CHAPTER 3

INNATE STIMULATORY CAPACITY OF HIGH MOLECULAR WEIGHT TRANSITION METALS Au (GOLD) AND Hg (MERCURY)

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Nickel, cobalt and palladium ions can induce an innate immune response by triggering Toll-like receptor (TLR)-4 which is present on dendritic cells (DC). Here we studied mechanisms of action for DC immunotoxicity to gold and mercury. Next to gold (Na₃Au(S₂O₃)₂⋅2H₂O) and mercury (HgCl₂), nickel (NiCl₂) was included as a positive control. MoDC activation was assessed by release of the pro-inflammatory mediator IL-8. Also PBMC were studied, and THP-1 cells were used as a substitution for DC for evaluation of cytokines and chemokines, as well as phenotypic, alterations in response to gold and mercury. Our results showed that both Na₃Au(S₂O₃)₂⋅2H₂O and HgCl₂ induce substantial release of IL-8, but not IL-6, CCL2 or IL-10, from MoDC, PBMC, or THP-1 cells. Also gold and, to a lesser extent mercury, caused modest dendritic cell maturation as detected by increased membrane expression of CD40 and CD80. Both metals thus show innate immune response capacities, although to a lower extent than reported earlier for NiCl₂, CoCl₂ and Na₂[PdCl₄]. Importantly, the gold-induced response could be ascribed to TLR3 rather than TLR4 triggering, whereas the nature of the innate mercury response remains to be clarified. In conclusion both gold and mercury can induce innate immune responses, which for gold could be ascribed to TLR3 dependent signalling. These responses are likely to contribute to adaptive immune responses to these metals, as reflected by skin and mucosal allergies.
INTRODUCTION

Metals are amongst the most notorious contact sensitizers clinically known. This counts in particular for the low-molecular weight transition metals nickel, cobalt and palladium. Still, in dentistry also mercury and gold have obtained a poor reputation as frequent causes for oral allergic complaints (Moller 2002; Moller 2010; Roberts and Charlton 2009; Evrard and Parent 2010). The latter metals are located next to each other in the periodic table as high molecular weight transition elements.

Cast gold alloys are the material of choice for most dental restorations in developed countries, like in Europe, United States and Japan. These alloys have been found to be ideal for these applications because of their corrosion resistance and biocompatibility. Next to their use in oral applications, gold-based alloys are also widely used in skin appliances, e.g. by jewellers for ear rings and piercing studs. These frequent uses of metallic gold are complemented by the medical use of gold salts for the local treatment of chronic inflammations such as in rheumatoid arthritis. Still, despite its biocompatibility, or even immunosuppressive capacities, gold has also obtained some disrepute as a contact allergen (Moller 2002; Ahlgren et al. 2002).

Mercury-based amalgam has been used for material fillings in dentistry for over a century. Its popularity for this application, however, has declined over recent years due its potential negative health effects. These may vary from autoimmune phenomena (Nielsen and Hultman 2002; Pigatto and Guzzi 2010; Rowley and Monestier 2005) to neurological problems (Kern et al. 2012; Mutter 2011). Yet, negative reports are still scarce or disputed, and certainly in developing countries amalgam fillings are still widely being used nowadays.

The focus of the present investigation was to analyze whether gold and mercury might induce innate immune responses. First dendritic cells DC generated from culturing peripheral blood monocytes were used. DC are central between innate and adaptive immune responses, and were found to be highly sensitive in revealing innate stimulatory capacities of the low-molecular weight transitional metals nickel, cobalt and palladium (Schmidt et al. 2010; Toebak et al. 2006; Rachmawati et al. 2013; Raghavan et al. 2012). Upon capturing antigens, DC begin to mature, and migrate to draining lymph nodes via afferent lymphatic vessels. This process is orchestrated by pro-inflammatory mediators, released from skin cells and DC, such as IL-8, IL-6, IL-1β and TNFα (Martin et al. 2011; Miyazawa et al. 2007; Toebak et al. 2006). Furthermore, it was studied whether observed responses could also be detected in freshly prepared PBMC, as well as in monocytic leukaemia cell line, THP-1 cells.

Innate stimulatory capacities were assessed by cytokine/chemokine levels (IL-8, CCL2, IL-6) and phenotypic alterations as detected by flow cytometric (FACS) analyses (CD40
and CD80). Following up on our previous report on nickel, cobalt and palladium-induced signalling (Rachmawati et al. 2013), TLR-2, 3 and 4 dependency of the gold and mercury-induced signalling was studied using HEK transfectant cell lines.

