Anti-Inflammatory Effect of Gold Nanoparticles Supported on Metal Oxides

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Anti-inflammatory effect of gold nanoparticles supported on metal oxides

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ABSTRACT

Gold (Au) can be deposited as nanoparticles (NPs) smaller than 10 nm in diameter on a variety of metal oxide (MOx) NPs. Au/MOx NPs have high catalytic performance and selective oxidation capacity which could have implications in terms of biological activity, and more specifically in modulation of the inflammatory reaction. Therefore, the aim of this study was to examine the effect of Au/TiO₂, Au/ZrO₂ and Au/CeO₂ on viability, phagocytic capacity and inflammatory profile (TNF- α and IL-1 β secretion) of murine macrophages. The most important result of this study is an anti-inflammatory effect of Au/MOx NPs depending on the MOx nature with particle internalization and no alteration of cell viability and phagocytosis. The effect was dependent on the MOx NPs chemical nature (Au/TiO₂ > Au/ZrO₂ > Au/CeO₂ if we consider the number of cytokines whose concentration was reduced by the NPs), and on the inflammatory mediator considered. The effect of Au/TiO₂ NPs was not related to Au NPs size (at least in the case of Au/TiO₂ NPs in the range of 3-8 nm). To the best of our knowledge, this is the first demonstration of an anti-inflammatory effect of Au/MOx NPs.

INTRODUCTION

Gold (Au) can be deposited as nanoparticles (NPs) smaller than 10 nm in diameter on a variety of metal oxide (MOx) NPs. Au/MOx NPs have attracted much attention due to their high catalytic performance for such as room temperature CO oxidation and selective oxidations in liquid phase¹². The catalytic activity of Au strongly depends on the kind of support. For example, although TiO₂ is almost inactive for CO oxidation, the deposition of Au NPs onto TiO₂ enables to catalyze CO oxidation at room temperature³⁴.

These data in the field of heterogeneous catalysis have firmly established that deposition of Au on MOx NPs increases dramatically the intrinsic catalytic activity of these NPs and this could also be the case for the biological activity. For example, Menchon and co-workers⁵ reported that Au/CeO₂ exhibits antioxidant activity against reactive oxygen species (ROS) in Hep3B and HeLa cell related lines due to a peroxidase activity. Since the inflammatory reaction is highly dependent on oxidative stress³, one can hypothesize that, in addition to their antioxidant properties, Au/MOx NPs can have anti-inflammatory effects. Such an effect could have important implications in terms of medical utilization of Au/MOx NPs. However, to the best of our knowledge no data on this effect is available in the current literature.

Therefore, in the present study we examined the effect of Au/MOx on cytotoxic and inflammatory response of macrophages, a key cell type involved in the inflammatory reaction. We used murine macrophages and investigated the roles of the size of Au NPs, the chemical nature of the supporting MOx NPs, and the kinetics of Au/MOx NPs interference with the inflammatory reaction.

RESULTS

No cytotoxic effect of Au/TiO₂ NPs on mouse macrophages

Since physico-chemical properties of Au/TiO₂ have already been extensively described¹³⁴, we have first compared biological effects of these Au/TiO₂ NPs versus TiO₂ NPs on mouse peritoneal macrophages. No cytotoxicity was observed by either of MTT (Figure 1a and b) or LDH release assays (Figure 1c and d) for TiO₂ and Au/TiO₂ NPs regardless of the concentration (1 to 100 µg.ml⁻¹), the exposure time (24 and 48h) and the size of Au NPs. By contrast, Ag/TiO₂ and Pt/TiO₂ displayed significant cytotoxic effects in murine RAW 264.7 macrophages at 100 µg.ml⁻¹ as compared to TiO₂, whereas Au/TiO₂ and Pd/TiO₂ did not show any cytotoxicity (Figure 2).

TiO₂ and Au/TiO₂ NPs (50 µg.ml⁻¹, 6h incubation) significantly and similarly increased the phagocytosis of fluorescent beads by peritoneal macrophages, showing an absence of modulation of TiO₂ NPs-induced macrophage activation by AuNPs (Figure 3).

TiO₂ and Au/TiO₂ NP are internalized in mouse peritoneal macrophages

Cellular uptake and intracellular morphology upon 24h exposure to TiO₂ and Au/TiO₂NPs were investigated in ultrathin sections of resin-embedded cells using TEM.

