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Effect of gold salt treatment on the receptor binding activity of monocytes and macrophages isolated from rats with adjuvant arthritis

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Summary. We investigated the effect of chrysotherapy on the Fc and complement receptor binding activity of peripheral blood (PB) monocytes and peritoneal (PE) macrophages isolated from normal rats and rats with adjuvant induced arthritis. The adjuvant induced severe disease in Dark Agouti (DA) rats and less marked disease in J. C. Lewis (JC) rats. Gold treatment reduced the disease in DA rats but exacerbated the disease in JC rats. PB monocytes generally exhibited increased receptor activity after adjuvant injection. Gold treatment resulted in a simultaneous reduction of the PB monocyte receptor activity and increased the PE macrophage receptor activity. This was considered to be due to a direct effect of gold, since the Fc receptor activity of PE macrophages increased after in vitro gold treatment.

Key words: Gold sodium thiomalate – Fc-receptors – Complement receptors – Adjuvant polyarthritis rats

Introduction

Gold I complexes have been used in the treatment of rheumatoid arthritis for over six decades. Although many studies have been carried out (see reviews in [1, 2]) the exact mechanism of action in suppressing and remitting the disease is unknown.

Mononuclear phagocytes not only play an important role in local destruction in chronically inflamed tissues in active rheumatoid arthritis but also regulate other cells of the immune system. The concept has long been advanced that the action of gold in rheumatoid arthritis may result from its capacity to alter the function of the mononuclear phagocytes at the site of inflammation [3–6]. This is strongly supported by ultrastructural studies which show a selective accumulation of gold in the lysosomes of the type A synovial cells and other macrophages of the synovium in patients treated by chrysotherapy [5, 7, 8]. Aurothiomalate also is actively taken up by peritoneal macrophages [4] and can alter the morphology of human peripheral blood monocytes [9]. Since gold accumulates in the long-lived macrophages regulating inflammation, the delayed response to gold therapy could be mediated by changes in macrophage function.

Gold compounds have been shown to inhibit a number of important functions of mononuclear phagocytes. These include phagocytosis [10, 11], chemotaxis [12, 13], enzyme production [4, 14, 15], intracellular killing of bacteria [16] and superoxide production [17, 18]. The result of investigations into the effect of gold on other macrophage functions, such as monokine production, are conflicting depending upon the experimental system used [19–21]. Many of these effects must be interpreted with caution as they either occur (a) in vitro at concentrations of gold higher than those found in the tissues and serum of patients undergoing chrysotherapy, or (b) with cell types unlike those present at inflammatory sites.

We have investigated, therefore, the effect of gold sodium thiomalate (GST) on recepter-binding activity of macrophages and monocytes isolated from rats with adjuvant-induced polyarthritis and have compared the in vivo findings with those in vitro using peritoneal macrophages.

Methods

Adjuvant disease

On Day 0, groups of male Dark Agouti (DA; 150–250 g) and J. C. Lewis (JC) (200–250 g) from the Institute of Medical and

Veterinary Science, Adelaide were injected intradermally near the tail base with 50 µl of finely ground, heat-killed delipidated human strain *Mycobacterium tuberculosis* (TBC; Tuberculin Section, Ministry of Agriculture, Fisheries and Food, Weybridge, UK) dispersed in squalane (SQ; Fluka) at a concentration of 10 mg/ml. On Day 14 after adjuvant injection, the polyarthritic disease was assessed by measurement of footpad thickness with a micrometer. Peripheral blood monocytes and peritoneal exudate cells were removed and asessed for receptor-binding capacity.

Drug treatments

Gold sodium thiomalate was given over a 14-day period by subcutaneous injection on alternate days commencing on Day 0 at 12.5 mg/kg body weight (32 µatoms Au/kg).

Isolation of rat peripheral blood (PB) monocytes and peritoneal (PE) macrophages

Rats were killed by cervical dislocation and peritoneal cells were harvested by washing the peritoneal cavity with 10 ml Hanks buffered saline (HBSS) supplemented with 10 units/ml heparin. Blood (5 ml) was taken by cardiac puncture and stored in heparinized tubes to prevent clotting. Peripheral blood monocytes where isolated by overlaying 10 ml of 1:1 diluted blood and saline onto Ficoll-Hypaque gradient centrifuged at 1 500 rpm (400 *g*) and the mononuclear cells where removed at the gradient interface. Isolated mononuclear cells were then washed three times in HBSS and resuspended in RPMI 1640 media (Flow Laboratories) supplemented with 10% foetal calf serum (FCS) and adjusted to a concentration of 4×10^6 per ml. Of the cell suspension 0.5 ml (2×10^6 cells) was placed in 16-mm wells of a 24-well culture tray (Costar) with 13-mm sterile glass coverslips at the bottom of each well. After incubation at 37 °C in 5% CO_2 for 1 h the cells not adhering to the glass coverslips were removed by washing three times with HBSS. More than 95% of the adherent cells stained positively for nonspecific esterase (NSE) [22]. Siliconized glassware was used throughout to prevent loss of cells due to adherence.