MATERIALS AND METHODS

Metal Chemicals

As metal allergens the following chemicals were used: nickel (II) chloride hexahydrate (NiCl$_2\cdot$6H$_2$O), sodium gold thiosulfate Na$_3$Au(S$_2$O$_3$)$_2$·2H$_2$O; Chemo technique Diagnostics, Vellinge, Sweden), mercuric chloride (HgCl$_2$; Riedel-de Haën, Seelze, Germany). LPS was obtained from Escherichia coli 055:B5 (Sigma, St Louis, MO, USA). NiCl$_2$, Na$_3$Au(S$_2$O$_3$)$_2$·2H$_2$O, HgCl$_2$, LPS were dissolved in H$_2$O as stock solutions and further diluted with culture medium just before use.

Peripheral blood mononuclear cells (PBMC)

PBMC were isolated from 40-50 ml freshly drawn peripheral venous blood of at least 4 different healthy donors without known metal allergies by Ficoll (Lymphoprep, Fresenius KabiNorge AS) density gradient centrifugation. Culture medium was Iscove's modified Dulbecco's medium (IMDM, Biowhittaker, Verviers, Belgium) with 10% heat-inactivated fetal calf serum (FCS, Hyclone, Logan USA), 1% penicillin streptomycin, 1% l-glutamine and 1% β-mercaptoethanol (2ME).

MoDC culture

MoDC were generated from freshly prepared PBMC. After 2 hours incubation of the PBMC in a humidified incubator, we removed the non-adherent cells by aspiration and washed once very gentle with 5 ml PBS. MoDC were generated as previously described (Bontkes et al. 2002). Briefly, adherent monocytes were cultured for 6-7 days in the humidified incubator in 10 ml IMDM medium supplemented with 10% FCS, 1% penicillin-streptomycin, 1% L-glutamine, and 1% 2ME, 1000 U/ml granulocyte-macrophage colony stimulating factor (GM-CSF Novartis, The Netherlands) and 20 ng/ml IL-4 (lot AG270911A: R&D systems Europe, Abingdon, UK). After 6-7 days, immature dendritic cells (iDC) were harvested and plated in 96 well flat tissue culture plates (Cellstar Greiner Bio-One, Frickenhausen, Germany) at approximately 5x10$^4$ cells per well.

THP-1 cells

A vial of cell passage 17 of THP-1 cells (ATCC, Rockville, USA) was kept at -80°C until thawing. The cells were cultured in 100 ml culture flasks at a density of 10$^6$ cells / 10 ml of RPMI 1640 containing 2 mM L-glutamine, 0.1 mg/ml streptomycin, 100 IU/ml penicillin, supplemented with 10% heated-inactivated FCS.
Metal toxicity experiments

In order to design appropriate concentration ranges of metals, the maximal non-toxic concentration was determined by the MTT reduction test (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Approximately 100 µl of cells (5x10^4/well) were plated in 96 well culture plates and exposed to increasing concentrations of the metals. After 24 hours incubation supernatants were removed and 50 µl of MTT solution (5mg/ml) was added per well. MTT solution was prepared freshly and dissolved with H_2O, filtered through a 0.22-µm filter. The plates were incubated in the dark at 37°C. After 2-3 hours of incubation, 50 µl DMSO (Merck, Darmstad, Germany) was added to each well and after shaking, the solution was measured using an ELISA reader at OD (optical density) 490 nm. The OD of the cells in the absence of metal was considered as 100%. Viabilities of exposed cells were determined by the formula: OD experimental sample/OD of control cells) x 100%.

Metals and LPS exposure

PBMC, iDC, THP-1 (5x10^4 cells/well) were exposed to LPS (100 ng/ml) and metals at concentrations between 0 and 750 µM. Plates were incubated at 37°C in 5% humidified CO_2. After 24 hours, supernatants were collected and stored at -20°C until measurement. Where indicated polymyxin B sulphate (25 µg/ml; Sigma-Aldrich, Inc St Louis USA) was mixed with the metal salt solutions in the culture wells before iDCs were added, to exclude possible involvement of endotoxin in metal-induced DC stimulation (Loutet et al. 2011; Roelofs et al. 2006). Complementary checks for LPS contamination were carried out with the Limulus amebocyte lysate (LAL) assays (Kinetic-QCL™ bulk kit, Lonza).

