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Inhibition of lymphocyte activation by gold sodium thiomalate

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1 Activation of lymphoid cells by both T and B cell mitogens was inhibited by gold sodium thiomalate (GST).

2 The action of GST did not appear to be exerted at early stages of lymphocyte activation.

3 Inhibition by GST was sustained throughout 4 days of culture.

4 The inhibitory effect of GST was reduced at low serum concentrations.

5 Sodium thiomalate and sodium chloroaurate were also able to inhibit lymphocyte activation.

Introduction

Gold sodium thiomalate (GST) and a number of other drugs containing sulphur-bonded gold atoms are among the few drugs capable of arresting the progress, or inducing remission, of rheumatoid arthritis. One of the features of the disease is the presence of activated lymphoid cells within the synovial tissue of involved joints (Panayi & Johnson, 1979; Janossy, Panayi, Duke, Bofill, Poulter & Goldstein, 1981) and it has been suggested that inhibition of this activation, possibly via interaction with accessory macrophages, may be responsible for the beneficial effect of these drugs (Lipsky & Ziff, 1977; Rosenberg & Lipsky, 1979). However, the precise mechanism of action is obscure and there is even some question as to whether the gold or thiol moiety is the active principle (Jellum, Aaseth & Munthe, 1977; Arrigoni-Martelli, Bramm & Binderup, 1978). In the absence of more effective drugs, further information on the mode of action of the gold drugs is clearly desirable and the present paper presents data indicating some of the basic pharmacology of the inhibitory action of GST in relation to lymphocyte activation.

Methods

Balb/c/Ola mice obtained from Olac in 1980 and maintained as an inbred line at the Clinical Sciences Building were used at between 8 and 16 weeks. Concanavalin A (Sigma) and lipopolysaccharide (Difco, E. coli) were prepared in phosphate buffered saline at $2\,mg\,ml^{-1}$ and $10\,mg\,ml^{-1}$ respectively. Gold sodium thiomalate, a generous gift from May and Baker, Dagenham, sodium thiomalate (Sigma) and sodium chloroaurate (BDH) were prepared in saline at 4 mg/ml or 1.2×10^{-1} M. All solutions were sterilized by filtration through 0.22 μm membranes and stored in small aliquots at −20°C.

The conditions of cell culture were as described below unless stated otherwise in the results section. A single cell suspension was prepared by teasing spleens apart with scalpel blades, passing the fragment through syringe needles and allowing tissue debris to settle. After being washed twice in Hanks' balanced salt solution the cells were counted in 0.2% nigrosine and white blood cell counting fluid before resuspension in RPMI medium (Gibco) and 10% foetal calf serum (FCS) (Flow, Lot 29041126 or Sera Lab Batch, 201025), selected to support optimal stimulation.

Quadruplicate cultures were set up in flat bottomed, 96 well tissue culture plates (Linbro or Costar). Constituents were added in 50 μl or 100 μl volumes to give a final volume of 200 μl with cells at $1 \times 10^6\,ml^{-1}$. Cultures were incubated in a humidified 5% CO_2 and air atmosphere at 37°C for 3 days. Eighteen hours before termination of each experiment, cultures were pulsed with 1 μCi [^{3}H]-thymidine at $500\,mCi\,mmol^{-1}$ (prepared by addition of 1.8 μmol thymidine (B.D.H.) to each 1 mCi of [^{3}H]-thymidine at $5\,Ci\,mmol^{-1}$ (Amersham, TRA–61)). Cells were harvested by washing onto glass fibre filters with distilled water, using a 'Titer-

Table 1 Effect of gold sodium thiomalate on activation of spleen cells by concanavalin A