Peroxidase staining

Cell preparations were stained for myeloperoxidase using the method of Kaplow [23].

Preparation of coated red blood cells

IgG coated red blood cells (IgGE). Red blood cells (rbc) from healthy normal rats (DA or JC) were washed three times in saline. They were then incubated with purified anti-rat rbc IgG (Cappel) for 1 h at 37 °C in HBSS. The concentration of IgG was lower than that needed for haemagglutination. These IgG-coated rbc were then washed three times in saline and diluted to 4×10^7/mL for use.

Complement (C') coated red blood cells (c'E). Rbc from healthy normal rats were coated with IgM in a similar manner defined for coating with IgG. IgM antibody was purified from anti-rat rbc antiserum (Cappel) by fractionation on a sepharose CL-6B column (Pharmacia). The IgM-coated rbc were then coated with complement by incubation in a 1/5 dilution of normal rat serum at 4 °C for 15 min and washed three times before use. IgM-coated cells did not bind to adherent cells.

Trypsinised red blood cells (TE). Trypsinised autologous rbc (5% v/v) were prepared by incubation of rbc with 1 mg/ml trypsin (Hopkins and Williams #883600) in Ca^{2+}/Mg^{2+} free HBSS for 15 min at 37 °C.

Receptor binding assay

Coated cells were washed three times before use and all rbc were used within 24 h of preparation; 0.5 ml (2×10^6) of coated rbc in RPMI 1640 media supplemented with 10% FCS were overlayed on each adherent cell monolayer and incubated at 37 °C for a further 1 h. Cultures were then washed three times to remove the nonbound rbc. Nontreated rbc did not bind to the adherent cells. Monolayers were fixed in 3% glutaraldehyde for 30 min, stained with Giemsa, mounted on coverslips, and then viewed under a microscope. With the aid of a graticule, the numbers of adherent rbc/monocyte were determined on each coverslip. At least five randomly chosen fields per coverslip were counted (50–100 cells/field). Assays were carried out in triplicate.

Results

Adjuvant induced arthritis

Figure 1 shows the severity of adjuvant-induced arthritis in DA and JC rats at day 14 as measured by increased hindpaw thickness. The adjuvant used in this study (Mtb/Squalane) induced severe polyarthritic disease in DA rats, evidenced not only by increased hindpaw thickness but also by nodule formation and the involvement of other joints [22]. However, the same adjuvant induced a significantly less marked disease in JC rats. While gold therapy resulted in a reduction of disease in DA rats, JC rats developed an enhanced disease when treated with GST. It was noteworthy that GST treatment of normal JC rats resulted in footpad thickening equal to that induced after adjuvant alone.

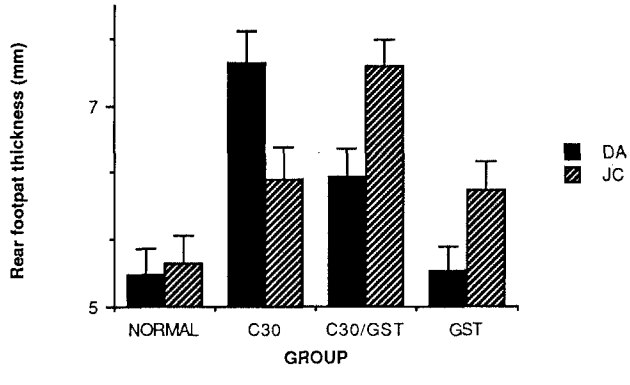

Fig. 1. Change in polyarthritic disease induced by injection of adjuvant was assessed by measuring the rearpaw thickness. Four groups of six to eight DA or JC rats were assessed 14 days after injection of adjuvant: normal untreated animals (NORMAL), animals injected with adjuvant alone (C30), animals injected with adjuvant and treated with GST (C30/GST), and normal animals treated with GST (GST). The results are expressed as the mean paw thickness ±SE

The effect of in vivo GST treatment on the recovery of adherent monocytes and macrophages

GST treatment of normal and adjuvant injected animals enhanced the recovery of adherent cells from the PE in both DA and JC rats at day 14 (Table 1). An increase in the number of cells containing peroxidase positive staining granules partially accounted for this enhanced recovery. GST treatment of normal animals enhanced the recovery of adherent monocytes from the PB particularly in JC rats. Similarly, injection of adjuvant enhanced the recovery of adherent monocytes particularly in JC rats. However, GST treatment enhanced the recovery of adherent monocytes from inflamed DA rats, yet decreased recovery from inflamed JC rats.