For metal exposures, cells were exposed to Na_3Au(S_2O_3)_2•2H_2O, HgCl_2 and NiCl_2 at 4 different serial dilutions, 750 µM (for HgCl_2 nM) as the highest concentration. Total volume in each well was 200 µl. iDC and PBMC (5x10^4 cells/well), supernatants were collected after 16-24 hours and kept at -20°C until cytokine/chemokine assessment (see below). Cell viability was tested in the cell pellet in the same plate (see below).

To explore involvement of mitogen-activated protein kinase (MAPK) pathways, notably with the p38 MAPK inhibitor SB203580, cells were cultured as above, but with and without addition of 20 µM SB203580 (InvivoGen, San Diego, USA) as from one hour at 37°C before starting metal and ligand exposures.

Assessment of TLR 2, 3 and 4 signalling with HEK293 transfectant cells

Human Embryonic Kidney (HEK) 293 TLR 2, 3 and TLR4/MD2 cells were cultured in T75 flasks in DMEM, 1% Glutamine, 1% pen/strep, 0.5 µg/ml G418 (Sigma-Aldrich, St Louis, MO, USA) and harvested upon confluence. Wild type HEK293 and HEK293 cells stably expressing human TLR2, TLR3 or TLR4-MD2, were a kind gift from D.T. Golenbock (University of Massachusetts Medical School, USA) to Y. van Kooyk/M. Verstege. Cells were split twice
a week until ready for use (Kuijf et al. 2010). Cells were plated at 1×10^5 cells/well in 100 µl medium in 96-wells flat bottom plates. After allowing cells to adhere for 1.5 to 2 hours 100 µl of metal salt solution was added to give final concentrations of 0, 250 and 500 µM (for HgCl₂ nM). As a positive control NiCl₂ was used. Cells were incubated for 24 hours at 37°C, and supernatants were harvested for IL-8 ELISA.

**Flow cytometry**

After 48 h exposure to metals, MoDC were washed in FACS medium (PBS containing 1% BSA). Expression of CD40 and CD80 was analysed by flow cytometry. Cell staining was performed using PE and APC-labelled monoclonal antibodies: mouse anti-human-CD40-PE and anti-human-CD80-APC (IgG₁ Pharmingen, B&D systems). Isotype controls assessing non-specific binding were monoclonal mouse IgG₁ APC and IgG₁ PE (Pharmingen, B&D systems). Cells were stained with antibodies for 30 minutes in the dark at room temperature, then washed. Flow cytometry was performed with BD-FACS Calibur and analysed using Cell Quest software. Mean fluorescence index (MFI) was calculated by formula: mean fluorescence intensity of DC stimulated with metals/mean fluorescence non-stimulated cells. The relative MFI was defined as fold increase over isotype control.

**Cytokine/chemokine production**

IL-4, IL-6, IL-8, IL-10, and IFNγ production was measured by Enzyme-linked immunosorbent assay (ELISA) with Peli-Kine ELISA kits (Sanquin, Amsterdam, The Netherlands) and for CCL2 by human CCL2 (MCP-1) ELISA ready set-Go (eBioscience, San Diego, USA) using 96-well microtiter plates (Nunc maxisorp microtiter plates, Nalge Nunc international), as per the manufacturer’s instructions. Absorbance was measured at 450 nm. The amount of cytokine/chemokine in the supernatant was assessed by using standard curves (lower detection limit of IL-4: 20.5 pg/ml ; IL-6: 11.5 pg/ml; IL-8: 15.4 pg/ml; IL-10: 7.7 pg/ml; IFNγ: 19.2 pg/ml; CCL2: 31.3 pg/ml). Data are presented in picograms or nanograms per ml.

**Data analysis**

The statistical significance of the effects of various metals on the secretion of IL-8 was analysed by using one way ANOVA and Kruskall-Wallis test (non parametric ANOVA), with statistic program GraphPad Prism Software version 6.0 (San Diego, CA, USA). P≤ 0.05 was considered statistically significant. All data are presented as mean ± SD.

**RESULTS**

**Cytotoxic effects of gold and mercury**

To study potential immunostimulatory effects of gold and mercury on mononuclear and blood cells (MoDC, PBMC and THP-1 cells), maximal non-toxic concentrations were
determined first. Cytotoxicity experiments were carried out using MTT reduction assays as a read-out. Cells were exposed for 24 hrs to increasing dosages of Na₃Au(S₂O₃)₂, HgCl₂ and NiCl₂ and concentration ranges between 0 – 750 μM were found to be appropriate for gold and nickel, whereas mercury required a 1000-fold lower dose range (0 - 750 nM). Of note, THP-1 cells were found to be slightly more sensitive to gold and nickel exposure than both primary cell types MoDC and PBMC (Figure 1).