TiO₂NPs and Au/TiO₂NPs (50 µg.ml⁻¹) were internalized in cells. The prevalent localization of NPs agglomerates was in cell vacuoles (Figure 4 a-c). In accordance with the previous paragraph showing a lack of cytotoxicity, no morphologic sign of cell damage was observed.

No inflammation induced by Au/TiO₂ NPs

Incubation of mouse peritoneal macrophages with TiO₂ NPs (50 µg.ml⁻¹, 6h), induced a significant increase in the concentration of TNF-α (Figure 5a) and IL-1β (Figure 5b), two main pro-inflammatory cytokines, in cell culture medium. This effect was not observed in the case of Au/TiO₂ NPs without any significant difference between the size of Au NPs (Figure 5).

Au/TiO₂ NPs attenuate LPS-induced inflammation

Having demonstrated that Au/TiO₂ NPs did not elicit an inflammatory reaction, we questioned whether Au/TiO₂ NPs could blunt inflammation induced in a model of pathophysiological relevance, such as macrophages exposure to *Escherichia Coli* LPS. Au/TiO₂ having 3 and 8 nm of Au NPs in diameters, abbreviated as Au3/TiO₂ and Au8/TiO₂, respectively, were used. Since no difference of anti-inflammatory effect was observed between Au3/TiO₂ and Au8/TiO₂, we investigated the effects of Au3/TiO₂ NPs (referred to as Au/TiO₂ in the following experiments). Mouse peritoneal macrophages were incubated with culture media 6h (control condition) or LPS 2h, and then the following 4 h with LPS alone or LPS plus TiO₂ or Au/TiO₂. Taking into account the results presented in the previous paragraph, TNF-α and IL-1β cell culture supernatant concentrations were measured as a surrogate of the inflammatory reaction. As expected, incubation of cells with LPS for 2 + 4h induced a significant increase as compared to the control condition (p<0.05, Figures 6a and b). Incubation of LPS followed by TiO₂ NPs did not significantly modify the effect of LPS alone. By contrast, this last effect was significantly attenuated by macrophage incubation with Au/TiO₂ NPs (p<0.05, Figures 6a and b). These results show that Au/TiO₂ can attenuate LPS induced inflammation, even when cells are incubated with these NPs after incubation with LPS.

Effects of Au/ZrO₂ and Au/CeO₂ NPs on LPS-induced inflammation

After showing the anti-inflammatory effect of Au/TiO₂ NPs, we investigated if this effect was related to the chemical nature of the MOx NPs supports. To answer to this question, we examined the effect of Au NPs deposited on either ZrO₂ or CeO₂ NPs.

No effect was observed with Au/CeO₂ NPs, whereas Au/ZrO₂ NPs attenuated LPS-induced IL-1β production without any effect on TNF-α concentration (Figures 6a and b). Therefore, we can

conclude that Au/MOx NPs possess an anti-inflammatory effect which depends on the nature of the MOx NPs and on the inflammatory mediator examined.

Is the anti-inflammatory effect of Au/MOx NP related to cytokines adsorption on their surface?

We investigated the mechanisms underlining the anti-inflammatory effect of Au/MOx NPs. Since NPs adsorb different proteins on their surfaces ⁶, we then investigated if the decreased concentration of LPS-induced TNF- α and IL-1 β concentration observed with Au/TiO₂ and Au/ZrO₂ (only for IL-1 β in this last case) was related to adsorption of these cytokines on these Au/MOx NPs. The results of these experiments showed that incubation of each one of these cytokines with the different Au/MOx NPs examined in this study did not change its respective concentration when compared to the MOx NPs without Au (Figure 7 a and b). This result excludes cytokines adsorption as a mechanism explaining the anti-inflammatory effect of Au/TiO₂ and Au/ZrO₂ NPs.

Is the anti-inflammatory effect of Au/MOx NP related to their antioxidant properties?

