The Anti-rheumatic Gold Salt Aurothiomalate Suppresses Interleukin-1β-induced Hyaluronan Accumulation by Blocking HAS1 Transcription and by Acting as a COX-2 Transcriptional Repressor*

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Gold compounds are among the oldest disease-modifying drugs and are still widely used today for treating rheumatoid arthritis. Despite decades of use, little is known about the mode of action of this class of drugs. Here we have demonstrated that aurothiomalate (AuTM) suppresses hyaluronan accumulation by blocking interleukin (IL)-1β-induced hyaluronan synthase-1 transcription. We have further demonstrated that, in fibroblast-like synoviocytes (FLSs), AuTM acts as a specific COX-2 transcriptional repressor in that IL-1β-induced COX-2 transcription is blocked, whereas COX-1 transcription and translation is unaffected. As a consequence, PGE2 levels released by FLS are dose-dependently reduced in cells exposed to AuTM. Of similar importance is the demonstration that AuTM does block NFκB-DNA interaction. In addition, two other transcription factors implicated in inflammatory events, namely AP-1 and STAT3, are blocked as well. The effect on NFκB likely explains the inhibition of COX-2 as well as that of HAS1, as both are genes that depend on the activation of NFκB. Interestingly, AuTM does not interfere with IL-1β-induced 16κβ degradation, in most cases a prerequisite for subsequent NFκB activation. Furthermore, evidence is presented that, in FLS, AuTM blocks NFκB-DNA interaction neither by binding to NFκB binding sites nor by interacting with activated NFκB proteins. Taken together, AuTM treatment of FLS blocks two of the most important pro-inflammatory events that are associated with rheumatoid arthritis. AuTM blocks the release of PGE2 and prevents the activation of NFκB, therefore blocking IL-1β-induced hyaluronan accumulation and likely a series of other pro-inflammatory NFκB-dependent genes.

There is mounting evidence that activated fibroblast-like synoviocytes (FLSs) play an exceptionally important role in the genesis and progression of rheumatoid arthritis (RA) (1). Although it is well established that a reactive immune system contributes to the inflamed synovium, e.g. by the release of pro-inflammatory cytokines such as tumor necrosis factor α and interleukin-1β (IL-1β), several clinical as well as experimental studies point at FLSs as a likely trigger of the pathogenesis of RA. The severe combined immunodeficiency mouse model of RA and a series of other experimental studies provide ample evidence that altered FLSs are capable of initiating RA in the absence of a functioning immune system (2, 3).

One characteristic feature of RA is the amount of hyaluronan (HA) found in the synovium (4, 5). It has long been known that HA effusion is indeed a very sensitive marker for dysfunctional, altered connective tissue (6). In joints, FLSs are the main source of the glycosaminoglycan molecule HA. HA forms only a fine layer in healthy joints. In RA-affected joints, however, total concentrations of HA can be enormous. Consequently, it has been suggested that plasma levels of HA could serve as a reliable marker for RA disease progression (7). Although controlled HA release is certainly essential for proper joint function, unfettered HA release is associated with a series of detrimental effects. HA fragments, readily created in an inflamed environment, possess chemoattractant properties, induce angiogenesis, and activate pro-inflammatory genes (8, 9). Among the many detrimental effects of HA fragments are the activation of NFκB, the stimulation of dendritic cells, as well as the activation of chemokines (10–15). HA surface expression also facilitates cell migration by acting as the major ligand for CD44 (16). In the later stages of RA, radicals released by activated cells will also drive HA degradation, further fueling inflammation. All of these factors make unfettered HA release highly undesirable but also point at HA-encoding genes as possible targets for medical intervention to interrupt the cycle leading to joint destruction.

In mammals, three genes encoding for plasma membrane protein are responsible for HA synthesis (17). As demonstrated earlier, in FLS, the hyaluronan synthase genes HAS2 and HAS3 are constitutively activated, and HAS1 can be readily induced by a series of cytokines (18). We have shown that the p38 mitogen-activated protein kinase plays an essential role in cytokine-induced HAS1 transcription and demonstrated the effect of phoretic mobility shift assay; RT, reverse transcription; CRE, cAMP-response element.
tiveness of glucocorticoids in reducing HA synthesis in FLS (18, 19). We further tested a series of drugs that have been successfully used to ameliorate RA. In the following, we tested the gold compound aurothiomalate (AuTM) for its effectiveness as an inhibitor of IL-1β-induced HA accumulation and studied the mechanisms that might account for the effectiveness of gold as one of the oldest disease-modifying antirheumatic drugs. The rational use of gold in medicine began in the early 20th century when Robert Koch discovered that a gold compound can kill the bacteria that cause tuberculosis. In 1929, Forester (believing that RA was related to tuberculosis) was the first to pioneer the use of gold injections as a treatment for RA (20). Despite their many side effects, gold compounds are still in use today and, as demonstrated again by a very recent report (21), can indeed be of great benefit for RA patients.