**Gold and mercury induced MoDC activation as detected by cytokine/chemokine secretion**

Subsequently, gold thiosulfate and mercury chloride were studied for their capacity to activate MoDC, as reflected by IL-8, CCL2 and IL-6 release. As a positive control nickel chloride was included as it is known to activate MoDC through direct TLR4 triggering. Although less impressive than nickel, gold also induces distinct IL-8 release at marginally toxic dosages, whereas mercury shows similar release, but at a lower level (Fig 2). In contrast to nickel, however, the stimulatory capacities of gold and mercury were not reflected by an appreciable increase in levels of other, less abundantly produced inflammatory (IL-6 or CCL2) or anti-inflammatory (IL-10) mediators.

**Figure 1. Cell viabilities after metal salt exposure.** Cell viability (%) relative to vehicle exposure is given after culturing MoDC, PBMC or THP-1 cells for 24 hours with increasing concentrations of gold thiosulfate and nickel chloride (in μM) or mercury chloride (in nM). Cell viabilities were assessed as outlined in Materials and Methods. Graphs represent means ± SD for 3 independent experiments.

**PBMC**

In order to further confirm the innate stimulatory capacities for gold and mercury, using the same approach, unseparated peripheral blood mononuclear cells (PBMC) were also studied. Robust production of IL-8 by PBMC could be observed for NiCl₂ exposure, albeit at a lower level than for MoDC. Again gold, and to a lesser extent mercury, showed distinct IL-8 release by PBMC (Fig 3). Production profiles for IL-6 were less clear, but supported the innate stimulatory capacities of gold and mercury. Additional ELISA’s carried out to detect CCL2, IFNg and IL-4 in PBMC supernatants did not reveal relevant signals for these cytokines. (data not shown).
THP1

To explore applicability of THP-1 cell line cells for studying metal-induced innate signaling, essentially similar experiments were carried out with these cells. Supporting the earlier data for MoDC and PBMC, not only NiCl$_2$ induced a dose-dependent increase in IL-8 secretion, but also both gold and mercury. Both high MW transition metals showed remarkably high levels of IL-8 release (Fig 3). Strongest responses were observed for mercury, which also induced appreciable IL-6 release in these cells.

Phenotypic analysis of metal-induced DC maturation

MoDC were exposed to the metal salts at maximally non-toxic dosages, i.e. 500 µM (nickel and gold), or 500 nM (mercury), whereas LPS was tested at 100 ng/ml. Exposure to LPS and NiCl$_2$ resulted in small but distinct increases in CD80 expression, whereas both heavy weight transitional metals only induced marginal increases in maturation markers which did not reach significance, except for gold-induced CD40 expression (Fig 4). In our hands THP1 cells were even less responsive in this regard, although again hints of both gold and mercury-induced CD40 expression could be observed.

Gold and mercury induced IL-8 secretion in HEK293 WT and TLR2, 3 and 4 transfectant cells

Further experiments were carried out to investigate whether, like for nickel, cobalt and palladium, TLR4 signalling might play a role in innate cell signalling to the high-molecular weight transitional metals. Besides the TLR4-transfected HEK293 TLR4/MD2 cell line, two additional HEK293 cell lines, transfected with TLR2 and TLR3 respectively, were tested. Next to gold and mercury salts, nickel and LPS were used as positive controls for TLR4-mediated cell activation. Lipoteichoic acid (LTA) and poly-IC were added as positive controls to the metal panel for testing TLR2 and 3 transfectants, respectively. Again, IL-8 release was utilized as read-out for down-stream signalling (Rachmawati et al. 2013).
Figure 2. IL-8, IL-6, CCL2 and IL-10 production after metal exposure of MoDCs. Immature MoDCs were exposed to increasing concentrations of gold thiosulfate and nickel chloride (in µM) or mercury chloride (in nM) for 24 hours. Bars represent mean ± SD from six independent experiments (n = 3 donors). Asterisks specify statistically significant (One way ANOVA and Kruskall-Wallis test (non parametric ANOVA)) differences in production of IL-8, IL-6, CCL2 and IL-10 at the given metal concentration as compared to the control medium (open bar): *p<0.05, **p<0.01, ***p<0.001. ND = not detectable.
Figure 3. IL-8 and IL-6 production after metal exposure of PBMC and THP-1. PBMC (A) and THP-1 cells (B) were exposed to increasing concentrations of gold thiosulfate and nickel chloride (in µM) or mercury chloride (in nM) for 24 h. Bars represent mean ± SD from three independent experiments (for PBMC n = 3 donors). Asterisks specify statistically significant (one way ANOVA and Kruskall–Wallis test (non parametric ANOVA)) differences in production of IL-8, IL-6 by cells at the given metal concentration as compared to the control medium (open bar): *p < 0.05, **p < 0.01, ***p < 0.001. ND = not detectable.
Figure 4. Phenotypic maturation after metal exposure of MoDC or THP-1 cells. MoDC (A) or THP-1 cells (B) were exposed for 24 h to LPS, gold thiosulfate and nickel chloride (500 µM) or mercury chloride (500 nM) as indicated. Bars represent mean ± SD from three independent experiments (for MoDC: n = 3 donors). Asterisks specify statistically significant (one way ANOVA and Kruskall–Wallis test (non parametric ANOVA)). MFI was calculated by mean fluorescence intensity of DC stimulated with metals as compared to mean fluorescence non-stimulated cells (open bar): *p < 0.05, **p < 0.01, ***p < 0.001.