We then examined if the anti-inflammatory effect of Au/TiO₂ and Au/ZrO₂ was related to antioxidant properties, as demonstrated in other conditions ⁷⁸. To investigate this issue, we measured the effect of Au/MOx NPs on intracellular reactive oxygen species (ROS) concentration. These experiments showed that intracellular ROS production induced by Au/TiO₂ and Au/ZrO₂ NPs was smaller than that of TiO₂ and ZrO₂ respectively, demonstrating an antioxidant effect of these NPs (Table 1), in accordance with their (partial) anti-inflammatory effect. This effect was more important in the case of Au/ZrO₂ compared to Au/TiO₂ (mean reduction of 32% vs 11% compared to ZrO₂ and TiO₂ respectively). This effect could be involved in the anti-inflammatory effect described previously. Interestingly, levels of ROS were lower in cells exposed to CeO₂ as compared to cells exposed to TiO₂ and ZrO₂, in accordance with antioxidant properties of CeO₂ described previously ⁹.

DISCUSSION

The most important result of this study is an anti-inflammatory effect of Au/MOx NPs depending on the MOx nature with particle internalization and no alteration of cell viability and phagocytosis. Interestingly, the lack of effect Au/TiO₂ on cell viability appears relatively specific for this combination of NPs, since Ag/TiO₂ and Pt/TiO₂ impaired macrophages viability. The effect of Au/TiO₂ NPs was not related to Au NPs size (at least in the case of Au/TiO₂ NPs in the range of 3-8 nm) and was dependent on the MOx NPs chemical nature (Au/TiO₂ > Au/ZrO₂ > Au/CeO₂ if we consider the number of cytokines whose concentration was reduced by the NPs) and on the inflammatory mediator considered. To the best of our knowledge, this is the first demonstration that Au/MOx present anti-inflammatory properties.

The effect of Au/MOx could be related to anti-inflammatory properties of Au NPs *per se*. Indeed, gold colloids have been thought to cure various diseases for many centuries, and recent studies have demonstrated that this effect is mediated by inhibition of NF- κ B activation¹⁰. Different studies showed a similar effect in the case of Au NPs *in vitro* and *in vivo*, this effect being usually mediated by antioxidant properties^{11,12,13,14,15}. However, other studies showed opposite results. For example, Ng and coworkers¹⁶ showed that incubation of human bronchial epithelial cells with 50 $\mu\text{g}.\text{ml}^{-1}$ of 20 nm Au NPs activated NF- κ B in bronchial epithelial cells. Close results were shown in other cell types^{17,18}. Therefore, an anti-inflammatory effect of Au NPs *per se* seems not a univocal mechanism explaining the anti-inflammatory effect of Au/MOx NPs. Moreover, attributing our results to the biological activity of Au NPs is questionable since most of the studies showing an effect of Au NPs on inflammation examined concentrations far above the ones to whom cells were exposed in the present study, taking into account that Au NPs represented only 1% of the mass of Au/MOx.

Adsorption of TNF- α and IL-1 β on Au/MOx surface could also explain their different anti-inflammatory activity. Indeed, NPs, including Au NPs, can adsorb different molecules on their surface, resulting in their inactivation and reduction in their concentration in the medium¹⁹. However, this mechanism seems unlikely in the present study since we did not observe any significant difference in TNF- α and IL-1 β levels when these cytokines were incubated with TiO₂ or ZrO₂ NPs and their respective Au counterparts.

Finally, the differences in the anti-inflammatory effect of Au/MOx NPs could be related to their different antioxidant properties. However, the anti-inflammatory effect of Au/MOx NPs was not paralleled by the intracellular antioxidant properties since both Au/TiO₂ and Au/ZrO₂ NPs reduced intracellular ROS concentration (with a more important effect of Au/ZrO₂ than Au/TiO₂) whereas only Au/TiO₂ attenuated both TNF- α and IL-1 β induction. Moreover, the absence of an anti-inflammatory effect of Au/CeO₂ was paralleled by an absence of an antioxidant effect of this NP as compared to CeO₂ NPs. Interestingly, in concordance with different studies, CeO₂ NPs exerted an antioxidant effect as compared to TiO₂ and ZrO₂ NPs (Table 1), but this effect was not accompanied by an anti-inflammatory effect. Collectively, these data suggest that in contrast with our initial hypothesis, an antioxidant effect does not appear as a mechanism globally explaining the effect of Au/TiO₂ and Au/ZrO₂. Indeed, an antioxidant effect could potentially explain the effect of Au/TiO₂ NPs but not the one of Au/ZrO₂ NPs because these last NPs had showed an antioxidant effect similar to Au/TiO₂ but attenuated only IL-1 β induction. Consequently, the effect of Au/ZrO₂ appears to depend on pathways inhibiting IL-1 β but not TNF- α expression.

