# Effect of Gold Preparations on the Development and Passive Transfer of Experimental Allergic Encephalomyelitis in Rats[1] (36678)

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(Introduced by Carl M. Pearson)

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Experimental allergic encephalomyelitis (EAE) is an example of an immunologically mediated disease which has been studied in some detail (9). In EAE both delayed hypersensitivity to basic myelin protein and complement-fixing antibody to brain extract occur. In rats EAE can be passively transferred to syngeneic recipients by lymph node cells or by thoracic duct lymphocytes taken from animals previously immunized with the encephalitogen (19).

Development of EAE in rats can be successfully prevented by the administration of many antineoplastic agents, such as cyclophosphamide (10), L-asparaginase (4), cytosine arabinoside (2), 6-mercaptopurine (16), and methotrexate (15). In addition, antiproliferative drugs such as cycloleucine (12) suppress EAE. Consequently, the concept has arisen in recent years that one criterion of potential immunosuppressive activity of a drug is its ability to prevent, or at least delay, the development of EAE at subtoxic doses (13).

Gold preparations are effective in the treatment of rheumatoid arthritis (1). Immune mechanisms are implicated in the expression of rheumatoid arthritis (20). In an attempt to find out whether or not the use of gold preparations (chrysotherapy) might be influencing the underlying immune mechanisms, we studied the effect of various gold preparations on the course of EAE development and on its passive transfer by lymph node cells.

*Methods.* Male Lewis or Dunning-Fischer rats weighing 200–250 g were housed in lots of three or four in plastic cages containing sawdust throughout the experiments. Under light ether anesthesia, both inguinal lymph nodes were exposed through a midline ventral incision, and injected with approximately 0.01 ml each of an emulsion of guinea pig spinal cord and complete Freund's adjuvant prepared exactly as descibed by Newbould (6). Incisions were closed with metal wound clips. None of these immunized animals showed any subsequent signs of adjuvant disease (arthritis), but the incidence of EAE was greater than 90%.

*Transfer of disease.* Highly inbred male Lewis rats were used principally for these experiments, usually with three donor animals being sacrificed per recipient. Inguinal, lumbar, periaortic, mesenteric, paratracheal, and axillary lymph nodes were dissected out of the donor animals on days 7, 8 or 9 after sensitization and placed in Hanks' solution. The nodes were gently rubbed over fine wire cloth (200 mesh), immersed in Hanks' solution. After a brief rapid centrifugation to remove tissue debris, the cell suspension was slowly centrifuged to collect the cells. The supernate was discarded and the cell pellet resuspended in either Hanks' solution, admixed with 0.2 vol 0.1 *M* sodium phosphate (pH 7.4) for incubation with gold preparations *in vitro* at 37° for 30 min; or in 4 ml of a mixture of Hanks' solution with 0.25 vol serum from donor animals, for infusion into the recipient animal. Gold-incubated cells were isolated by centrifugation before resuspension in the infusion medium. Suspensions

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containing 3 to 5 $\times$ $10^8$ viable node cells were injected intravenously into the syngeneic recipients via a cannula inserted into the left femoral vein of each recipient animal. Viability of the node cells was checked with trypan blue. Recipients were observed daily for the subsequent 2 weeks.

*Evaluation of disease.* Only overt signs of paralysis were evaluated. Animals were weighed and examined daily. Onset of paralysis is defined as the first day on which flaccid paralysis of the tail was noted. Progression to hind limb and sphincter paralysis was noted in severly afflicted animals and correlated closely with weight loss.

*Gold preparations and their administration.* Animals were treated in groups of four with any given dose of a particular gold preparation. Solutions of sodium aurothiomalate (ATM) for injection were prepared by 6:1 saline dilution of Myochrysine (Merck, Sharp and Dohme, York, PA). Aurothioglucose suspended in sesame oil (Solganal, Schering Corp., Bloomfield, NJ) was used undiluted. Aqueous aurothioglucose (ATG) was prepared as a solution of the crystalline preparation (K & K Labs, Hollywood, CA) in 5% sodium acetate (w/v) and sterilized by filtration through a Millipore filter. Crystalline sodium aurothiosulfate (ATS, Sanochrysin, Ferrosan, Copenhagen, Denmark) was dissolved aseptically in saline. These solutions were prepared afresh and kept at 2° throughout the duration of the experiment. SK&F 36914 (triethylphosphino gold chloride) was suspended in sterile water for oral dosing, or dissolved in a 3:2 ethanol–water solution for intramuscular injection, daily. Preparations were injected through 25 gauge needles into hamstring or gluteal muscles in a dose of either 8 mg drug/kg. (*i.e.*, 4 mg of gold/kg) daily or 25 mg/kg every third day (q3d). In addition, groups of animals received SK&F 36914 as a suspension by oral gavage. Control animals received daily saline injections intramuscularly.

