J Mater Sci: Mater Med (2010) 21:1289–1295
DOI 10.1007/s10856-009-3941-8

Titanates deliver metal ions to human monocytes

John C. Wataha · David T. Hobbs · Jacqueline J. Wong · Sami Dogan · Hai Zhang · K.-H. Chung · Mark C. Elvington

Received: 30 June 2009 / Accepted: 6 November 2009 / Published online: 26 November 2009

Abstract Amorphous peroxotitantes (APT) are insoluble titanium-based particles that bind a variety of metal compounds with high affinity; these particles could be sequestered locally in a solid phase to deliver metal-based drugs. Previous studies have confirmed the 'biodelivery' of metals from metal–APT complexes to fibroblasts, but not monocytes. Our goal in the current study was to use monocytic cytokine secretion to assess delivery of gold or platinum-based compounds from APT to human THP1 monocytes. Cytokine secretion was not triggered by APT alone or metal–APT complexes. In monocytes activated by lipopolysaccharide (LPS), APT alone enhanced or suppressed IL1β or IL6 secretion, yet TNFα secretion was unaffected. Complexes of APT and Au(III) or *cis*-platin altered LPS-activated IL6 or IL1β secretion most, TNFα least. Our results suggest that the APT deliver metals to monocytes.

1 Introduction

Amorphous peroxotitanates (APT; $H_vNa_wTi_2O_5{\cdot}x\text{-}H_2O[_yH_zO_2]$, $v + w = 2$ and $z = 0$–2) are metal oxides with binding affinity for a variety of metal ions including ions of strontium, the actinides obtained from the reprocessing of spent nuclear fuels, hazardous metals found in mining and industrial wastewaters, and noble metals such as gold and platinum [1, 2]. Because APT particulates are not readily soluble in biological environments, they may have utility to deliver metal ions from a sequestratable metal-loaded-APT phase. Because all current metal-based drugs have significant systemic toxicity, metal–APT complexes might be used to deliver relatively high local concentrations of a metal-based drug to target tissues with reduced systemic adverse effects, or capture metal ions from body tissues into the sequestratable phase.

Previous studies of the biological effects of native APT particles have documented little cytotoxicity toward fibroblasts and monocytes (Fig. 1) [3]. Metal–APT complexes deliver metal ions to fibroblasts and suppress mitochondrial activity (Fig. 1) at metal concentrations that have no metabolic effect by themselves, suggesting some sort of facilitated delivery of these metals by the complexes [2, 4]. Yet, the same experiments with monocytes have not demonstrated any 'biodelivery'; monocytic mitochondrial activity was not suppressed after exposure to a variety of metal–APT complexes (Fig. 1) [4]. These latter experiments have left open the question of whether complexes of metal with APT are capable of delivering metal compounds or ions to monocytes in the same fashion as fibroblasts. Such delivery is potentially important to the development of metal-based drugs because of the central role monocytes and macrophages play in wound healing, chronic inflammation, and biological response to materials [5, 6].

Monocytic cytokine secretion is an important 'arm' of monocytic function. Because of their particulate nature, unloaded APT have a potential to activate or alter monocytic cytokine secretion, yet do not appear to trigger or alter TNFα secretion from monocytes (Fig. 2) [3]. However, the full scope of the effects of APT alone on

J. C. Wataha (✉) · J. J. Wong · S. Dogan · H. Zhang · K.-H. Chung
Department of Restorative Dentistry, University of Washington School of Dentistry, D770 Health Sciences Bldg, 1959 NE Pacific Street, Box 357456, Seattle, WA 98197-7456, USA
e-mail: jwataha@gmail.com