Table 1. Recovery of adherent monocytes and macrophages. Adherent cells were isolated from the PB and PE (as described in Methods) from groups of four to six DA or JC rats 14 days after injection of adjuvant. The numbers of adherent cells were determined by counting, with the aid of a graticule, at least 5 randomly chosen fields per coverslip. The four groups assessed were: normal untreated animals (NORMAL), animals injected with adjuvant alone (C30), animals injected with adjuvant and treated with GST (C30/GST) and normal animals treated with GST (GST). Greater than 95% of the adherent cells stained positive for nonspecific esterase in all groups

DA rats	Peritoneal cavity		Peripheral blood
	Total[a]	Perox.+VE[b]	Total[c]
NORMAL	39.5 ±10.5	(4.2)	1.12±0.20
C30	33.5 ±14.3	(9.4)	1.92±0.20
C30/GST	84.5 ±12.2	(17.8)	4.14±0.88
GST	56.0 ±15.6	(13.2)	1.52±0.36
JC rats			
NORMAL	33.9 ± 5.9	(2.8)	0.58±0.14
C30	52.1 ± 5.7	(9.4)	2.66±0.31
C30/GST	71.3 ±16.6	(17.5)	1.90±0.32
GST	121.0 ±32.5	(38.4)	2.48±0.12

[a] Numbers of adherent cells ($\times 10^5$) obtained from the peritoneal cavity
[b] Numbers of adherent cells ($\times 10^5$) obtained from the peritoneal cavity containing peroxidase positive staining granules
[c] Numbers of adherent cells ($\times 10^5$) obtained per ml of peripheral blood

The effect of in vivo GST treatment on receptor expression

PE macrophages were isolated from DA and JC rats 14 days after injection of adjuvant. The ability of these cells to bind IgGE, c'E and TE was used to assess the Fc receptor, complement receptor and nonimmune receptor binding activity respectively (Fig. 2). Injection of adjuvant alone had no significant effect on the binding activity of any of the receptors on PE macrophages at day 14. However, GST treatment of normal and adjuvant-injected animals resulted in a significant increase in the activity of all receptors when compared to untreated animals.

The Fc receptor activity of PE macrophages and PB monocytes was compared in the same groups of DA and JC rats (Fig. 3). GST treatment did not affect the Fc receptor activity of PB monocytes in normal rats, but rats injected with adjuvant showed a significant increase in Fc receptor activity. This increase was much greater in the JC rat. GST treatment of both DA and JC rats injected with adjuvant reduced the Fc activity of PB monocytes to normal levels.

The effect of GST treatment on Fc receptor activity of PE macrophages and PB monocytes was observed during the 14 days after injection of adjuvant was followed (Fig. 4). The Fc binding activity of PB monocytes was always substantially less than PE macrophages. The Fc receptor activity of JC PE macrophages following GST treatment increased to more than twice normal levels within 2 days (having received one GST injection) and was main-

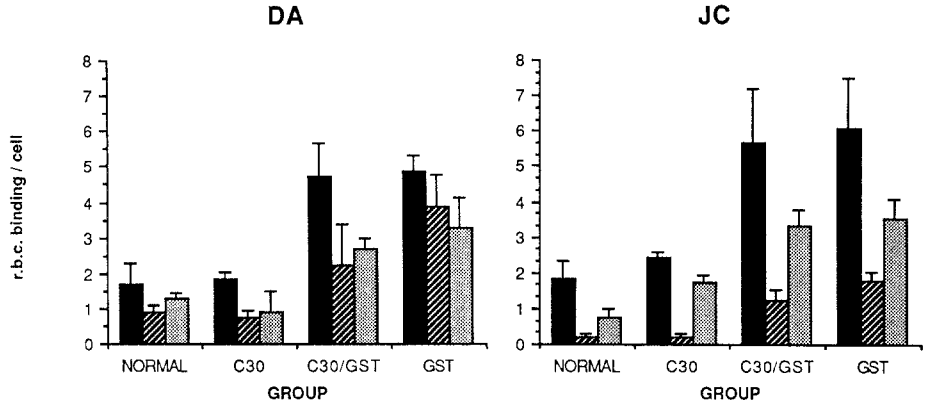

Fig. 2. The receptor binding activity of PE macrophages was assessed by measuring the binding of IgG-coated (■), complement-coated (▨) and trypsinised autologous rbc (▩) (as described in Methods). Macrophages were isolated from DA and JC rats from the same four groups as in Fig. 1. The results are expressed as the mean number of rbc binding per cell ±SE