Despite the long term use of gold compounds as a remedy for RA, little is known about the molecular mechanisms that account for AuTM effects. Here we have studied the effectiveness of AuTM as a suppressor of IL-1β-induced HA accumulation in cells that are of significant importance for this disease. With regard to the mechanisms of AuTM-mediated anti-inflammatory effects, our experimental data support the concept that AuTM acts as a COX-2 transcriptional repressor and blocks the activation of a series of transcription factors involved in inflammatory responses.

**EXPERIMENTAL PROCEDURES**

**Reagents**—If not stated otherwise, reagents such as IL-1β, AuTM, and cell culture media, etc. were from Sigma (Vienna, Austria). HA enzyme-linked immunosorbent assay (ELISA) was from Corgenix, (Westminster, Colorado). PGE2 ELISA and COX-2 antibodies were from Cayman Chemical Company Europe, (Tallinn, Estonia). Phosphospecific and non-phosphospecific antibodies (c-Jun, c-Fos, STAT3, IκBα) were from Cell Signaling Technology, (Beverly, MA). Oligonucleotides for electrophoretic mobility shift assay (EMSA) experiments and antibodies for supershifts were from Santa Cruz Biotechnology, (Santa Cruz, CA).

**Cell Culture**—Human FLSs (type-B synoviocytes) were purchased from Dominon Pharmakine, (Derio, Bizkaia, Spain) or were isolated and cultured in our laboratory as previously described (18). In brief, FLSs were propagated in T75 tissue culture flasks or culture dishes (Iwaki, Funabashi, Chiba, Japan) in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 1-glutamine, and 50 units/ml penicillin/streptomycin. For experiments, FLSs were detached using trypsin and transferred to 6- or 24-well plates (Iwaki, Funabashi). For ELISA experiments, FLSs were cultured in 24-well plates, in 6-well plates for reverse transcription (RT)-PCR and in 10-cm dishes for EMSA experiments, respectively.

**SDS-PAGE and Western Blotting**—Cells were washed twice in ice-cold phosphate-buffered saline and subsequently dissolved in SDS sample buffer (5 mmol/liter Tris/HCl (pH 6.8), 2% w/v SDS, 10% glycerol, 50 mmol/liter di-thiothreitol, 0.01% w/v bromphenol blue). Aliquots of whole cell protein extracts (10–25 μl/well) were separated on minigels. Proteins were blotted onto polyvinylidene difluoride membranes (Amersham Biosciences) using a semidry apparatus (Bio-Rad) and processed exactly as described in the provided protocols (Cell Signaling Technology). Proteins were made visible using LumiGLO (New England Biolabs, Beverly, MA) and Kodak BioMax MR films or the chemiluminescence detection device GeneGnome (Syngene, Cambridge, UK).

**RNA Isolation and RT-PCR**—RNA isolation, RT-PCR, quality controls, and quantifications were done as described previously (18, 22). Small aliquots of RNA were used to check the quality of RNA using agarose gel and ethidium bromide or Vistagreen (Molecular Probes, Eugene, OR) for visualization. First strand cDNA synthesis was performed exactly as described by the supplier of the RT-PCR kit (Amersham Biosciences). Aliquots were used for PCR. Primer sequences and experimental conditions for conventional block cycler RT-PCR as well as for real time RT-PCR have been described in detail elsewhere (22, 23). Each RT-PCR experiment included a dissociation curve to verify the specificity of the amplicon as well as no-template controls.