D. T. Hobbs · M. C. Elvington
Savannah River National Laboratory, Savannah River Nuclear Solutions LLC, Aiken, SC, USA

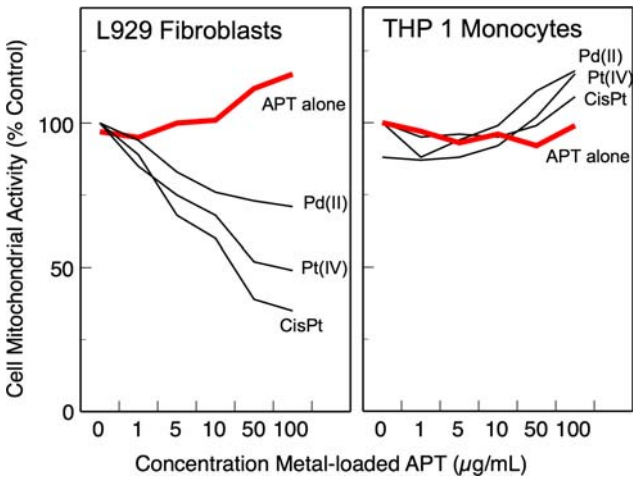

Fig. 1 Amorphous peroxotitanate (APT) loaded with metal ions or compounds transfer sufficient metal to fibroblasts to suppress mitochondrial activity, but such transfer has not been observed for monocytes. APT particles alone have little significant effect on mitochondrial activity for fibroblasts or monocytes [4]

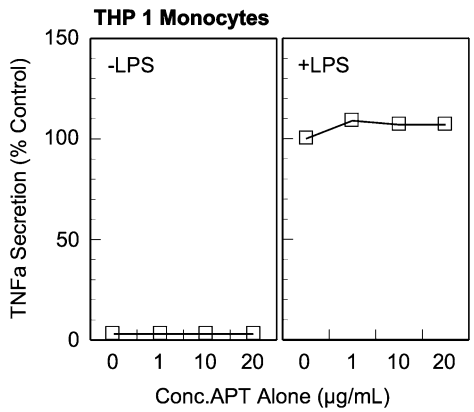

Fig. 2 APT particles alone have not been reported to activate secretion of TNFα from THP1 monocytes (*left*) or alter lipopolysaccharide-induced TNFα secretion from monocytes (*right*) [3]

monocytic cytokine secretion is unknown. Any such effects would be an important consideration when assessing the ability of metal–APT complexes to suppress or enhance monocytic cytokine secretion during service as agents of drug delivery.

In the current study, we assessed the effect of unloaded, native APT particles on cytokine secretion beyond the TNFα data currently available. Then, because previous studies have shown that metals alone may enhance or suppress monocytic cytokine secretion at sublethal levels [7], a second goal of the current study was to determine if metal–APT complexes could deliver metal compounds at sufficient concentrations to affect changes in monocytic cytokine secretion. In this manner, we hoped to demonstrate that metal–APT complexes facilitate metal ion delivery to monocytes as well as fibroblasts. We focused on compounds of gold, platinum, and palladium that have known or suggested therapeutic uses in medicine [8, 9].

2 Materials and methods

2.1 APT loading with metal compounds

Amorphous peroxotitanates (APT) were synthesized from monosodium titanates (Optima Chemical Group, LLC Douglas, GA) to form particles ranging in average diameter from 0.1 to 10 μm as described previously [1]. APT particles were loaded with Au(III), Auranofin® (AF), *cis*-platin, Pt(IV) or Pd(II) (Table 1) by combining 0.25 g of APT suspended in 1.4 g of water (pH = 6.9) with 10 ml of a phosphate-buffered saline solution containing the desired metal ion or metal compound (Tables 1, 2). Because we wanted to maximize metal loading to the APT particles, the concentrations of metals in the loading solutions were maximized (limited by solubility in PBS) to provide the greatest driving force to load metal onto the APT particles [4]. Thus, no effort was made to normalize loading concentrations among the metals in the current study. After mixing at ambient temperature for 48 h, the metal–APT materials were separated from the parent solutions by centrifugation (RCF = $1200\times g$, 3 min), after which the solid phases were rinsed quickly with 6 portions of chilled PBS (4°C; pH = 7.4), then stored as moist solids with water contents of approximately 75 wt%. Maintaining moist solids greatly increased the ability to re-suspend the particles in solution during cell-culture experiments.

The amount of metal compounds loaded onto the APT was determined by measuring the difference in metal concentrations of the metal loading solutions before and after contact with the APT solids. Metal ion concentrations in the solutions before and after contact with the APT solids were determined using inductively coupled plasma

Table 1 Metal compounds, sources

Metal species	Source compound	Manufacturer
Au(III)	$HAuCl_4{\cdot}3H_2O$ MW = 393.83	Sigma-Aldrich (St. Louis, MO)
Auranofin (AF)	Auranofin MW = 679.49	Alexis Biochemicals (Lausen, Switzerland)
Pd(II)	$PdCl_2$ MW = 177.33	Johnson Matthey, Inc. (West Chester, PA)
Pt(IV)	$PtCl_4$ MW = 336.89	Johnson Matthey, Inc. (West Chester, PA)
*cis*Pt	*cis*-$[PtCl_2(NH_3)_2]$ MW = 790.56	Alfa-Aesar (Ward Hill, MA)