Treatment of actively immunized animals was initiated 1 day prior to sensitization with encephalitogen. The recipients of the node cells were only treated with gold preparations after cell transfer, the first dose being given later on the same day. A second group of recipients were treated with ATM 25 mg/kg im daily either on days 1–4 or 4–7 inclusive after node cell transfer.

*Determination of serum gold concentrations.* Serum gold concentrations were determined in groups of rats receiving sodium aurothiomalate (ATM) either in doses of 8 mg/kg daily or 25 mg/kg every third day. Both groups received the same total dose of ATM. Injections were scheduled so that both groups received their respective final injections 5 days prior to obtaining blood by cardiac puncture for analysis. Blood was allowed to clot spontaneously. Gold content of the serums was determined by atomic absorption spectrophotometry using an internal standard (5).

*Results. Effect of gold preparations on the course of EAE.* All gold preparations tested consistently delayed, but did not prevent signs of the disease, namely paralysis and weight loss. Onset of paralysis was consistent-

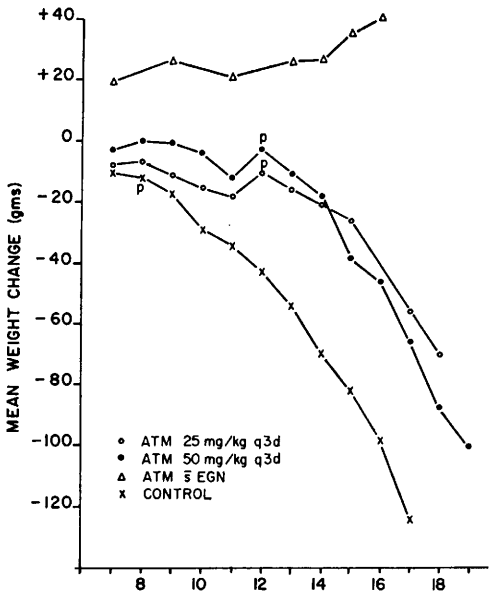


FIG. 1. Effect of aurothiomalate (ATM) given every third day on the course of EAE in Lewis rats; p marks the onset of paralysis. Animals given aurothiomalate but not encephalitogen did not lose weight (upper curve). (Abscissa = days after injection with encephalitogen.)

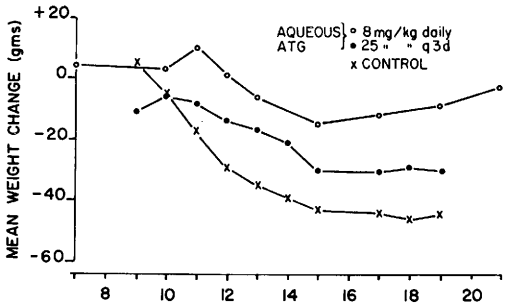


FIG. 2. Comparison of the effect of daily vs "every-third-day" injections of aqueous aurothioglucose on the development of EAE. The same total dose was given each group of treated Dunning-Fischer rats. Note the delay of paralysis by 6 days in the group treated daily. (Abscissa = days after injection with encephalitogen.)

ly delayed by 2 to 6 days, and weight loss was less severe in the gold-treated group when compared to sensitized animals receiving saline injections (Fig. 1). Animals treated with sodium aurothiomalate but not given encephalitogen gained weight. Daily injections gave superior results to the injection of 3 times the amount of gold "every third day" (Fig. 2). On this latter schedule (q3d), aurothioglucose administered as a suspension in sesame oil proved more effective than the aqueous drug (Fig. 3). In order of diminishing efficacy in delaying paralysis (and ameliorating weight loss) the preparations were: ATG > ATM ≡ SK&F 36914 > ATS (Fig. 4). SK&F 36914 was equally potent given either po or im. Treatment of sensitized animals with im monosodium thiomalate (4 mg/kg/day), or sodium thiosulfate (8 mg/kg/day) was completely ineffective, indicating that the gold component in the ATM and ATS was essential for a therapeutic effect.