Concanavalin A ($\mu g\,ml^{-1}$)	*Concentration of gold sodium thiomalate* ($\mu g\,ml^{-1}$)			
	None	3	10	30
None	293 ± 57	305 ± 176	178 ± 103	178 ± 34
0.1	594 ± 342	526 ± 43	276 ± 40	106 ± 8
0.3	9552 ± 684	7522 ± 505	4347 ± 364	1034 ± 177
1	28958 ± 2203	18880 ± 1430	8787 ± 1253	2454 ± 424
3	79752 ± 9171	37835 ± 3743	10808 ± 4338	3084 ± 277
10	3522 ± 85	3345 ± 511	2707 ± 261	1003 ± 70

Values represent the mean ± s.e. of quadruplicate cultures.

tek' cell harvester. Radioactivity was assessed by placing the dried filters in a toluene based scintillation mixture and counting in a scintillation spectrometer. Results are expressed as counts per minute ($ct\,min^{-1}$) of recovered radioactivity or as percentage inhibition, calculated by the following formula:

$$\frac{\text{test cells ct min}^{-1} - \text{unstimulated cells ct min}^{-1}}{\text{activated cells ct min}^{-1} - \text{unstimulated cells ct min}^{-1}}$$

Results

The data shown in each figure or table are from single experiments representative of at least four similar experiments.

Depression of mitogen activation of spleen cells

Table 1 shows the results of activating lymphocytes with concanavalin A (Con A) and the effect of adding

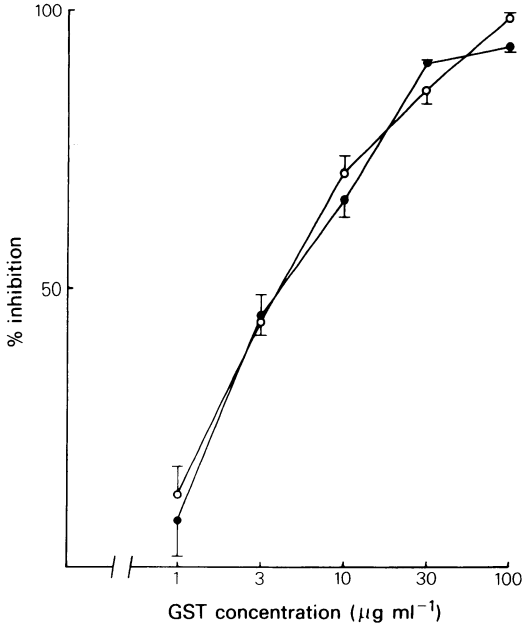

Figure 1 Inhibition of concanavalin A – (Con A) and lipopolysaccharide – (LPS) induced activation of lymphocytes by gold sodium thiomalate (GST). Spleen cells were activated with Con A $1\,\mu g\,ml^{-1}$ (●) or LPS $30\,\mu g\,ml^{-1}$ (○). Background [^{3}H]-thymidine incorporation was $603\,ct\,min^{-1}$ and mitogen stimulation in the absence of GST gave 32550 and $6607\,ct\,min^{-1}$ for Con A and LPS respectively.

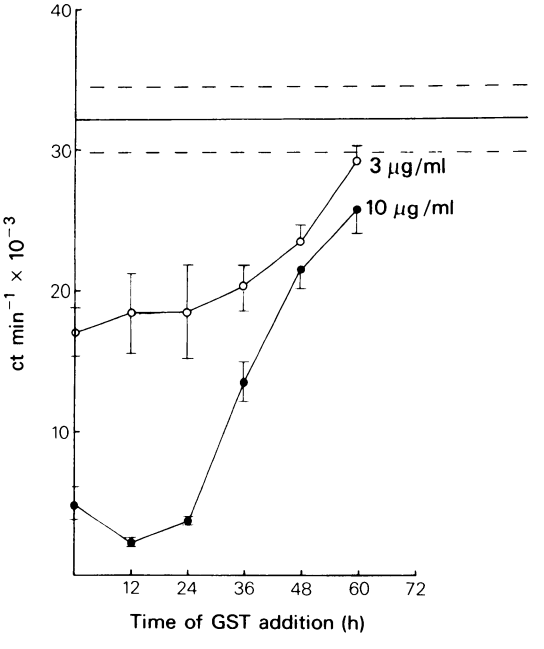

Figure 2 Addition of gold sodium thiomalate (GST) to concanavalin A (Con A) activated lymphocytes at different times during the incubation. Spleen cells were activated with Con A $1\,\mu g\,ml^{-1}$ and GST was added in $5\,\mu l$ at the times indicated, to a final concentration of 3 (○) or $10\,\mu g\,ml^{-1}$ (●). The solid and broken horizontal lines indicate the response where no GST was added ± s.e.