Table 2 Degree of metal–APT loading

Metal ion	Metal loading solution conc.[a] (μM)	APT loading[b] (picomol/μg-APT)	Max. metal delivery from metal–APT complexes (μM)[c]
Au(III)	2,800	110 (11)	11
AF	470	18 (1.8)	1.8
Pd(II)	27,400	20 (2)	2
Pt(IV)	15,100	123 (12)	12
*cis*Pt	6,100	227 (23)	23

[a] Initial metal concentration used to prepare metal–APT materials

[b] Based on solution analyses before and after contact with APT

[c] Based on 100% delivery metal ion when metal–APT concentrations was 100 μg/ml and delivered into 200 μl cell-culture medium

emission spectroscopy (ICP-ES). Metal loading on APT was reported as the number of picomoles (picomol) of metal per μg of APT (Table 2).

2.2 Cell-culture and exposure of cells to APT or metal–APT complexes

THP1 monocytes (ATCC TIB202, American Type Culture Collection, Manassas, VA) were exposed to APT alone or metal–APT complexes for 72 h to assess their ability to induce or alter cytokine secretion from these cells, which orchestrate wound healing and chronic inflammatory responses [5]. THP1 monocytic cells were cultured in suspension in RPMI 1640 cell-culture medium (InVitrogen-Gibco, Carlsbad, CA) and 10% fetal bovine serum, with 2 mmol/l glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin, all at pH 7.2 (InVitrogen-Gibco). Cells were plated in 24 well format with 250,000 cells per well in 1 ml ($n = 6$ per condition). Immediately after plating, 50 μl/well of a solution designed to deliver a final concentration of 0, 10, 50, or 100 mg/l of the metal–APT complex was added and swirled into the cultures. Cultures were incubated at 37°C for 66 h, after which 1 μg/ml of lipopolysaccharide (LPS, *E. coli*, serotype 0111:B4, Sigma) was added to half of the cultures ($n = 3$) for 6 h. Next, cultures were processed to assess cytokine secretion into the cell-culture medium. Experiments were repeated to assure reproducibility of the methods. Because the population doubling time of THP1 was approximately 40 h in our cultures, 66 h was the longest time we could use without risking effects of nutrition depletion or pH change in the culture. The 6 h LPS exposure time was chosen to allow sufficient time for protein synthesis and secretion to occur.

2.3 Measurement of cytokine secretion (ELISA)

After exposure to the APT or metal–APT complexes, the cells and medium from each culture well were transferred to individual 1.5 ml microfuge tubes, then centrifuged ($1000\times g$; 5 min). The supernatants (900 μl) were transferred to a second microfuge tube, avoiding the cell pellet, and the supernatants were frozen at −20°C until assay.

The supernatants were assayed for three cytokines (IL1β, IL6, and TNFα) known to direct wound healing and inflammatory responses and known to be secreted from THP1 monocytes [6]. Enzyme-linked immunosorbant assays (ELISA) were used to measure cytokine levels (R&D Systems, St. Paul, MN) in the supernatant media. IL1β and IL6 were measured without dilution, but TNFα medium was diluted with PBS by 1:5 (vol/vol) prior to assay, based on pilot experiments and experimental replicates. Cell-culture medium was used as a zero-value blank solution, and known concentrations of the cytokines were tested to assure appropriate responsiveness of the assay system. Cytokine levels were normalized to the +LPS controls for each cytokine, and cytokine secretion among the groups for each metal–APT complex were compared using one-way ANOVA and Tukey pairwise multiple comparison intervals, with α set at 0.05.

2.4 Measurement of leaching from metal–APT complexes

Because one possible mechanism of action of the metal–APT complexes involved extracellular leaching of the metal or metal compound from the complex, we assessed this possibility for the Au(III)–APT complex. We focused on Au(III) because it had significant effects on monocytic cytokine secretion and has been investigated for its ability to alter monocytic cytokine secretion [7]. From a chemical viewpoint, release of Au(III) from the Au(III)–APT complex would be likely to occur given both the relatively high metal loading of the complex and the propensity of Au(III) to bind with chloride ions in aqueous solutions and form the very soluble, anionic tetrachloro gold(III) complex, $[Au(Cl)_4]^-$.