Numerous mechanisms control the production and activity of IL-1 β , including the processing of the 31-kDa, inactive IL-1 β precursor into the bioactive, 17-kDa cytokine *via* intracellular protein complexes termed the inflammasomes²⁰. The most intensely studied inflammasome is the NLRP3 inflammasome. Various stimuli activate the NLRP3 inflammasome: bacterial structures such as muramyl dipeptide or LPS, bacterial RNA, β -glucan, double-stranded RNA, etc. The release of ROS has been repeatedly reported to mediate NLRP3 inflammasome activation by various stimuli, including LPS, but this has been surrounded by controversy²¹. Other proposed mechanisms responsible for NLRP3 inflammasome activation are *i*) translocation to mitochondria²², *ii*) release of mitochondrial DNA or cardiolipin²³, *iii*) release of lysosomal cathepsins into the cytoplasm²⁴, or *iv*) calcium-dependent phospholipase 2 activation²⁰. Interference with one or several mechanisms could explain attenuation of LPS-induced IL-1 β expression by Au/ZrO₂ NPs. However, investigating these possibilities needs further studies.

This study presents 2 main limitations. First, we only measured 2 cytokines as a surrogate of inflammatory markers. Even if these cytokines are broadly representative of the inflammatory reaction and are one of the very early ones in the inflammatory cascade, and we investigate primary murine macrophages (a key cell involved in inflammation), a wider analysis of mediators of this reaction is necessary to validate the present results. Second, we investigated only 3 MOx NPs supports. Although we choose supports widely characterized in terms of catalytic properties when combined with Au NPs, investigating a broader panel of MOx could allow a better characterizing the anti-inflammatory properties of Au/MOx NPs. It has to be noted, however, that we compared cytotoxicity of Au/TiO₂ with other metallic NPs deposited on TiO₂ (Ag, Pt and Pd) and we showed that only Au/TiO₂ and Pd/TiO₂ did not impaired cell viability at the concentrations examined, providing an additional argument for the beneficial effects of Au/TiO₂ NPs.

In conclusion, this study showed that Au/MOx possess anti-inflammatory properties in macrophages. This effect could have potential applications in the clinical setting since it does not interfere with physiological parameters of these cells such as viability and phagocytosis.

METHODS

Nanoparticles preparation

Au NPs with two different mean diameters (3 and 8 nm) supported on pristine TiO₂ NPs (Nippon Aerosil Co., Ltd., AEROXIDE P25) were prepared, termed Au3/TiO₂ and Au8/TiO₂, respectively. Au3/TiO₂ were prepared by deposition-precipitation³ followed by calcination at 300 °C for 4h. Au8/TiO₂ was prepared by solid grinding with dimethyl Au (III) acetylacetonate and by calcination at 300 °C²⁵. Au/ZrO₂ and Au/CeO₂ were purchased from Haruta Gold Inc©. The primary particle sizes of

Au₃/TiO₂, Au/ZrO₂, and Au/CeO₂ were 26 nm, 11 nm, and 7.3 nm respectively, estimated from their specific surface areas of the MOx NPs. The secondary particle sizes of Au₃/TiO₂, Au/ZrO₂, and Au/CeO₂ were 5.5 μm, 0.9 μm, and 3.6 μm respectively, determined by dynamic light scattering method. Using atomic absorption spectroscopy, the actual Au loadings for Au₃/TiO₂, Au₈/TiO₂, Au/ZrO₂, and Au/CeO₂ were 0.96, 1.18, 0.97, and 0.96 wt%, respectively. The mean diameters of Au NPs were estimated by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) to be 3.3±1.4 nm for Au₃/TiO₂, 7.8±2.7 nm for Au₈/TiO₂, and 3.3±1.8 nm for Au/ZrO₂. In the case of Au/CeO₂, it was difficult to observe Au NPs because Au was mostly deposited as single atoms and the atomic number of Ce is close to that of Au.