As shown in fig 5, in contrast to nickel, neither gold nor mercury induced activation signals in the TLR4/MD2 transfectant. Also TLR2-mediated signalling was not involved, as was clear from testing the same panel in TLR2 transfectant cells, that did respond well to the positive control LTA. Interestingly, gold was strongly active in the TLR3 transfectant cells, which also showed specific reactivity to poly-IC as the internal positive control. The respective ligand-induced effects were clearly due to the presence of TLR4/MD2, TLR3 and TLR2 receptors, since none of the metals, nor the control ligands induced responses in wild type, non transfectant HEK293 cells (fig 5).
**Figure 5. IL-8 production after metal exposure of HEK WT**

(A), TLR4/MD2 (B), TLR 3 (C) and TLR 2 (D) transfectant cells. HEK293 WT and transfectant cells were exposed for 24 h to LPS (50 ng/ml), LTA (50 ng/ml), poly-IC (50 ng/ml), gold thiosulfate and nickel chloride (500 µM) or mercury chloride (500 nM) (grey bars) or culture medium as indicated. Results are shown from three independent experiments (values are mean ± SD). For statistical analysis, the highest dose values were compared with the medium control (one way ANOVA and Kruskall–Wallis test (non parametric ANOVA): *p < 0.05, **p < 0.01, ***p < 0.001).
DISCUSSION

Inflammation is a protective response by the body to ensure removal of detrimental stimuli, as well as a healing process for repairing damaged tissue (Medzhitov 2008). Inflammation is caused by various factors such as microbial infection, tissue injury and exposure to toxic materials. The primary contributor to acute inflammation is innate immune reactivity, which can also recruit adaptive immune responses. Whereas parenchymal and stromal cells, such as epithelial cells, endothelial cells and fibroblasts, contribute to innate immunity, pivotal roles are played by innate immune cells including macrophages and DC. These cells express high levels of germline-encoded pattern recognition receptors (PRRs) which are responsible for sensing the presence of microorganisms. The recognized structures are often conserved among microbial species, and called pathogen associated molecular patterns (PAMPs). The most prominent family of PRRs comprises transmembrane proteins, the Toll-like receptors (TLRs). Recent evidence indicates that one of this family member, i.e. TLR4 is also triggered by distinct low-molecular weight transition metals, such as nickel, cobalt and palladium (Schmidt et al. 2010; Rachmawati et al. 2013; Raghavan et al. 2012). This finding has shed new light on clinical and experimental experiences with these metals showing remarkable activities in inducing innate as well as adaptive immune responses. Here, we considered that some other, high molecular weight, transition metals are widely used in dental applications, i.e. gold and mercury, but also gained serious disrepute from immunotoxic capacities. The present study, therefore, was set up to further explore the capacities of the latter metals to activate TLR-mediated cell signalling.

As reported earlier, TLR-signalling in DC is most readily detected by pro-inflammatory cytokine/chemokine (notably IL-8, and to a lesser extent CCL2 and IL-6) release, next to augmented expression of distinct surface molecules reflecting maturation and adaptive immune stimulatory capacity (CD80 and CD40) (Toebak et al. 2006). In particular IL-8 is abundantly produced, which actually led to its early identification as a major interleukin, whereas the costimulatory molecules are pivotal in providing help to adaptive immune cells (Munroe 2009). The present study confirms the release of IL-8 as a most sensitive read-out for innate immune signalling, whereas the most remarkable finding was that gold salt was very effective in this regard which, using TLR transfectant cell lines, could be ascribed to triggering TLR3. The gold salt used for testing was gold-thiosulphate, which contains gold ions at their lowest oxidation state, thus representing the first ions generated upon oxidation of metallic gold. This salt is also used for skin testing gold contact allergy, as well as used for anti-rheumatoid arthritis treatment.