Gold desorption was evaluated by preparing a fresh batch of Au(III)-loaded APT as described above. After rinsing the Au(III)-loaded APT solids six times with chilled PBS solution, we suspended the APT solids (ca. 1.0 g) in 40 ml of fresh Au(III)-free PBS solution in a 50 ml centrifuge tube. This suspension was mixed on a tumbler at ambient laboratory temperature for 504 h. Periodically, we removed the centrifuge tube from the tumbler and centrifuged the suspension at 3000 rpm for 5 min. We then transferred 200–500 μl aliquots of the clear supernatant liquid into a small volume of dilute nitric acid solution. The acidified samples were analyzed by inductively coupled plasma-emission spectroscopy (ICP-ES) to determine the gold content.

3 Results

3.1 APT particles alone altered LPS-activated secretion of IL6 and IL1β

APT particles alone did not activate detectable secretion of IL6, IL1β, or TNFα from THP1 monocytes at any dose up to 100 μg/ml (Figs. 3, 4, 5, −LPS conditions). When LPS activation followed APT (alone) exposure, TNFα secretion was unaltered (Fig. 4). However, APT alone caused a dose-dependent suppression of IL6 secretion (Fig. 3), reaching 30–35% at concentrations of 50–100 μg/ml APT. For IL1β, the effects of APT were biphasic; APT caused a 30–40% increase in IL1β secretion at 10–50 μg/ml, but a 10% (statistically insignificant) suppression of secretion at 100 μg/ml.

3.2 Metal–APT complexes altered LPS-activated IL6 and IL1β secretion

Metal–APT complexes with Au(III), AF, Pd(II), Pt(IV), and *cis*Pt did not activate monocytic cytokine secretion at any APT concentration up to 100 μg/ml (Figs. 3, 4, 5). However, these complexes had distinct effects on the

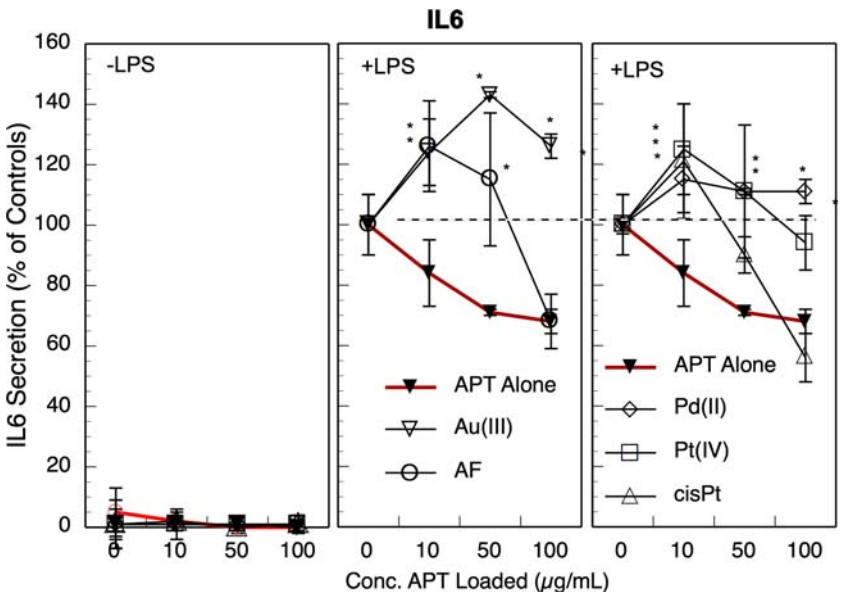

Fig. 3 Monocytic IL6 secretion in response to APT alone or metal–APT complexes. Human THP1 monocytes were exposed in culture to APT particles loaded with Au(III), Auranofin (AF), Pd(II), Pt(IV), or *cis*-Platin (*cis*PT) (see Table 2) for 72 h and treated (*center and right panels*) or not (*left panel*) with lipopolysaccharide (LPS, *E. coli*, serotype 0111:B4, 1 μg/ml) for the last 6 h. APT was added alone as a control (*heavier weight line*). Error bars indicate standard deviations ($n = 3$ per condition). Asterisks indicate statistical differences between the metal–APT conditions and APT alone (two-sided *t*-tests, $\alpha = 0.05$)

