Unique properties of auranofin as a potential anti-rheumatic drug

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Abstract
Gold salts, auranofin (AF), aurothiomalate (ATM) and aurothioglucose (ATG) displayed immunosuppressive action in a series of in vitro assays which mimic the cell-cell interactions thought to occur in rheumatoid arthritis. The gold salts inhibited phytohaemagglutinin (PHA)-induced thymidine incorporation and γ-IF production by peripheral blood mononuclear cells, as well as IL-2-induced proliferation of PHA-blasts. The separate addition of IL-2 and γ-IF partly reversed the anti-proliferative effects of ATM and ATG; however, the addition of IL-1 had no effect. ATM and ATG inhibited PHA-stimulated IL-1 production by mononuclear cells but not spontaneous or LPS-induced IL-1 production by adherent monocytes. It was concluded that ATM and ATG inhibited lymphocyte function and lymphocyte-amplification of macrophage function.

The anti-proliferative effects of AF were partly reversed by IL-2 but not by γ-IF or IL-1. AF inhibited PHA-stimulated IL-1 production by mononuclear cells as well as spontaneous and LPS-induced production by adherent cells. It appeared that AF inhibited lymphocyte and macrophage function directly. AF also displayed potential anti-inflammatory activity in that it inhibited PGE₂ and collagenase production by proteolytically dispersed rheumatoid synovial cells.

Introduction
Rheumatoid arthritis is a disease of chronic immunologically-mediated inflammation. It may result from a specific or polyclonal response to an unknown antigen. The disease is characterised by excessive antibody production which may be the result of B-lymphocyte hyperactivity driven by unrestrained T-lymphocyte helper/induced cells. Present in the joint synovial tissue are macrophages and macrophage-like synovial cells which express Ia determinants of the Major Histocompatibility Complex. Antigen presented in association with surface Ia markers can serve as a stimulant in the mixed lymphocyte reaction. The importance of lymphocytes in the pathogenesis of rheumatoid arthritis is supported by profound clinical improvement seen in rheumatoid patients following depletion of recirculating lymphocytes either by lymphapheresis [1] or thoracic duct drainage [2]. The development of adjuvant arthritis in rats has also been delayed by depletion of T-cells using anti-pan T-cell monoclonal antibodies (W3/13) [3]. Implication of the macrophage or antigen-presenting cell has come from a study in which antibodies against Ia (HLA-DR) were found to alleviate the symptoms of rheumatoid arthritis [4]. The expression of Ia determinants on macrophages can be stimulated by lymphocytes through the production of γ-interferon (γ-IF) [5]. Ia expression is thought to be implicated in macrophage production of in-
terleukin (IL)-1 [6]. IL-1 is an amplifying factor in IL-2 receptor expression and IL-2 production in antigen and mitogen driven lymphocyte proliferation [7]. IL-2 functions as the growth factor supporting T-cell proliferation. If the pathogenesis of rheumatoid arthritis stems from macrophage-lymphocyte interactions, then the above cytokines may play a modulating role in the disease. It follows that pharmacological agents which regulate the release or action of these cytokines may modulate the course of rheumatoid arthritis. Gold compounds are one of the few classes of drugs which retard the progress of the disease [8]. Aurothiomalate (ATM) and aurothioglucose (ATG), both injectable gold salts, are considered therapeutically equivalent. Auranofin (AF), an orally active gold preparation, appears to be slightly less potent, although clinical experience with this new agent has so far been limited [9]. It is, however, reported to be less toxic. We have studied the effects of these gold compounds in a series of in vitro assays which mimic the cell-cell interactions thought to occur in the rheumatoid synovium. All three gold preparations modified lymphocyte amplification of macrophage function while AF, in addition, affected macrophage function directly. AF also inhibited the generation of inflammatory mediators from rheumatoid synovial cells in culture.

Materials and Methods

Auranofin (Smith Kline and French Labs Ltd) was dissolved in absolute alcohol at 20 mg/ml and further diluted in Dulbecco's Modified Eagles Medium (DMEM) to the required concentrations. Aurothiomalate (10% Myocrisin, May and Baker Ltd) was diluted in DMEM as required. Aurothioglucose (Sigma) was dissolved at 8 mg/ml in DMEM and sterilized by membrane filtration (0.22 μm Millex) prior to dilution in DMEM. The cytokine preparations used were purchased from the sources indicated: interleukin-1 (Genzyme, UK, 100 u/ml), γ-interferon (Ventrex, USA, 100 u/ml), T-cell growth factor (Associated Biomedics Systems Inc, USA). The isolation and culture of peripheral blood mononuclear cells were essentially as described in our previous paper in these Proceedings. Drugs and cytokine preparations were added at the beginning of the culture period. γ-IF in the culture supernatant was measured directly after 72 h using a Centocor RIA kit. Adherent monocytes were prepared by incubating for 2 hours at 37°C and non-adherent cells removed by repeated washing. Drugs were then added and incubation continued for a further 48 hours.

Adherent synovial cell cultures (RASC) were established essentially as described by other workers [10], using synovectomy material obtained from rheumatoid patients undergoing corrective surgery. Adherent synovial cells (3 × 10⁵/ml), maintained in DMEM supplemented with 10% heat-inactivated foetal calf serum (HIFCS) release large amounts of prostaglandin E₂ and collagenase during primary culture. Upon sub-culturing, the production of mediators declines to low or undetectable levels. These quiescent cells were used for assay of IL-1 action through stimulation of PGE₂ release. After 48 h incubation periods at 37°C in 5% CO₂, the cell-free supernatants were removed and the PGE₂ content measured as previously described [11]. Mononuclear cell supernatants were assayed on synovial cells at 1 in 10 and 1 in 25 dilutions. At these dilutions, the effects of drugs and PGE₂ production by mononuclear cells were insignificant.

In collaboration with Dr W. Harvey (Eastman Dental Hospital, London), synovial cell supernatants were assayed for collagenase by digestion of ³H-acetylated rat skin collagen fibrils, using trypsin to activate latent enzyme.

Results

All three gold salts, AF, ATM and ATG, inhibited both sub-optimal (0.1 μg/ml) and optimal (1.0 μg/ml) PHA-stimulated ³H-thymidine incorporation in peripheral blood mononuclear cells as seen in Figure 1. The gold salts were more effective against sub-optimal than optimal PHA-induced stimulation.

AF (IC₅₀ 0.84 μg/ml) was the most potent inhibitor, being approximately 160 and 480 times more active on a weight basis than ATM (IC₅₀ 138 μg/ml) and ATG (IC₅₀ 405 μg/ml) respectively.

Exogenous IL-2 (T-cell growth factor, 1 u/ml) partly reversed the inhibitory effects of the gold salts upon sub-optimal PHA-stimulated cells as illustrated in Figure 2a. Recombinant IL-2 (100 u/ml) had a similar effect (results not shown). The effect was notable against concentrations of gold