Actually, the latter feature of gold, i.e. immunosuppression, would appear contradictory to its innate immune stimulatory activity, which has intrigued clinicians and (immuno) toxicologists for many years (Buckley et al. 2011; Merchant 1998). Gold (I) salts (auranofin,
sanocrysin) may exert immunosuppressive actions through inhibiting IkappaB kinase activation and/or through pro-apoptotic activities (Jeon et al. 2003; Kim et al. 2004). Gold-induced release of the immunosuppressive mediator IL-10 may not play a role in this feature, as observed here. Still, pro-inflammatory activity can be clearly exerted through direct triggering of TLR3, and increased release of downstream mediators. The net outcome of these seemingly contradictory effects may depend on ill-defined secondary factors. At any rate, the latter pro-inflammatory activity can be expected to contribute to the frequent development of adaptive immune responses to gold (Martin et al. 2011). How sufficient gold ions reach the intracellular locale of TLR3, i.e. lysosomal surfaces, for triggering is as yet unclear. Alternatively, small numbers of extracellular TLR3 receptor molecules suffice for this purpose. Since gold (I) can be further oxidized inside phagocyte lysosomal compartments and resulting gold (III) represents a major hapten in gold allergy (Goebel et al. 1995), these effects may act synergistically in sensitization. Exposure to gold salt indeed also led to augmented expression of co-stimulatory molecules, although this effect was less pronounced than observed with nickel. Anyhow, the gold paradox is not unique, since also e.g. steroids are known to exert both immunosuppressive and immunostimulatory effects (Baeck and Goossens 2012).

While mercury does not present a paradox, its toxicities are undisputed. It is a non-essential metal in the human body, whereas it is ubiquitously distributed in the environment. Certainly because of its superior mechanical features and wide availability, the usage of mercury in dental amalgams has found its way across all continents. But, because of its toxicity and the appearance of competitive dental composite materials, the use of dental amalgams has now become extinct in affluent societies. Major toxic activities include its irreversible reactivity with selenium, an essential dietary element required by selenoenzymes (Reeves and Hoffmann 2009). These enzymes prevent and reverse oxidative damage in cells, e.g. in the brain and endocrine organs. Here, we observed an hitherto not reported effect of mercury, i.e stimulation of innate immunity. Given our dental focus, we did not include organic mercury in these experiments. Like with gold, for mercury salt this triggering of innate immunity was most clearly revealed by IL-8 release. Using the available limited panel of TLR transfectants we could not identify the triggering receptor(s) involved.

In fact, these might also belong to any of the alternative PRR families, i.e. C-type lectin receptors (CLR), retinoic-acid inducible gene (RIG)-I-like receptors (RLR) or NOD-like receptors (NLR). Preliminary experiments on the pathways involved, using the p38 MAPK blocker SB203580, resulted in strong (70-80 %) suppression of both gold and mercury-induced IL-8 production by THP-1 cells [data not shown], indicating that the innate signalling by mercury, like for gold, involves p38 MAPK phosphorylation. Anyhow, the innate triggering capacity of mercury as revealed in the present experiments is likely to contribute to its irritant properties, causing e.g. pustular lesions, and to its sensitizing capacity.
2011), as well as to its putative role in the induction of autoimmunity (Nielsen and Hultman 2002).

CONCLUSIONS

To conclude, the present study adds the high molecular weight transitional metals gold and mercury to the panel of metals showing distinct innate immune stimulatory capacities. For gold, evidence was obtained for a role of TLR3 in activation of mononuclear cells. This activation was robust, and could be detected in freshly prepared monocyte-derived DC, as well as in unseparated PBMC and in monocytoïd cell line THP1 cells. Of course, clinical relevance of these findings relates to the local release of the metal ions tested, i.e. Au (I) and Hg (II), from alloys or amalgams. Application of alloys and amalgams with lowest release of such ions would reduce the immunotoxic risks as outlined. For gold, appropriate alloys have been identified, whereas for amalgams continued use for dental applications seems contra-indicated.

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