Metal NPs other than Au, namely Ag, Pt, Pd, supported on TiO₂ (loading: 1wt%) were prepared by impregnation. TiO₂ was suspended in aqueous AgNO₃, H₂PtCl₆·6H₂O, or PdCl₂, followed by evaporation to dryness at 70 °C. The remaining paste was reduced in 10% H₂ in N₂ at 300 °C for 4h.

Cells isolation and incubation with nanoparticles

Peritoneal macrophages and macrophages from the RAW 264.7 cell line were used in the different experiments.

Peritoneal macrophages. C57BL/6 mice were injected intraperitoneally with 1 ml of 3% Brewer thioglycollate (Sigma-Aldrich, B2551). After 96 h, peritoneal cells were harvested by lavage with 0.67% phosphate buffered saline (PBS). After a soft centrifugation, cells were maintained in Dulbecco's modified Eagle medium (DMEM) 4,5 g.L⁻¹ glucose supplemented with 10 % fetal calf serum (FCS) and 1 % penicillin and 1% streptomycin. Then mice peritoneal macrophagic cells were exposed for 6–48 h to 1–100 μg/mL particles of Au/TiO₂, Au/ZrO₂, Au/CeO₂, and MOx alone.

RAW 264.7 macrophages. The RAW 264.7 cells were cultured using a medium having the same composition as above and were exposed for 24 h to 1–100 μg/ml nanoparticles of Au/TiO₂, Ag/TiO₂, Pt/TiO₂, Pd/TiO₂ and TiO₂.

Cellular viability

The viability of cells was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-1) assay, and the quantification of the release of lactate dehydrogenase (LDH).

Phagocytosis assay

RAW 264.7 cells (ATCC) were exposed to 50 μg.mL⁻¹ CNT for 6 h, together with Latex beads-rabbit IgG-FITC complex, as per the manufacturer's instructions (Phagocytosis assay Kit; Cayman Chemical, 500,290). Fluorescence was measured at λ_{exc} = 485 nm, and λ_{emi} = 535 nm²⁶.

Transmission electron microscopy (TEM)

Macrophages submitted to different exposure were fixed overnight at 4 °C using TEM-grade fixative solution of 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. The samples were washed and stored in 0.1 M sodium cacodylate buffer and kept at 4 °C until processing. Sample embedding was performed in epoxy resin according a standard protocol.

Inflammatory responses

Inflammatory response was evaluated by the quantification of cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β in cell supernatant, measured by enzyme-linked immunosorbent assay (ELISA).

Measurement of oxidative stress by DCFH-DA assay

Endogenous ROS were quantified by oxidation of 2',7'-dichlorofluorescein diacetate (DCFH-DA) into 2',7'-dichlorofluorescein (Sigma, Saint Quentin Fallavier, France). Briefly, cells were cultivated in six-well culture plates and treated with 50 $\mu\text{g}\cdot\text{ml}^{-1}$ NPs. Cells were also treated with 250 μM H_2O_2 as a positive control (data not shown). Cells were incubated with 20 μM DCFH-DA for 30 min at 37 °C and fluorescence recorded for 90 min. Results were expressed as the mean ratio of fluorescence recorded every 15 min during the 90 min period.

Measurement of TNF- α and IL-1 β adsorption to NPs

Adsorption of TNF- α and IL-1 β adsorption to NPs was performed by ELISA as described previously described²⁷.

Statistical Analysis

JASP software (version 0.11.1, <https://jasp-stats.org/>) was used to analyze quantitative data. Non-parametric ANOVA was used to compare multiple groups. Paired comparisons with Mann-Whitney test were performed if the differences using ANOVA were statistically significantly different. Data were presented as mean values $\pm$ standard error of the mean (SEM), and results were considered statistically significant if $p < 0.05$.

Study approval for animal experiments

The Institutional Animal Care and Use Committee approved experimental procedures on mice (Autorisation De Projet Utilisant Des Animaux A Des Fins Scientifiques APAFIS authorization #14914-2018042515599016). All experiments were carried out in accordance with relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHORS CONTRIBUTION

TF prepared and analyzed NPs, performed chemical experiments and critically reviewed the manuscript. TF, MZ and DE performed biological experiments. TM, MH, and SL critically reviewed the manuscript and worked on its final elaboration. TI contributed to the design of the study, critically reviewed the manuscript and worked on its final elaboration. JB designed the study and wrote the first and final drafts of the manuscript.