**EMSA**—Nuclear extracts from FLSs were prepared essentially as described previously (24). The consensus oligonucleotides purchased from Santa Cruz Biotechnology or from Promega (Mannheim, Germany) were as follows: AP-1 (5′-CCG TTG ATG AGT CAG CCG GAA-3′), AP-2 (5′-GAT CGA ACT GAC CGC CCG CCG GCC GT-3′), NFκB (5′-AGT TGA GGG GAC TTT CCC AGG C-3′), CAMP response element (5′-AGA GAT TGC CTG ACG TCA GAG TAG-3′), and SP-1 (5′-ATT CGA TCG GGG GCG GGC GAG C-3′). The double-stranded oligonucleotides were end-labeled using T4 polynucleotide kinase and [γ-32P]ATP. After labeling and purification by chromatography, 5 μg of nuclear extract was incubated with 100,000 cpm of labeled probe in the presence of 1.5 μg of poly(dI-dC) at room temperature for 20 min followed by separation of this mixture on a 6% polyacrylamide gel in Tris/glycine/EDTA buffer at pH 8.5. For supershift and competition experiments, nuclear protein extract (5 μg) was added to tubes containing all of the binding buffer components (100 mM HEPES (pH 7.9), 250 mM NaCl, 5 mM EDTA, 5 mM 2-mercaptoethanol, 0.5% Tween 20, 25% glycerol) including poly(dI-dC). Where indicated, 1 μl of unlabeled oligonucleotides or 1 μl (2 μg) of an antibody solution was added together with nuclear extract. The resulting reaction mixtures were incubated for 30 min at room temperature after which labeled oligonucleotides were added. For nonspecific competition, 7 pmol of double-stranded oligonucleotides were used. The following antibodies (Santa Cruz Biotechnology) were used for supershift experiments: NFκB p50 (C-19) sc-1190X, NFκB p52 (K-27X sc-298X, NFκB p65 (A)X sc-109X, c-Rel (B-6)X sc-695X, STAT3 (H-190)X sc-7179X).

**HA Measurements**—Aliquots of culture medium were removed, centrifuged (5 min at 2000 × g), and tested for the presence of HA via a procedure provided by Corgenix (Westminster, Colorado). Optical density values were used to calculate HA levels using a universal assay calculation program (AssayZap, Biosoft, Cambridge, UK).

**Quality Control and Data Analysis**—Special care was taken to terminate PCR reactions on the conventional block cycler in the log phase of amplification. As demonstrated earlier (18), a series of cycles were routinely tested to define optimal PCR
AuTM Inhibits HA in a Dose-dependent Manner—As demonstrated earlier, IL-1β is a strong inducer of HAS1 transcription and HA release in FLS (18). First, we tested the effect of AuTM on IL-1β-induced HA accumulation by HA-specific ELISA. FLSs, grown to high density, were incubated with 0, 25, 50, and 100 μM AuTM for 24 h. Where indicated, IL-1β (5 ng/ml) was added for 16 h. Supernatant was harvested, centrifuged, and analyzed for the presence of HA by HA-specific ELISA. Pretreating FLS with 25 μM AuTM (Au25 + IL-1) was sufficient to significantly inhibit IL-1β-induced HA release. Increasing AuTM to 50 or 100 μM resulted in more pronounced inhibitory effects. MED, untreated cells.

RESULTS

AuTM Inhibits HA in a Dose-dependent Manner—As demonstrated earlier, IL-1β is a strong inducer of HAS1 transcription and HA release in FLS (18). First, we tested the effect of AuTM on IL-1β-induced HA accumulation by HA-specific ELISA. FLSs, grown to high density, were incubated with 0, 25, 50, and 100 μM AuTM for 24 h. Subsequently, FLSs were stimulated with IL-1β (5 ng/ml) for 16 h. HA levels were quantitated by ELISA. Shown in Fig. 1 is a representative experiment that demonstrates that preincubation with AuTM blocks IL-1β-induced HA release dose-dependently. Compared with FLS treated with only IL-1β, levels of HA were significantly lower in FLS pretreated with 25 μM AuTM (n = 3, p = ≤0.05). Increasing the concentration of AuTM further resulted in further reduction of IL-1β-induced HA release. However, preincubating FLS with AuTM for 30 min or 2 or 6 h, respectively, was not sufficient to significantly affect HA levels in the supernatant of FLS exposed to IL-1β (data not shown).

AuTM Influences IL-1β-induced HAS1 mRNA Transcription—As shown in Fig. 2, compared with IL-1β-treated FLS, HAS1 steady state mRNA levels are lower in AuTM-pretreated cells. FLSs were treated with 10 and 100 μM AuTM for 24 h. Where indicated, IL-1β (5 ng/ml) was added for 10 h. HAS1 and actin mRNA were amplified on a block cycler and separated on an agarose gel. Gel sections and a graph resulting from the quantification of gel sections on the FluorImager 595 device are shown. As demonstrated here, 100 μM AuTM almost completely abolished the IL-1β effect on HAS1 mRNA, and 10 μM were still sufficient to significantly lower IL-1β-induced mRNA levels. At the concentrations used, no effect of AuTM on actin mRNA levels was noted.