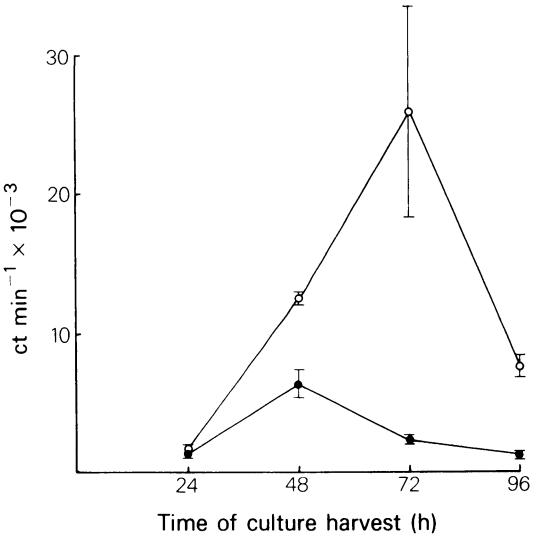

Figure 3 The effect of gold sodium thiomalate (GST) on the kinetics of concanavalin A (Con A)-activation of lymphocytes. Spleen cells were activated with Con A 3 μg ml^{-1} in the presence (●) or absence (○) of GST at 10 μg ml^{-1}. Quadruplicate cultures from each group were harvested at 24, 48, 72 or 96 h after a 4 h pulse of [^{3}H]-thymidine.

GST to the cultures. Inhibition occurs throughout the range of Con A concentrations that stimulate thymidine incorporation. Concentrations of GST causing inhibition of the response varied to some extent between experiments but the ID_{50} was generally between 3 and 30 μg ml^{-1} under the conditions described (Figure 1).

That inhibition is not restricted to T lymphocyte activation is also indicated in Figure 1 where activation by lipopolysaccharide (LPS), a selective B cell mitogen (Greaves & Janossy, 1972), is shown to be similarly inhibited.

Variation of gold sodium thiomalate effects with time

Addition of GST to cultures at various periods after culture initiation shows that it need not be present during the early stages to produce its inhibitory effects (Figure 2). When GST addition is delayed for 12 or even 24 h there is still a similar degree of inhibition and most of the action appears to be exerted between 24 and 60 h. The data also indicate that GST is not acutely toxic to lymphocytes and does not act by inhibiting [^{3}H]-thymidine uptake directly.

Maximal uptake of [^{3}H]-thymidine induced by Con A occurs at 2–3 days under the conditions employed and it could be suggested that GST alters the time at which [^{3}H]-thymidine uptake is maximal rather than resulting in an absolute reduction of activation. That this is not so is shown by the data in Figure 3 where inhibition is evident during days 2–4.

Effect of serum concentration

Because the gold and thiomalate dissociate when GST is administered *in vivo*, with the gold becoming rapidly protein bound (Jellum *et al.*, 1977; Davis & Barraclough, 1977; Danpure, Fyfe & Gumple, 1979; Van de Stadt & Abbo-Tilstra, 1980) it seemed possible that the presence of serum would reduce its activity. It was therefore unexpected to find that the inhibitory activity was less at reduced serum concentrations, indicating that serum binding might actually be necessary for the inhibition (Table 2). The change in serum concentration also alters the profile of the Con A dose-response. Whether this is due to protein binding or a change in concentration of growth factors is not clear. The absence of inhibition by GST at low serum concentrations again indicates the lack of a direct cytotoxic effect.