All authors approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

Figure Legends

Figure 1. Cytotoxicity of Au nanoparticles (NPs) with two different diameters (3 and 8 nm respectively) supported on pristine TiO₂ (Au₃/TiO₂ and Au₈/TiO₂ respectively) and TiO₂ NPs alone on mouse peritoneal macrophages. Cell viability was measured using the MTT assay (panels a and b) and LDH relargage (panels c and d) after 24 and 48h incubation (panels a and c and b and d respectively). Data are expressed as percentage of control condition (without NPs, 100% is the reference value) and are represented as mean $\pm$ SEM of 6 independent experiments. Positive controls are doxorubicine (DOXO, 1, 5 and 10 $\mu\text{g}.\text{ml}^{-1}$) and TritonX-100 (10 vol. %). * significantly different from control ($p < 0.05$).

Figure 2. Cytotoxicity of Au, Ag, Pt, Pd NPs supported on pristine TiO₂ NPs and pristine TiO₂ NPs alone on murine RAW 264.7 macrophages. Cell viability was measured using the WST-1 assay after 24 h incubation. Data are expressed as percentage of control condition (without NPs, 100% is the reference value) and are represented as mean $\pm$ SEM of 6 independent experiments. Positive control is cisplatin (1, 5 and 10 $\mu\text{g}.\text{ml}^{-1}$). * significantly different from control ($p < 0.05$), # significantly different from control ($p < 0.01$)

Figure 3. Effect of Au₃/TiO₂, Au₈/TiO₂ and TiO₂NPs (50 $\mu\text{g}.\text{ml}^{-1}$, 6h incubation) on phagocytosis of fluorescent beads by mouse peritoneal macrophages. Data are expressed as arbitrary units (AU) of fluorescence intensity and are represented as mean $\pm$ SEM of 6 independent experiments. * significantly different from control ($p < 0.05$).

Figure 4. Transmission electron microscopy views of mouse peritoneal macrophages incubated 24h with 50 $\mu\text{g}.\text{ml}^{-1}$ of Au₃/TiO₂, Au₈/TiO₂ and TiO₂ NPs.

The scale bar in images in column a is 2 microns, and in columns b and c is 0.2 microns. As observed in the images, the prevalent localization of NPs agglomerates was in cell vacuoles. No morphologic sign of cell damage was observed.

Figure 5. Effect of Au₃/TiO₂, Au₈/TiO₂ and TiO₂NPs (50 $\mu\text{g}.\text{ml}^{-1}$, 6h incubation) on TNF- α and IL-1 β secretion by mouse peritoneal macrophages (panels a and b respectively). Data are expressed as picograms/ml in cell culture medium and are represented as mean $\pm$ SEM of 6 independent experiments. # significantly different from control ($p < 0.01$)

Figure 6. Effect of Au deposited on different metal oxide NPs on LPS induced TNF- α and IL-1 β secretion by mouse peritoneal macrophages (Panels a and b respectively). Cells were incubated with culture media 6h (control condition, C) or LPS 2h, and then the following 4 h with LPS alone or LPS plus the different NPs (50 $\mu\text{g}.\text{ml}^{-1}$). * significantly different from C ($p < 0.05$), ** significantly different from LPS 2h – TiO₂ 4h ($p < 0.05$), & significantly different from LPS 2h – ZrO₂ 4h ($p < 0.05$)

Figure 7. TNF- α and IL-1 β adsorption on Au deposited on different metal oxide NPs. NPs (50 $\mu\text{g}.\text{ml}^{-1}$) were incubated during 6h with TNF- α (150 $\text{pg}.\text{ml}^{-1}$) and IL-1 β (100 $\text{pg}.\text{ml}^{-1}$) in culture media, then the cytokines were quantified in the media after centrifugation.