AuTM Affects PGE2 Levels in FLS—We found earlier (33) that PGE2 is one of the most potent inducers of HAS1. We believed AuTM might affect PGE2 levels released by FLS and therefore tested whether AuTM exerts its effect on HA and HAS1 mRNA accumulation by interfering with PGE2 release. Levels of PGE2 in IL-1β-stimulated FLS were measured and compared with PGE2 levels in cells pretreated with 20, 50, and 100 μM AuTM for 24 h. Shown in Fig. 3 are representative data demonstrating that in unstimulated FLSs, PGE2 levels are below the detection limit, whereas the exposure of FLS to IL-1β (5 ng/ml) for 8 h resulted in the release of 863 ± 67 pg of PGE2. Pretreating FLS with 20 μM AuTM did not result in significantly lower levels of PGE2 in the supernatant of these cells. However, increasing AuTM concentrations to 50 and 100 μM, respectively, resulted in significantly lower PGE2 levels (48 ± 5 and 72 ± 8% inhibition, respectively).

AuTM Suppresses COX-2 but Has No Effect on COX-1 Translation—To account for the lower PGE2 levels observed in FLS, COX-1 as well as COX-2 protein levels were tested in cells exposed to 10 and 100 μM AuTM for 24 h and compared with untreated and IL-1β (5 ng/ml)-treated cells. COX-1 protein is readily detectable in whole cell protein extracts of FLS, as demonstrated by the Western blot experiment shown in Fig. 4. More importantly, COX-1 protein levels are not altered either through exposure of FLS to IL-1β or through the exposure of
AuTM Does Not Prevent IL-1β-induced IκBα Degradation—NFκB activation is of relevance for IL-1β-induced COX-2 and HAS1 activation (23). Enzymatic degradation of IκBα is in most cases a prerequisite for subsequent NFκB translocation, and a series of anti-inflammatory drugs have been shown to exert their beneficial effects by blocking IκBα degradation. FLSs were left untreated or were treated with AuTM (0, 12, 25, 50, and 100 μM) for 24 h. Subsequently, IL-1β (5 ng/ml) was added for 20 min. As shown in Fig. 6, IκBα protein is readily detected in resting FLS (medium) and is completely degraded in response to IL-1β treatment (Fig. 6, lane 0 plus IL-1). More importantly, AuTM, even at the concentration of 100 μM, did not prevent IL-1β-induced IκBα degradation.

AuTM Does Not Alter mRNA Levels of Jun or Fos Nor Does It Prevent Phosphorylation of c-Jun and STAT3—To account for the effects of AuTM seen on transcription factor-DNA interactions in EMSA experiments, a series of experiments were done that might elucidate the underlying mechanisms of this phenomenon. AuTM exerts its effect only after prolonged incubation, therefore down-regulation of AP-1-binding proteins was one possible explanation. However, real time RT-PCR experiments (data not shown) proved that mRNA levels of Jun and Fos were unaltered in FLS exposed to AuTM (66 and 100 μM) for up to 48 h, the latest time point at which mRNA levels were analyzed. Similarly, neither protein levels of c-Fos nor phosphorylation of c-Jun in response to IL-1β treatment nor the phosphorylation of STAT3 was significantly altered in FLSs pretreated with AuTM for up to 48 h. Shown in Fig. 7 is an experiment in which FLSs were incubated with AuTM (30, 44, 66, and 100 μM) for 48 h, after which IL-1β (5 ng/ml) was added.
where indicated. The labels “MED” and “IL-1” indicate the position of untreated and IL-1β-only-treated cells.

In FLS, AuTM Does Not Prevent NFκB-DNA Interactions by Binding to the NFκB Oligonucleotide or by Binding to NFκB Proteins—A series of transcription factors were blocked as a result of AuTM exposure; therefore, a series of experiments were performed to test whether AuTM prevents NFκB-DNA interaction by binding to DNA or to members of the NFκB protein family. FLSs were stimulated with IL-1β (5 ng/ml) for 45 min, subsequently nuclear proteins were extracted and used in EMSA experiments. Shown in the upper section of Fig. 8A is an experiment in which labeled NFκB oligonucleotides were incubated with the indicated amounts of AuTM for 24 h prior to stimulation with IL-1β for 45 min. Subsequently, nuclear protein extract was added, mixed, and incubated for an additional 15 min at room temperature. Subsequently, nuclear protein extract was added, mixed, and incubated for an additional 15 min at room temperature. Thereafter, samples were loaded on a 6% native gel, separated, and exposed to film. As shown in this section of Fig. 8, even preincubating NFκB oligonucleotides with 100 μM AuTM does not alter subsequent NFκB binding.