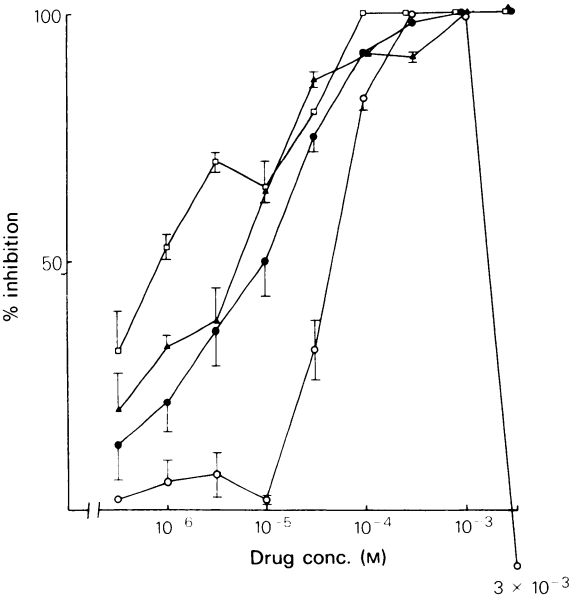

Figure 4 Inhibition of concanavalin A (Con A)-induced lymphocyte activation by gold sodium thiomalate (GST) (●), sodium thiomalate (○) and sodium chloroaurate (□). Spleen cells were activated with Con A 3 μg ml^{-1} in the presence of various drug concentrations as shown. Background [^{3}H]-thymidine incorporation was 284 ct min^{-1} and activation in the absence of drugs gave 11853 ct min^{-1}. The effect of adding an equimolar mixture of sodium thiomalate and sodium chloroaurate is shown as (▲).

Table 2 Effect of serum concentration on the inhibition of concanavalin A (Con A)-induced lymphocyte activation by gold sodium thiomalate (GST)

Serum concentrations	*Con A* ($\mu g\,ml^{-1}$)	*GST concentrations* ($\mu g\,ml^{-1}$) None	3	10	30
	None	744 ± 114	1001 ± 290	554 ± 93	645 ± 97
1.25%	1	74970 ± 7056	83057 ± 9104	80538 ± 3503	66135 ± 6381
	3	403 ± 61	404 ± 233	628 ± 94	299 ± 172
	None	910 ± 127	1207 ± 271	548 ± 84	768 ± 74
2.5%	1	156309 ± 4396	163517 ± 3901	143739 ± 6082	136090 ± 5170
	3	6943 ± 1892	5493 ± 1792	8754 ± 4110	2951 ± 473
	None	548 ± 63	619 ± 63	527 ± 473	511 ± 89
5%	1	76018 ± 2176	50021 ± 1744	45134*	17535 ± 5373
	3	18715 ± 6693	28094 ± 5545	18313 ± 2621	5317 ± 3069
	None	577 ± 23	540 ± 65	472 ± 78	475 ± 102
10%	1	31740 ± 2204	24138 ± 1757	10085 ± 484	3778 ± 160
	3	35063 ± 5991	17930 ± 1281	10381 ± 1976	2754 ± 153

Values represent the mean ± s.e. of quadruplicate cultures. * Mean of two values only.

Inhibition by thiomalate and sodium chloroaurate

The dissociation of gold and thiomalate in serum and the question as to which part of the drug is most important in treatment of rheumatoid arthritis raises the question of which moiety is most important in lymphocyte activation. As shown in Figure 4, GST, sodium thiomalate and sodium chloroaurate each inhibited the response although sodium thiomalate was least active on a molar basis.

A most striking effect was the highly reproducible reversal of inhibition by thiomalate at high concentration which is an indication of the absence of toxicity of this drug towards lymphocytes.