Table 1

Nanoparticle	Mean values $\pm$ SEM	Statistical significance
Au/TiO ₂	12068 $\pm$ 499	*
TiO ₂	13547 $\pm$ 340	
Au/ZrO ₂	9170 $\pm$ 109	**
ZrO ₂	13538 $\pm$ 129	
Au/CeO ₂	12743 $\pm$ 340	
CeO ₂	12583 $\pm$ 255	#

Reactive oxygen species (ROS) production in RAW 264.7 cells after expositions to different NPs (n=7).

The final concentration of NPs was 50 $\mu\text{g} \cdot \text{ml}^{-1}$. Amounts of ROS production were evaluated by the CM₂-DCFDA. * $p < 0.05$ vs TiO₂, ** $p < 0.01$ vs ZrO₂, # $p < 0.05$ vs TiO₂ and ZrO₂.

Figures

Figure 1

Cytotoxicity of Au nanoparticles (NPs) with two different diameters (3 and 8 nm respectively) supported on pristine TiO₂ (Au₃/TiO₂ and Au₈/TiO₂ respectively) and TiO₂ NPs alone on mouse peritoneal macrophages. Cell viability was measured using the MTT assay (panels a and b) and LDH relargage (panels c and d) after 24 and 48h incubation (panels a and c and b and d respectively). Data are expressed as percentage of control condition (without NPs, 100% is the reference value) and are represented as mean $\pm$ SEM of 6 independent experiments. Positive controls are doxorubicine (DOXO, 1, 5 and 10 $\mu\text{g}.\text{ml}^{-1}$) and TritonX-100 (10 vol. %). * significantly different from control ($p < 0.05$).

Figure 2

Cytotoxicity of Au, Ag, Pt, Pd NPs supported on pristine TiO₂ NPs and pristine TiO₂ NPs alone on murine RAW 264.7 macrophages. Cell viability was measured using the WST-1 assay after 24 h incubation. Data are expressed as percentage of control condition (without NPs, 100% is the reference value) and are represented as mean $\pm$ SEM of 6 independent experiments. Positive control is cisplatin (1, 5 and 10 $\mu\text{g} \cdot \text{ml}^{-1}$). * significantly different from control ($p < 0.05$), # significantly different from control ($p < 0.01$)

Figure 3

Effect of Au₃/TiO₂, Au₈/TiO₂ and TiO₂NPs (50 µg.ml⁻¹, 6h incubation) on phagocytosis of fluorescent beads by mouse peritoneal macrophages. Data are expressed as arbitrary units (AU) of fluorescence intensity and are represented as mean ± SEM of 6 independent experiments. * significantly different from control (p < 0.05).

Figure 4

Transmission electron microscopy views of mouse peritoneal macrophages incubated 24h with 50 $\mu\text{g.ml}^{-1}$ of Au_3/TiO_2 , Au_8/TiO_2 and TiO_2 NPs. The scale bar in images in column a is 2 microns, and in columns b and c is 0.2 microns. As observed in the images, the prevalent localization of NPs agglomerates was in cell vacuoles. No morphologic sign of cell damage was observed.

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Effect of Au3/TiO2, Au8/TiO2 and TiO2NPs (50 $\mu\text{g}.\text{ml}^{-1}$, 6h incubation) on TNF- α and IL-1 β secretion by mouse peritoneal macrophages (panels a and b respectively). Data are expressed as picograms/ml in cell culture medium and are represented as mean $\pm$ SEM of 6 independent experiments. # significantly different from control (p < 0.01)

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Effect of Au deposited on different metal oxide NPs on LPS induced $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ secretion by mouse peritoneal macrophages (Panels a and b respectively). Cells were incubated with culture media 6h (control condition, C) or LPS 2h, and then the following 4 h with LPS alone or LPS plus the different NPs ($50 \mu\text{g}.\text{ml}^{-1}$). * significantly different from C ($p < 0.05$), ** significantly different from LPS 2h – TiO₂ 4h ($p < 0.05$), & significantly different from LPS 2h – ZrO₂ 4h ($p < 0.05$)

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TNF- α and IL-1 β adsorption on Au deposited on different metal oxide NPs. NPs (50 $\mu\text{g}.\text{ml}^{-1}$) were incubated during 6h with TNF- α (150 $\text{pg}.\text{ml}^{-1}$) and IL-1 β (100 $\text{pg}.\text{ml}^{-1}$) in culture media, then the cytokines were quantified in the media after centrifugation.