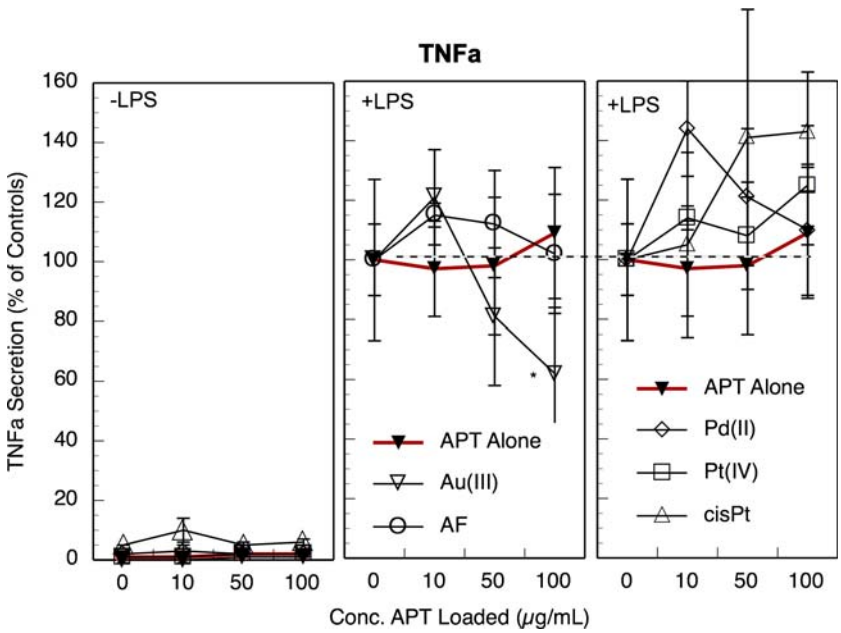

Fig. 4 Monocytic TNFα (TNFa) secretion in response to APT alone or metal–APT complexes. Human THP1 monocytes were exposed in culture to APT particles loaded with Au(III), Auranofin (AF), Pd(II), Pt(IV), or *cis*-Platin (*cis*Pt) (see Table 2) for 72 h and treated (*center and right panels*) or not (*left panel*) with lipopolysaccharide (LPS, *E. coli*, serotype 0111:B4, 1 μg/ml) for the last 6 h. APT was added alone as a control (*heavier weight line*). Error bars indicate standard deviations ($n = 3$ per condition). Asterisk indicates statistical difference between the metal–APT conditions and APT alone (two-sided *t*-tests, $\alpha = 0.05$)

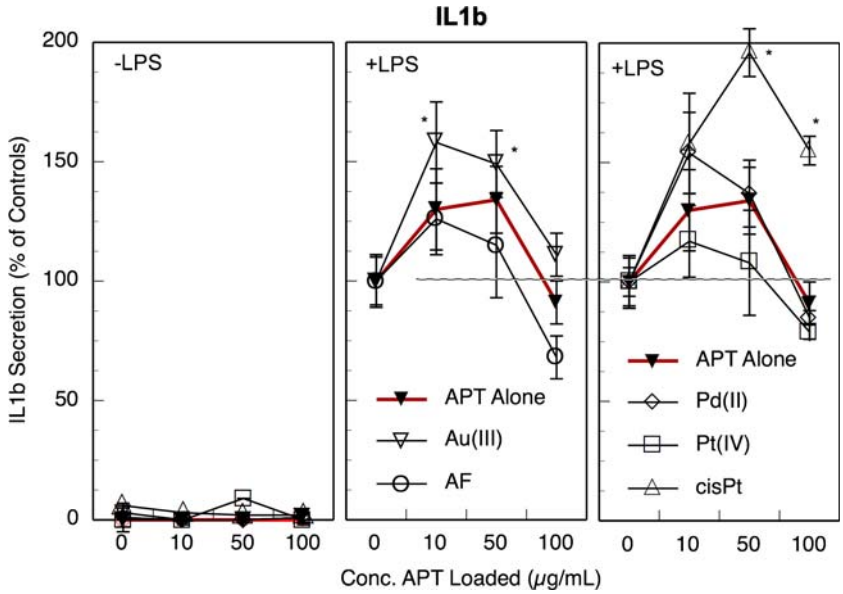

Fig. 5 Monocytic IL1β (ILb) secretion in response to APT alone or metal–APT complexes. Human THP1 monocytes were exposed in culture to APT particles loaded with Au(III), Auranofin (AF), Pd(II), Pt(IV), or *cis*-Platin (*cis*PT) (see Table 2) for 72 h and treated (*center and right panels*) or not (*left panel*) with lipopolysaccharide (LPS, *E. coli*, serotype 0111:B4, 1 μg/ml) for the last 6 h. APT was added alone as a control (heavier weight line). Error bars indicate standard deviations ($n = 3$ per condition). Asterisks indicate statistical differences between the metal–APT conditions and APT alone (two-sided *t*-tests, $\alpha = 0.05$)

secretion of IL6, IL1β, and TNFα when monocytes were activated by LPS after exposure to metal–APT complexes. These effects are described for each type of metal–APT complex in the following paragraphs.