Similarly, as shown in the lower section of Fig. 8B, preincubating nuclear extracts with up to 100 μM AuTM for 1 h at RT was without significant effect on subsequent NFκB-DNA interactions. As in Fig. 8A, this time after preincubating nuclear protein extract with AuTM for 1 h at RT, oligonucleotides were
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RA came from an animal study demonstrating that prolonged injection of HA in healthy animals can cause all of the classical signs of RA (28). In addition to many clinical reports questioning the practice of HA injections, another animal study designed to demonstrate the beneficial effects of HA injections also provides evidence of potentially profound adverse effects of such measures (29).

Three genes are known to encode hyaluronan synthase (30). HAS1 is the only gene that is not active in resting FLS. However, this gene is readily up-regulated in response to a series of cytokines (18). We demonstrated that IL-1β-induced HAS1 activation depends on the activation and translocation of the transcription factor NFκB (23). Within the hyaluronan synthase family, HAS1 is therefore the only gene that shares a dependence on NFκB activation for its up-regulation with a large group of other molecules that are characterized by their pro-inflammatory properties (31). In further support of HAS1 being a gene with pro-inflammatory properties is the recent demonstration that it mediates HA-CD44-mediated cell migration (32).

Figs. 1 and 2 demonstrate that AuTM modulates HA release in FLS by interfering with HAS1 transcription. This by itself would be desirable in affected joints of RA patients and might explain some of the beneficial effects of this drug. With regard to the mode of action of AuTM-mediated HA inhibition in FLS, there seem to be at least two mechanisms that account for the observed phenomenon. First, the presented data support the concept that AuTM blocks IL-1β-induced HAS1 activation by blocking COX-2. As demonstrated, COX-2 protein levels are dose-dependently inhibited, whereas AuTM has no affect on COX-1 protein levels. That COX-2 is relevant in this context is evidenced by the demonstration that PGE2 is one of the most potent activators of HAS1 transcription (33). Secondly, in FLS, AuTM interferes with the translocation of a series of transcription factors, among them NFκB, a transcription factor that has been shown to be essential for the activation of IL-1β-induced HAS1 transcription and many other pro-inflammatory genes (23, 31, 34, 35). Although the inhibitory action of AuTM on NFκB activation might be attributed to its effect on PGE2, other mechanisms must be at work as well. Not only is NFκB affected by AuTM in a dose-dependent manner, but so are AP-1 and CRE-BP, two transcription factors that are highly activated in unstimulated FLS. In addition, STAT3-DNA interactions, absent in resting FLS and induced in IL-1β-stimulated FLS, are affected as well. Interestingly, IkBα degradation induced by IL-1β, in most cases a prerequisite for the release and the subsequent translocation of NFκB into the nucleus, is not affected by AuTM. These findings make it unlikely that events upstream of IkB account for the observed effects of AuTM in FLS.

DISCUSSION

In this manuscript, we present data that show that AuTM is able to block IL-1β-induced HA release in FLSs by blocking HAS1 mRNA transcription in a dose-dependent manner. Our interest in HA stems from the fact that the most prominent and early signs of RA are swollen joints. One of the characteristics of RA-affected joints is the large amount of synovial fluid containing HA and HA degradation products. Although HA has been considered to be unequivocally beneficial by many physicians, there are clear signs that HA degradation products exert a series of unwanted effects (4, 26, 27). The strongest support for our working hypothesis that unfettered HA might be detrimental in
interact with double-stranded DNA has been demonstrated (36). More importantly, gold has also been shown to interact with DNA bases in a sequence-specific manner (Ref. 37 and references therein), and it has been shown to be capable of diffusing into the nucleus or passing the nuclear envelope (38).

We did test the possibility that AuTM might interact with DNA, therefore blocking subsequent binding of transcription factors in a sequence-specific way. As shown in Fig. 8, such mechanisms are unlikely to account for the effects of AuTM on NF-κB-DNA binding in this cell type, as even 100 μM AuTM did not interfere with such interactions.