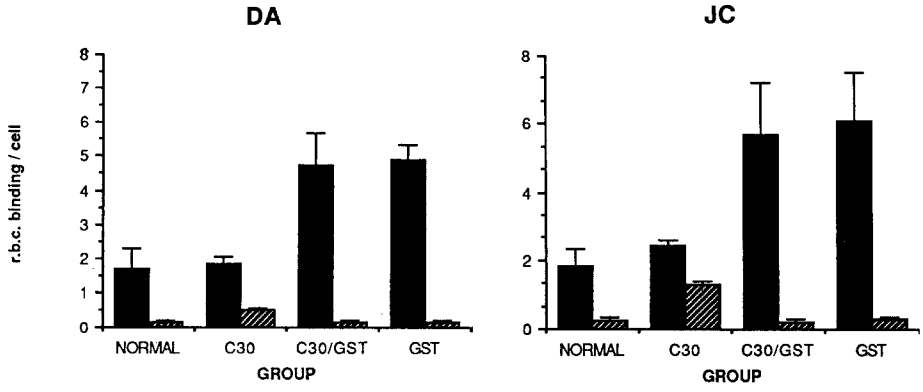

Fig. 3. The Fc receptor activity of PB monocytes (▨) and PE macrophages (■) was compared in the same four groups of DA and JC rats (Figs. 1 and 2). The results are expressed as the mean number of rbc binding per cell ±SE

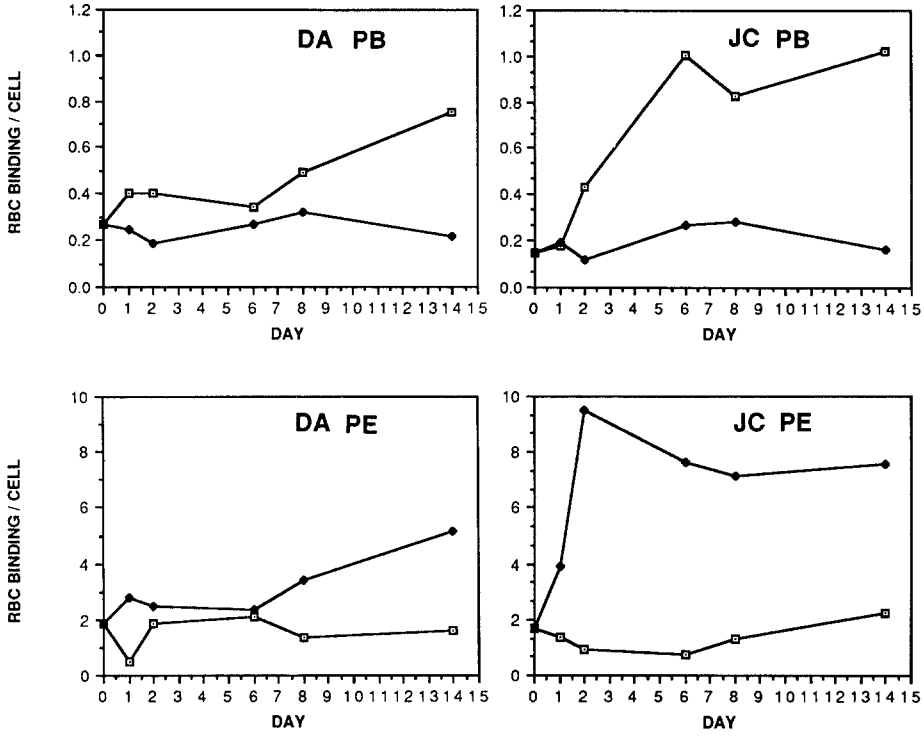

Fig. 4. The changes in Fc receptor activity of the PB monocytes and PE macrophages observed during the 14 days after injection of adjuvant in untreated (-□-) and gold-treated (-◆-) DA and JC rats. Groups of four animals were assessed on days 0, 1, 2, 6, 8, and 14. The results are expressed as the mean number of rbc binding per cell