Au(III)–APT complexes markedly enhanced the secretion of IL6 versus APT alone at all doses of APT applied, suggesting that Au(III) was delivered to the monocytes in some fashion (Fig. 3). Au(III)–APT increased IL1β secretion over APT alone, although the effects were not statistically significant except at 10 μg/ml (Fig. 5). Au(III)–APT complexes at 50 and 100 μg/ml suppressed TNFα secretion from LPS-activated monocytes versus APT alone, but suppression was only statistically significant at 100 μg/ml (Fig. 4).

AF–APT complexes had less pronounced effects than Au(III)–APT on LPS-activated cytokine secretion and APT alone (Figs. 3, 4, 5). When monocytes were activated by LPS post-APT exposure, IL6 secretion was enhanced and reversed the suppression caused by APT alone. IL1β and TNFα secretion were not significantly different from APT alone (Figs. 4, 5). Pt(IV)–APT complexes caused modest, but significant reversals of IL6 suppression induced by APT alone in LPS-activated monocytes (Fig. 3). The effects of Pt(IV) on IL1β and TNFα were not significantly different than those observed for APT particles alone up to 100 μg/ml (Figs. 4, 5). The results for Pd(II)–APT complexes (Figs. 3, 4, 5) were similar to those for Pt(IV)–APT complexes.

*Cis*Pt–APT complexes had effects on the activation of monocytes that were distinct from all other metal compounds tested (Figs. 3, 4, 5). These complexes reversed the suppression of IL6 secretion induced by APT alone at 10 μg/ml, but enhanced the suppression of IL6 at 100 μg/ml. Although the results were not always statistically significant, *cis*Pt–APT enhanced IL1β secretion from activated monocytes to levels beyond those observed for the other complexes (Fig. 5), and unlike other metal compounds, appeared to enhance TNFα secretion from activated monocytes at 50 and 100 μg/ml (Fig. 4).

3.3 Au(III)–APT did not release significant levels of Au(III) over 21 days

Because Au(III)–APT complexes caused changes in cytokine secretion that represented many of the other metal–APT complexes (Figs. 3, 4, 5) and because loading of Au(III) was among the most efficient (Table 2), we investigated the release of Au(III) from Au(III)–APT complexes over time (Fig. 6). Within the range of detection limits of the techniques, no Au(III) was detected in the PBS solution over the 21-day test.

4 Discussion

Previous studies have not reported adverse effects of APT particles alone on cell function or metabolism [3, 5]. Specifically, APT alone did not suppress monocytic mitochondrial activity at concentrations up to 100 μg/ml (Fig. 1) [10], or trigger or alter secretion of TNFα from

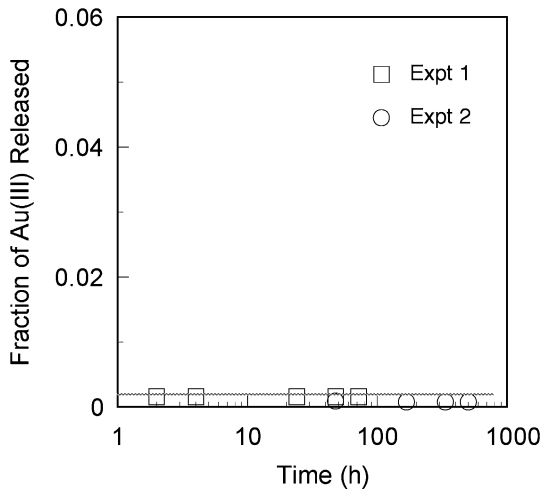

Fig. 6 Au(III) release from Au(III)–APT complexes. Au(III)-loaded APT was suspended in a PBS solution for up to 21 days. Aliquots of leachant were periodically taken and analyzed after removing any insoluble solids by ICP-ES to determine the concentration of Au(III) in solution

THP1 monocytes (Fig. 2) [10]. In the current study, we have observed distinct changes in LPS-activated IL1β (Fig. 5) and IL6 secretion (Fig. 3) after APT exposure while confirming previous negative results for TNFα (Fig. 4). The current results strongly suggest that APT particles are not biologically neutral with regard to monocyte activation and interact through unknown mechanisms with monocytes. Furthermore, because APT modified the secretion of IL1β, IL6, and TNFα each differently, the results in Figs. 3, 4, 5 suggest a complex mechanism of action rather than general suppression or stimulation of cell function by APT particles.

