added in optimal concentrations as previously determined. Two-way MLCs were carried out between unrelated donors at 1×10^6 cells per donor and assayed at day 3. All tests were done in triplicate.

Accepted for publication July 15, 1976

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Results and discussion

Gold sodium thiomate (GTM) significantly inhibited, in a dose-related fashion, transformation of lymphocytes stimulated by mitogens, PPD, and by other lymphocytes in the MLC (Table 1). These experiments repeated multiple times with various donors all showed similar degrees of inhibition by gold at the concentrations listed in Table 1. Gold sodium thiosulphate (GTS) also significantly inhibited lymphocyte transformation to an extent

Table 1 Transformation inhibition of stimulated lymphocytes by gold thiomate (GTM)

Stimulating agent	GTM concentration ($\mu\text{g/ml}$)	TI*†	Inhibition (%)
PHA-3 γ ‡	0	258.6 $\pm$ 7.4	—
	10	220.7 $\pm$ 2.6	15
	50	109.7 $\pm$ 5.3	58
	100	54.0 $\pm$ 5.5	79
Con A-25 γ	0	92.5 $\pm$ 6.4	—
	10	65.1 $\pm$ 9.3	30
	50	56.0 $\pm$ 23.3	40
	100	20.1 $\pm$ 20.0	79
PWM-15 γ	0	123.8 $\pm$ 2.2	—
	10	112.8 $\pm$ 4.0	9
	50	72.3 $\pm$ 4.9	42
	100	58.4 $\pm$ 3.8	53
PPD-25 γ	0	10.2 $\pm$ 6.2	—
	10	7.2 $\pm$ 3.4	29
	50	4.1 $\pm$ 7.3	60
	100	4.6 $\pm$ 2.4	55
MLC	0	17.0 $\pm$ 3.5	—
	10	13.6 $\pm$ 12.1	24
	50	5.4 $\pm$ 11.2	71
	100	3.1 $\pm$ 4.9	82

*Transformation index (TI):

Stimulated ^3H counts—nonstimulated ^3H counts/nonstimulated ^3H counts.

† $\pm$ % Standard error of stimulated mean.

‡Purified PHA-P obtained from Wellcome Reagent, Ltd.

PHA = phytohaemagglutinin; Con A = concanavalin A;

PWM = pokeweed mitogen; PPD = purified protein derivative;

MLC = mixed lymphocyte culture.

comparable to that of GTM, while thiomalic acid and sodium thiosulphate did not significantly inhibit (Table 2). GTM and GTS are not directly toxic to the lymphocytes in the concentrations used as assessed by (1) trypan blue uptake of cells incubated with or without gold, (2) gold effect on ^3H thymidine uptake by nonstimulated lymphocytes, and (3) reversibility of the inhibitory effect of GTM after washing (Table 3).

The mechanism of the transformation inhibition by gold is unclear. Cultures assayed on consecutive days up to 7 days show similar degrees of inhibition (e.g. in the experiment 62% inhibition at day 1 and 46% at day 7), indicating that it does not merely delay the lymphocyte response to mitogen. It appears to act early in the stimulatory process as gold added before the mitogen was more inhibitory than gold added after the mitogen (Table 4). In fact in the experiment shown, gold was not inhibitory at all when added 6 hours after the mitogen. It does not act by direct binding to mitogen as (1) increasing the mitogen concentration in the culture, or (2) preincubation of mitogen with gold and then removal

of gold by dialysis before addition of the mitogen to the culture does not overcome the inhibitory effect of gold in the former (1), or decrease the stimulation by mitogen in the latter case (2) (dialysis data shown in Table 5). Nor does gold appear to inhibit mitogen incorporation by lymphocytes as repeated experiments failed to show inhibition by gold of ^{125}I -labelled Con A incorporation by cells (Table 6). Thus it appears that gold inhibits a process of early lymphocyte amplification but has no effect on binding to mitogen or cellular uptake of mitogen.

In support of the data presented here, Harth *et al.* (1976) have also recently reported inhibition by gold

Table 2 GTM and gold thiosulphate (GTS) inhibition of Con A* stimulated lymphocytes

Compound added	Concentration (mol/l)	TI	Inhibition (%)
Experiment 1			
0	—	141.8 ± 7.7	—
GTM	5 × 10 ⁻⁵ †	112.0 ± 3.9	21
	10 ⁻⁴	71.7 ± 19.0	49
	5 × 10 ⁻⁴ †	46.7 ± 26.1	67
GTS	5 × 10 ⁻⁵	124.6 ± 1.5	12
	10 ⁻⁴	41.3 ± 10.5	71
	5 × 10 ⁻⁴	6.1 ± 14.2	96
Experiment 2			
0	—	82.8 ± 14.8	—
GTM	10 ⁻⁴	36.9 ± 7.9	55
	5 × 10 ⁻⁴	22.0 ± 3.3	73
GTS	10 ⁻⁴	27.3 ± 14.8	67
	5 × 10 ⁻⁴	6.4 ± 14.3	92
Thiomalic acid	10 ⁻⁴	73.8 ± 10.0	11
	5 × 10 ⁻⁴	84.1 ± 6.8	0
Na thiosulphate	10 ⁻⁴	68.7 ± 13.1	17
	5 × 10 ⁻⁴	79.6 ± 5.3	4

*25γ added to stimulated cultures.