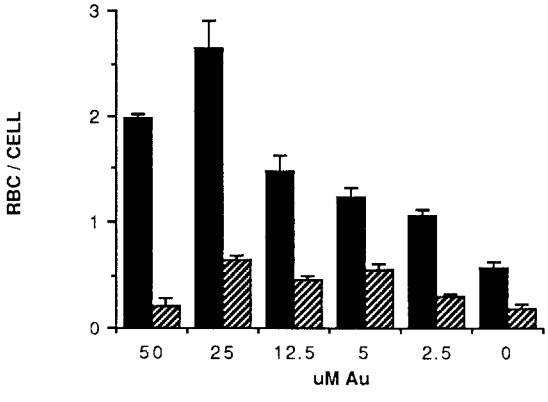

Fig. 5. PE macrophages from normal JC rats were incubated with GST for 24 h and their Fc-binding activity assessed as previously described. The total number of rbc binding (■) and the number of rbc phagocytosed (▨) were determined. The results are expressed as the mean ±SE of four experiments

tained at levels greater than four times normal thereafter. DA rats did not respond as rapidly or to the same degree, with Fc receptor activity not reaching more than twice normal levels until day 8, and being maintained at this level until day 14.

The effect of in vitro GST treatment on receptor expression

PE macrophages were isolated from normal JC rats and incubated with GST for 24 h. The Fc receptor binding activity was then assessed (Fig. 5). GST at a concentration of 2.5 μ*M* caused a significant increase in Fc receptor activity, with a peak effect noted at 25 μ*M*. At concentrations exceeding 50 μ*M*, GST was found to be toxic to the cells when assessed by trypan blue exclusion. During the 1-h

incubation the number of red blood cells phagocytosed also increased significantly with GST at a concentration of 5–25 μM. Similar results were obtained when PE macrophages from normal DA rats were studied.

Discussion

It has been reported recently that gold therapy can have ambivalent effects on adjuvant-induced polyarthritis in rats, being dependent upon the strain of rat used [24]. In the study reported here the effect of polyarthritis and GST treatment on macrophage and monocyte receptor function in DA and JC rats has been investigated. Increased Fc receptor activity was observed with peripheral blood monocytes from inflamed rats of both strains. This is consistent with human studies which have demonstrated that increased receptor activity is associated with active rheumatoid arthritis [25–27].

In rats injected with adjuvant, the arthritogen (mycobacterium) leaves the site of injection via the draining lymphatics within the first few days [28]. It is then quickly disemminated throughout the body, probably via the thoracic duct and blood stream. Labelled material has been identified in the knee joint and in other sites of rats injected with adjuvant containing labelled mycobacteria [28]. Macrophages and monocytes are considered to play an important role in the clearance and degradation of the material. The reduced susceptibility of the JC rat strain (as compared to the DA) to adjuvant arthritis could be a reflection, therefore, of its ability to respond to the injection of adjuvant by an increase in the receptor activity and the numbers of peripheral blood monocytes during the first few days after injection. This increased receptor activity would allow enhanced uptake and degradation of the arthritogen.

Increased receptor activity could be important at the site of inflammation in the removal of immune complexes and particulate matter that may otherwise stimulate inflammation by complement fixation. However, excessive receptor activity could result in activation of macrophages thereby stimulating the release of products that enhance inflammation. Gold treatment of JC rats may result in overstimulation of resident macrophages, such as in the peritoneal cavity, and enhancement of disease. The more moderate response elicited by GST in the DA rat may be sufficient to remove particulate matter and immune complexes without overstimulating the macrophages.

The observation that GST increased the Fc receptor activity of PE macrophages in vivo within 24 h at concentrations of gold similar to those observed in the serum of patients [29] and animals [30] undergoing gold treatment, is in accord with previous findings [31]. The effect of GST is unlikely to be mediated by other cell types as it occurred rapidly and the homogeneity of the cell population was maintained.

Gold treatment may induce changes consistent with an enhancement of maturation of mononuclear phagocytes. Promonocytes in the bone marrow mature to become monocytes of the PB which, in turn, mature to become macrophages in the tissues [32]. As they mature, more cells express Fc and complement receptors [33]. Since PE macrophage are unlikely to divide [32], the increased numbers of these cells isolated from gold-treated rats may be due to an influx of cells caused by maturation of PB monocytes. In addition the increased numbers of peroxidase-positive staining cells indicates that these cells are recently derived from the PB since resident PE macrophage do not contain peroxidase-positive staining granules [34].

Changes in macrophage receptor activity may help explain the variability of response in two different rat strains to adjuvant and gold therapy. The basis for the genetic variability of response is under further investigation. We have suggested that gold therapy may affect the maturation of cells of the monocyte/macrophage lineage. The subsequent changes in cell function may be markedly different depending apon the site from which the cells were isolated. The majority of studies (particularly those involving patients) use cells isolated from the peripheral blood to determine the effect of various antirheumatic drugs. These findings may not reflect the action antirheumatic drugs may have upon the function of cells at other sites, particularly in the inflamed joint.

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