In past work, metal–APT complexes did not suppress THP1 monocyte mitochondrial activity (Fig. 1), despite confirmation that metals alone suppressed such activity [4]. In contrast, similar experiments reported suppression of fibroblast mitochondrial function by metal–APT complexes. Previous results suggested that either insufficient metal was loaded onto the APT particles to affect monocytes, or that APT did not facilitate delivery of metals to monocytes in the same manner as fibroblasts [4]. The current results show unequivocally that metal–APT complexes carry sufficient amounts of metal to alter monocyte cytokine secretion (Figs. 3, 4, 5). Therefore, biodelivery of metals to monocytes from metal–APT complexes is likely. As with the results for APT alone, the effect of each metal was different among the cytokines, reinforcing an emerging hypothesis that the effects of both the APT and metal–APT complexes do not globally suppress or activate cells.

We selected the widely used THP1 cell line as a model for our current work, yet prudence dictates that consideration be given to possible differences in the secretory function of THP1 versus native monocytes. Differences between THP1 and freshly isolated human monocytes have been reported previously, although general trends are similar [11]. Thus, although we believe that the current work is predictive of general trends in the effects of APT and APT–metal complexes on monocytes, we suggest that native monocytes may respond differently at a detailed level, and should be investigated further.

Published work has reported that Au(III) alone enhances LPS-induced IL6 secretion, and to some extent, IL1β secretion from THP1 monocytes [7]. These effects were similar to those observed in the current study for Au(III)–APT complexes (Figs. 3, 5), suggesting that the complexes delivered sufficient Au(III) to enhance cytokine secretion. However, the concentrations of Au(III) alone required to enhance cytokine secretion was 25–75 μM, which was 2–7-fold greater than could be delivered by the Au(III)–APT complexes (Table 2), even if all the loaded Au(III) was released extracellularly at the maximum concentration of APT (100 μg/ml). Thus, it appears unlikely that extracellular release of Au(III) from APT fully accounted for the effects observed in Figs. 3 and 5.

Several possible mechanisms of delivery of Au(III) to monocytes by APT exist: either the Au(III) is being 'delivered' at the cell membrane surface or after ingestion of metal–APT particles by the monocytes (Fig. 7). Our data here supports at least some role for facilitated delivery of Au(III) to cells by APT. This possibility is further supported by a lack of measurable extracellular release of Au(III) from APT (Fig. 6), although these data are preliminary. A similar analysis can be made for AF–APT delivery (Table 2) [7]. However, in the case of AF, the potential release of AF from the loaded APT is within the same range as necessary for AF alone to cause enhanced IL6 secretion [7]. Thus in the case of AF, one cannot rule out an extracellular delivery mechanism; AF release from

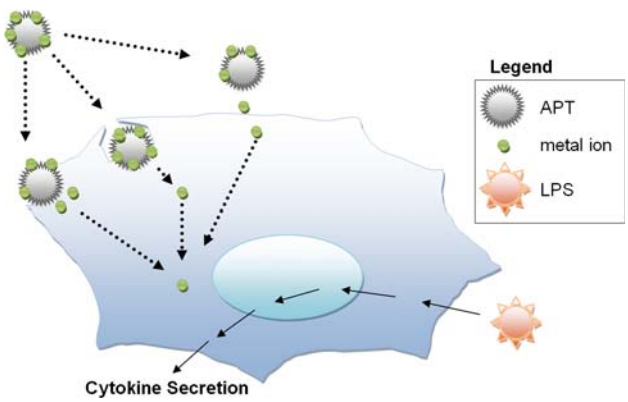

Fig. 7 Possible mechanisms of metal delivery to monocytes by APT. Dotted arrows signify possible fates of the metal–APT complexes; solid arrows signify the cytokine secretion activation pathway

AF–APT complexes has yet to be studied. For Pd(II), few effects on cytokine secretion have been observed for Pd(II) alone, and few were observed for Pd(II)–APT complexes. Insufficient data are available in the literature for Pt(IV) and *cis*Pt to make similar analyses. In the current work, we did not investigate specific mechanisms of metal compound delivery to monocytes, focusing initially on if such delivery is possible. Based on our data, further work into these mechanisms appears warranted.