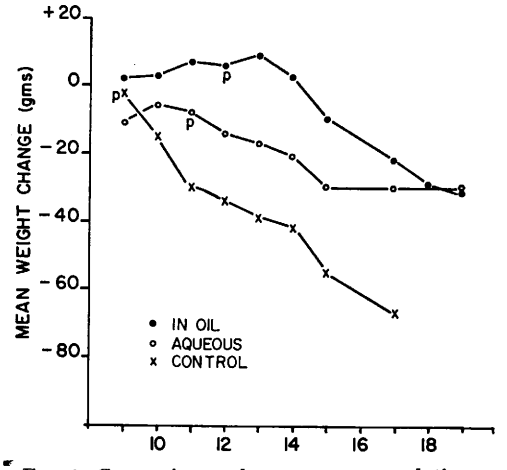


FIG. 3. Comparison of an aqueous solution vs sesame oil suspension of aurothioglucose given every third day (q3d) in dose of 25 mg/kg to Dunning-Fischer rats. Controls received no injections; p marks the onset of paralysis. (Abscissa = days after injection with encephalitogen.)

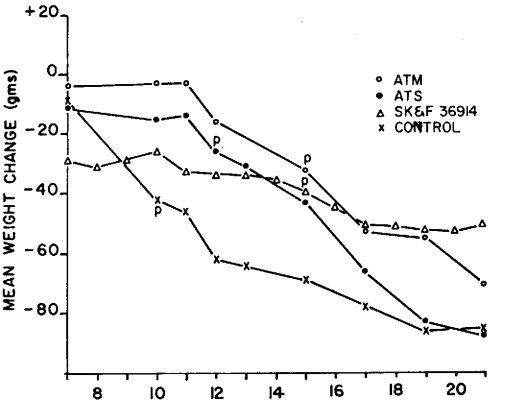


FIG. 4. Effect of sodium aurothiomalate (ATM), sodium aurothiosulfate (ATS), and SK&F 36914 at dose of 8 mg/kg daily on EAE in Dunning-Fischer rats. Controls received daily saline injections. Note delay in the onset of paralysis (p) by 5 days in the ATM- and SK&F 36914-treated groups. (Abscissa = days after injection with encephalitogen.)

*Effect of gold preparations on passive transfer of EAE.* EAE is readily transferred to virgin syngeneic rats by lymph node cells harvested from actively immunized donor animals between days 6 and 10 following inoculation with encephalitogen (8). In these experiments, we treated each of the following with gold compounds:

a. animals donating cells;
b. animals receiving cells; and
c. the cells themselves in transit.

*A. Donors treated with gold.* The donor animals were treated from the day prior to injection of encephalitogen until the time of sacrifice, with ATM 8 mg/kg im daily. Cells from these animals transferred the disease as readily as those from untreated donors. Fur-

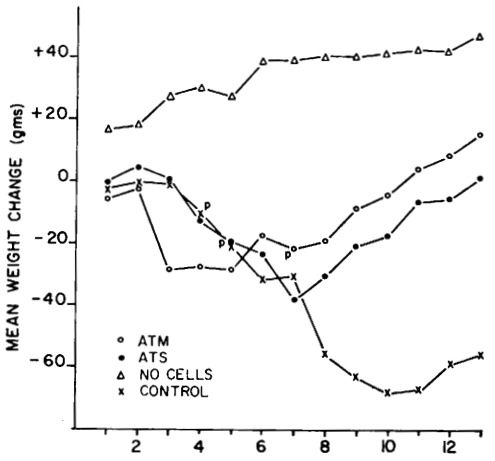


Fig. 5. Effect on passively transferred disease of treating recipients of sensitized lymph node cells (LNC) with sodium aurothiomalate (ATM) or sodium aurothiosulfate (ATS). Controls received daily saline injections. Gold-treated recipients had clinically milder disease reflected in less weight loss. p = time of onset of paralysis. (Abscissa = days after LNC transfer.)

thermore, the axillary and periaortic nodes from the gold-treated donors appeared grossly to be no less hypertrophied than those of the untreated sensitized littermates.

*B. Recipients treated with gold compounds.* When animals receiving the sensitized node cells (from untreated donors) were treated with either sodium aurothiomalate or aurothioglucose 25 mg/kg on the day of transfer and 8 mg/kg daily thereafter, the onset of paralysis was delayed by only 1 to 3 days, but the ensuing weight loss was significantly less than that suffered by the control animals which had received the same number of node cells and then been treated only with daily saline injections (Fig. 5). When recipients were treated with ATM 25 mg/kg/day on days 1–4 after node cell transfer, they failed to develop paralysis, and they maintained normal weight. Recipients treated with the same dose on days 4–7 became paralyzed 3 days later than, but lost approximately the same weight as, undrugged recipients.

*C. Sensitized node cells incubated with gold preparations at time of transfer.* When lymph node cells from sensitized, undrugged donors were incubated *in vitro* with the various water soluble gold preparations in concentrations attainable in serum, no significant beneficial effect was observed in the recipients. (Fig. 6).