That auranofin, another gold-containing disease-modifying antirheumatic drug, does affect a series of NFκB-dependent genes in endothelial cells has been demonstrated previously (39). This group, using endothelial cells, reported protection of tumor necrosis factor-α/poly(ADP-ribose) polymerase cell-mediated cytotoxicity by this gold compound (40). However, auranofin and AuTM were not equally effective in that AuTM diminished only CD62E (a NFκB-dependent gene) expression, whereas auranofin suppressed both CD54 and CD62E. Another possible explanation for the observation that AuTM does not interfere with IL-1β-induced IkBα degradation but nevertheless blocks NFκB activation are gold-protein interactions as reported by others (41, 42). As demonstrated in Fig. 8B, it is unlikely that AuTM exerts its effect in FLS by binding directly to activated NFκB proteins, because preincubation of nuclear protein extracts of stimulated FLS with AuTM had no effect on subsequent DNA-protein binding in EMSA experiments.

Yet another possibility to account for the observed effects of AuTM on NFκB-DNA interactions might be that AuTM alters expression levels of NFκB inhibitors, such as the NFκB precursors p100 and p105 and BCL-3 and/or IkB. Such a modus operandi is again unlikely, because real time PCR as well as Western blot experiments showed no significant change in mRNA and protein levels of p50, p52, p65, IkBα, and BCL-3 (data not shown). Furthermore, as shown in Fig. 9, it is the classical p50/p65 protein complex that is activated in FLS and p52, a protein with the potential to act together with RelB as a repressor (43), not detectable in this cell type when stimulated with IL-1β.

Furthermore, gold has been shown to be capable of inducing certain heat shock proteins (44). Because this class of proteins have been shown to possess potent anti-inflammatory and NFκB inhibitory effects (45, 46), AuTM-mediated activation of HSP70 and HSP32 (HO-1) was monitored for up to 48 h. Such data revealed that AuTM was without noticeable effect, whereas at the same time, FLS readily responded to short term hyperthermia with the activation of HSP70 and HSP32 (data not shown).

In general, gold is considered to be an inert metal without biological function; however, gold does have a series of biolog-
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Gold has further been demonstrated to activate a copper-specific promoter, and it was therefore speculated that gold is mimicking Cu(I) and Ag(I) in the metal binding sites of such promoters (49). Subsequently, this group speculates that gold might act by activating copper transport proteins, therefore removing excess copper that might generate reactive oxygen species in diseases such as RA. A model summarizing the demonstrated effects and mechanism of gold treatment is shown in Fig. 10.

Despite the use of gold in the treatment of RA since 1929 and the fact that gold compounds were some of the first drugs to demonstrate retardation of radiographically verifiable damage, many questions still remain unanswered. With the exception of a few studies, uptake, distribution, and effects of gold compounds in FLS have not been investigated (50–53). It is still not known whether gold uptake in FLS differs from that of other cell types or whether such uptake is cell cycle-specific. Neither is there information available with regard to intracellular concentrations of gold, e.g. in the course of treatment of RA patients in cells that are relevant to disease progression such as FLS. Extracellular levels of AuTM in RA patients might not be informative if levels of gold in FLS fluctuate as demonstrated, e.g. in U937 cells. Gold uptake in these cells was demonstrated to be 27% but increased to >70% during differentiation (54). Similarly, undifferentiated HL-60 cells did not take up colloidal gold, but 68.6% of cells did so during maturation (54). Such vast differences in uptake might also rest with differences among the diverse gold compounds. For example, auranofin has been reported to block NFkB transactivation by blocking IkBα degradation in RAW 264.7 cells, whereas in the same cells, auroglucose and AuCl₄⁻ were without any effects on this transcription factor (55).

In summary, AuTM treatment of FLS blocks two of the most important pro-inflammatory events that are associated with RA. AuTM blocks the release of PGE2 and prevents the activation of NFkB therefore blocking IL-1β-induced HA accumulation and likely a series of other pro-inflammatory NFkB-dependent genes. Experimental data seem to exclude direct interactions of AuTM with DNA and NFkB proteins in FLS. However, the effect on PGE2 is most likely a consequence of AuTM-mediated inhibition of COX-2 transcription, because the activation of this gene has been shown to depend on the activation of NFkB (56). Similarly, the undesired HAS1 activation and subsequent HA release by activated FLS is also blocked by AuTM.

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