Caution should be used when comparing the delivery efficiencies of different metal–APT complexes in the current study because of the different loading concentrations and loading saturations (Table 2). We chose to maximize loading of the APT with each metal compound to optimize our chances of observing effects of the loaded complexes on THP1 function. These different loading saturations unfortunately confounded most comparisons among metal compounds. Future work should attempt to load the APT with the same loading concentrations, and other studies should compare loaded APT complexes with similar loading levels to allow comparison of the efficiencies of cellular delivery. The effect of oxidation state of the metal ion also could be considered.

The degree of metal loading to the APT undoubtedly is important to ultimately optimizing metal delivery for therapeutic use. Ascertaining the appropriate degree of metal loading is difficult because therapeutic levels of these metals are not known. Establishing therapeutic levels has been impeded by an inability to use the native metal ions systemically to avoid toxicity. However, our data suggest that sufficient metal ion delivery can be facilitated with the degree of loading in the current study to alter cytokine secretion, which is a first step in ultimately determining appropriate therapeutic effects.

The current work is preliminary, but does support a hypothesis that metal–APT complexes deliver metal compounds to monocytes as well as fibroblasts to affect cellular function. From the variety of monocytic responses observed in the current study, metal–APT complexes might be used to boost or suppress monocytic cytokine secretion, depending on the nature of the metal compounds and cytokine 'target'. A great deal of further work is necessary to refine such possibilities, but our current work suggests that such work may be fruitful.

Acknowledgments The authors thank the University of Washington and the Savannah River National Laboratory LDRD Program for their support of our work. The authors also sincerely thank Ms. Petra Lockwood for her excellent technical help with the assays.

References

1. Nyman M, Hobbs DT. A family of peroxo-titanate materials tailored for optimal strontium and actinide sorption. Chem Mater. 2006;18:6425–35.
2. Davis RR, Hobbs DT, Kahshaba R, Sehkar P, Seta FN, Messer RLW, et al. Titanate particles as agents to deliver gold compounds to fibroblasts and monocytes. J Biomed Mater Res A Galleys. January 2009 [Epub ahead of print].
3. Davis RR, Lockwood PE, Hobbs DT, Messer RLW, Price RJ, Lewis JB, et al. In vitro biological effects of sodium titanate materials. J Biomed Mater Res B. 2007;83:505–11.
4. Wataha JC, Hobbs DT, Lockwood PE, Davis RR, Elvington M, Lewis JB, et al. Peroxotitanates for biodelivery of metals. J Biomed Mater Res B Appl Biomater. 2009;91:489–96.
5. Anderson JM, Miller KM. Biomaterial biocompatibility and the macrophage. Biomaterials. 1984;6:5–10.
6. Auger MJ, Ross JA. The biology of the macrophage. In: Lewis CE, McGee JO, editors. The macrophage. Oxford: Oxford University Press; 1992.
7. Wataha JC, Lewis JB, Volkmann KR, Lockwood PE, Messer RLW, Bouillaguet S. Sublethal concentrations of Au(III), Pd(II), and Ni(II) differentially alter inflammatory cytokine secretion from activated monocytes. J Biomed Mater Res B. 2004;69:11–7.
8. Kean WF, Hart L, Buchanan WW. Auranofin. Br J Rheumatol. 1997;36:56–72.
9. Abratt RP, Pontin AR, Sarembock LA, Barnes RD, Livingstone RR. *Cis*-platin combination chemotherapy for non-seminomatous germ-cell tumours of the testis. S Afr Med J. 1987;72:468–9.
10. Hobbs DT, Messer RLW, Lewis JB, Click DR, Lockwood PE, Wataha JC. Adsorption of biometals to monosodium titanate in biological environments. J Biomed Mater Res B. 2006;78:296–301.
11. Heil TL, Volkmann KR, Wataha JC, Lockwood PE. Human peripheral blood monocytes vs. THP-1 monocytes for in vitro biocompatibility testing of dental materials components. J Oral Rehabil. 2002;29:401–7.