†5 × 10⁻⁵ mol/l GTM approximately 10 μg/ml; 5 × 10⁻⁴ mol/l approximately 150 μg/ml.

Table 3 Reversible GTM inhibition of transformation*

Lymphocytes washed or not washed at 24 hours of culture†	+ or - addition of GTM	TI	Inhibition (%)
Not washed	—	227.7 ± 11.8	—
Not washed	+	113.8 ± 3.1	50
Washed	—	203.8 ± 2.8	—
Washed	+	212.6 ± 4.8	0

*PPHAP 3γ with or without GTM 100 μg/ml added at time 0.

†Indicated cultures washed 2x with Hanks's BSS.

Table 4 Effect of varying times of addition of GTM on transformation inhibition

Time GTM 100 μg/ml added in relation to Con A addition*	Inhibition (%)
24 h before	60
1 h before	57
1 h after	37
6 h after	0
24 h after	0

*Con A 25γ added at 24 hours after culture initiation.

Table 5 Effect of preincubation of Con A and GTM

Preincubated compounds*	Final Con A concentration	TI	Inhibition (%)
Con A	25	275.3 ± 8.4	—
Con A and GTM	25	348.1 ± 2.5	0
Con A	2.5	25.6 ± 6.2	—
Con A and GTM	2.5	33.0 ± 3.6	0

*Con A alone or Con A and GTM incubated together for 24 hours at 37°, then dialysed to remove unbound gold and then added to cultures to produce the final Con A concentration. GTM concentration before dialysis was 100 μg/ml with 25γ Con A and 10 μg/ml with 2.5γ Con A. GTM 100 μg/ml added to Con A solution after incubation and dialysis caused a 52% decrease in TI.

Table 6 Effect of GTS on incorporation of ^{125}I Con A by lymphocytes*

GTS concentration (mol/l)	^{125}I counts × 10 ³ /culture†	Inhibition (%)
0	241.3 ± 16.0	—
5 × 10 ⁻⁵	220.1 ± 2.5	8‡
10 ⁻⁴	234.8 ± 8.4	3‡
5 × 10 ⁻⁴	212.0 ± 6.2	12‡

*GTS added to cultures at time 0; 25γ ^{125}I Con A added at 24 hours; cultures incubated 1 hour at 4°C then cells washed 4 × with Hanks's BSS and cells counted for ^{125}I incorporation.

†Mean ± standard error of triplicate tests.

‡Not significantly different (P=0.05) from control. Other experiments performed with lower ^{125}I Con A concentrations, differing incubation times and temperatures of lymphocytes with ^{125}I Con A and gold all failed to show inhibition.

(2.5 to 10 $\mu\text{g/ml}$) of lymphocytes stimulated by PHA and in the MLC. They also noted inhibition by gold of cell-mediated lympholysis.

The *in vitro* data presented here and in the report by Harth *et al.* suggests a possible *in vivo* mechanism for the therapeutic effect of gold. *In vivo* gold may alter the responsiveness of lymphocytes to disease associated antigens. While the gold concentrations in this study are higher than those found in sera of gold treated patients, the highest concentration used in this study is approximately one-third the concentration found in lymphatic tissue (about 300 μg gold/g lymph node wet weight), reported by Gottlieb *et al.* (1972) in an autopsy study of a patient who had received 5 g gold thioglucose. In view of the delayed therapeutic response to gold it appears that this tissue accumulation rather than sera concentration may be the more important factor in determining its effect.

One *in vivo* corollary was also tested. It was postulated that poor and good clinical responses to gold therapy by rheumatoid arthritis patients might reflect poor and good *in vitro* responses to the inhibitory effects of gold on their lymphocytes. The lymphocytes of 5 poor and 5 good responders to gold therapy of more than 4 months' duration were studied. *In vitro* stimulation by PHA and Con A was inhibited to the same degree by varying concentrations of gold in the poor responders, good responders,

and healthy controls. Thus, in these initial tests poor and good clinical responses to gold therapy could not be explained on the basis of different peripheral blood lymphocyte responsiveness to the inhibitory effect of gold.

Supported in part by a grant from the US Public Health Service (GM 15759).

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