*Serum gold levels.* The mean serum gold concentration in a group of 4 Lewis rats receiving sodium aurothiomalate 25 mg/kg im every third day was 3.58 ± 0.50 μg/ml. This contrasts with a mean gold level of 1.50 ± 0.30 μg/ml in a group of four litter mates treated with im aurothiomalate 8 mg/kg daily ($p < .05$). Both groups received the same total quantity of aurothiomalate, and both groups received their last injection of the drug 5 days before serum was withdrawn for analysis.

*Discussion.* Our studies also included observations on the effect of gold treatment on adjuvant arthritis in both Wistar and Fischer rats. Newbould (7) and more recently, Walz *et al.* reported that ATM (17) and SK&F 36914 (18) ameliorated the adjuvant-induced arthritis. However, Jessop and Currey (3) could not confirm that ATM had any benefit upon the development of adjuvant arthritis. While we have noted reductions in both paw inflammation and biochemical indices of disease (serum fibrinogen and glycoproteins) and preservation of serum albumin in some gold-treated groups of adjuvant injected rats, we were not able to reproduce these effects of ATM, ATS and ATG in other groups of animals on different occasions.

In contrast, we found that the intramuscular administration of gold compounds *consistently* delays the onset, and much diminishes

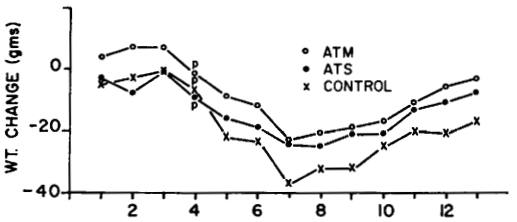


FIG. 6. Development of EAE in recipient animals after the passive transfer of lymph node cells (LNC) incubated *in vitro* with and without sodium aurothiomalate (ATM) or sodium aurothiosulfate (ATS). p = time of onset of paralysis. (Abscissa = days after LNC transfer.)

the severity, of EAE in both Lewis and Dunning-Fischer rats. In cell transfer experiments, gold treatment of animals donating node cells or treatment of the cells themselves *in vitro* failed to significantly modify subsequent development of disease in the recipients. However, treatment of the recipient animals after receiving node cells (from undrugged donors) both delayed and ameliorated the subsequent expression of the EAE.

Gold-treated donors of node cells exhibited gross lymph adenopathy. This differs from the response of animals treated with conventional immunosuppressive agents, such as cyclophosphamide, methotrexate, and cycloleucine; all of which profoundly depress the lymphadenopathy, following inoculation with encephalitogen. This is consistent with the fact that gold merely ameliorates the EAE whereas these more powerful drugs can completely suppress its development.

The macrophage has been postulated to be a target for gold drugs by Persellin and Ziff (11) who found that soluble gold is taken into the macrophage. It is clear, however, that any involvement of node macrophages in initially "processing" the encephalitogen is not affected by the gold treatment in these experiments. The subsequent role of the macrophage in mediating tissue injury might be regulated by the gold drug however, especially if such cells were to first become "sated" with the gold. In the present experiments, any macrophages involved in the expression of the disease would have been exposed to these gold preparations for 10 days or more.

Gold preparations might thus act, partly or wholly, as an anti-injury agent in target tissue. In rats, sodium aurothiomalate partially inhibits the paw edema immediately following adjuvant injection (7), and the pleural effusion induced by carrageenan (14) and could therefore be considered to have certain anti-inflammatory properties.

*Summary.* 1. Treatment of actively sensitized animals with various gold preparations delays the onset of the characteristic paralysis of EAE and appreciably diminishes the severity of the disease, as reflected in reduced weight loss of the gold-treated animals.

2. When the EAE was passively transferred by sensitized lymph node cells; treatment of the animals donating the cells prior to transfer, or incubation of the "pathophoric" node cells with gold preparations *in vitro*, did not retard the subsequent development of the disease in the recipient animals.

3. Treatment of recipient rats at, and after, the time of transferring sensitized lymph node cells, both delayed and attenuated the passively transferred disease.

4. Gold preparations apparently exert their beneficial effect upon EAE at a stage after the initial sensitization with encephalitogen.

5. Daily intramuscular treatment with 8 mg/kg aurothiomalate gave lower serum gold levels, but more effectively suppressed the disease, than treatment with 25 mg/kg aurothiomalate every third day.

6. Triethylphosphino gold chloride (SK& F 36914), a new lipophilic gold preparation, given either im or po, was approximately as effective as equal doses of sodium aurothiomalate in delaying the onset and decreasing the severity of the EAE.

7. Sodium aurothioglucose in sesame oil suspension (Solganal) was more effective than equal doses of aqueous aurothioglucose when given every third day. However, on the daily injection regimen, aqueous aurothioglucose protected animals more efficiently than the oily suspension.

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