Discussion

Determination of atomic gold levels in the serum of rheumatoid arthritis patients undergoing chrysotherapy with GST indicate that 3 to 4 $\mu g\,ml^{-1}$ may be present one week after injection of a standard weekly dose (50 mg) intra-muscularly, with levels of 5 to 7 $\mu g\,ml^{-1}$ during the first two days (Gottlieb, 1981). These levels are equivalent to 6 to 8 $\mu g\,ml^{-1}$ or 10 to 14 $\mu g\,ml^{-1}$ of GST respectively. The data presented here indicate that these concentrations of GST can inhibit lymphocyte activation *in vitro*. Inhibition occurred even in the presence of optimal concentrations of mitogen, although Lipsky & Ziff (1977) found that inhibition of human lymphocyte activation was more effective when sub-optimal mitogen stimulation was used. Inhibition of both Con A- and LPS-induced activation indicates inhibition of a function necessary for both B and T lymphocyte activation.

The results of adding GST at intervals after culture initiation suggest that it does not act at a very early stage of lymphocyte activation but may influence an event occurring after initial triggering of the cells, such as the production of factors necessary for lymphocyte proliferation. Lies, Cardin & Paulus (1977) found that inhibitory effects were not observed if GST was added later than 6 h from the start of the culture. Lipsky & Ziff (1977) also suggested that an early stage of activation was affected, although failure to add GST until 18 h appeared to have no less an effect where less than 100 $\mu g\,ml^{-1}$ GST was used.

The observation that an increased serum concentration allows greater inhibition by GST is interesting in that protein binding of drugs would generally be expected to reduce their activity. These results may relate to the observation of Griffin & Steven (1982) who showed that albumin could act as a carrier in exchange reactions, allowing transfer of gold from GST to the active centre of trypsin. The activity of trypsin was thereby inhibited, although GST in the absence of a protein, or some other carrier, had little effect. The ability of this enzyme to cleave fluorescein-labelled peptides from collagen was also inhibited by sodium thiomalate, at concentrations approximately an order of magnitude greater than GST, indicating that the ability of both compounds to have a similar effect is not peculiar to lymphocyte activation.

That GST, sodium thiomalate and sodium chloroaurate were each inhibitory is interesting in view of the reports that thiomalate may be an active anti-arthritic agent (Munthe & Jellum, 1980; Munthe, personal communication) and reports that other thiol compounds may be therapeutically useful (Jaffe, 1980). Thiomalate has been reported to in-

hibit mouse lymphocyte function at concentrations above $6\,\mu g\,ml^{-1}$ (Jennings, Macrae & Gorczynski, 1979). Lipsky & Ziff (1977) and Lies *et al.* (1977) reported that thiomalate did not inhibit human lymphocyte function although McCormack & Palmer (1980) found that it did at concentrations of $19\,\mu g\,ml^{-1}$ and above, whilst GST and sodium choroaurate needed to be at $50\,\mu g\,ml^{-1}$.

Use of sodium chloroaurate as a control for the action of thiol-free metal may be criticized on the ground that its oxidation state is +3 whereas that of GST is +1. However, in the presence of a three fold excess of thiol it is suggested that gold (III) will behave as gold (I) (Sadler, 1982). Since RPM1 media contains approximately 2×10^{-4} M cystine this would allow gold (III) to behave as gold (I) at concentrations less than 1.3×10^{-4} M, even without the contribution of serum thiol groups and the thioether groups of methionine also present in the media.

Thiols have been shown to have a considerable capacity to enhance lymphocyte activation (Chen & Hirsch, 1972; Broome & Jeng, 1973; Opitz, Lemke & Hewlett, 1978) and examples of molecules which may be important in lymphocyte activation and which contain or are affected by thiols include immunoglobulin antigen-complexing factor (Paraskevas, 1980), soluble immune response supressor (Pierce & Aune, 1980) and 2-mercaptoethanol-activated serum factor (Opitz *et al.*, 1978). It therefore seems plausible that GST, and possibly some of the other anti-rheumatic drugs containing thiol groups, may interact with the sulphydryl or metal-sensitive sites of an enzyme, receptor, or mediator involved in the activation of lymphocytes.

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