CTAB-coated gold nanorods elicit allergic response through degranulation and cell death in human basophils

Article *in* Nanoscale · June 2012

DOI: 10.1039/c2nr30435j · Source: PubMed

7 authors, including:

Anthony Ka Lun Cheung
New B Innovation Ltd
13 PUBLICATIONS **188** CITATIONS
SEE PROFILE

Huanjun Chen
Sun Yat-Sen University
109 PUBLICATIONS **5,385** CITATIONS
SEE PROFILE

H.P. Ho
The Chinese University of Hong Kong
146 PUBLICATIONS **2,009** CITATIONS
SEE PROFILE

Chun Kwok Wong
The Chinese University of Hong Kong
258 PUBLICATIONS **7,767** CITATIONS
SEE PROFILE

Nanoscale

Cite this: DOI: 10.1039/c2nr30435j

www.rsc.org/nanoscale

COMMUNICATION

CTAB-coated gold nanorods elicit allergic response through degranulation and cell death in human basophils†

Ka Lun Cheung,[a] Huanjun Chen,[b] Qiulan Chen,[c] Jianfang Wang,[b] Ho Pui Ho,[c] Chun Kwok Wong[d] and Siu Kai Kong*[a]

Received 24th February 2012, Accepted 21st May 2012
DOI: 10.1039/c2nr30435j

The effect of CTAB (cetyltrimethylammonium bromide)- or PEG (polyethylene glycol)-coated gold-nanorods (Au-NRs) on the non-IgE mediated allergic response was studied. We found that the CTAB-Au-NRs released more allergic mediators such as histamine and β-hexosaminidase from human basophil KU812, a common model for studying allergy, after 20 min incubation. Also, the CTAB-Au-NRs induced more apoptosis than the PEG-Au-NRs in KU812 24 h after treatment. These short- and long-term effects were not solely due to the CTAB residues in the supernatant desorbed from the Au-NRs.

Gold nanoparticles (Au-NPs) have been receiving a lot of attention recently because of their size and unique optical properties. Their unique and adjustable physical and chemical properties, including surface/volume ratio, surface charges, magnetic and optical characteristics,[1] allow them to be used for many new therapeutic applications[2,3] such as microbial killing,[4] drug[5,6] and nucleic acid delivery.[7–9]

Despite the wide interests in the biomedical field in using Au-NPs, there are increasing concerns regarding the health and safety issues of using Au-NPs in humans and animals. Noticeably, the fabrication procedures of Au-NPs involve CTAB (cetyltrimethylammonium bromide), which disrupts the cell membranes[10] and therefore is highly cytotoxic.[11] To reduce the toxicity, CTAB coating can be replaced by PEG (polyethylene glycol) through an ion exchange process. PEG is much more biocompatible as a result of its non-immunogenicity, non-antigenicity,[12–14] and chemical inertness.[12] Also, PEG provides a stable anchor for biomolecule conjugation.[15] For example, PEG was used to cross-link DNA on Au-NPs for tumour-targeted gene therapy, or recombinant enzyme methioninase for cancer therapy.[16]

Among various Au-NPs, gold nanorods (Au-NRs) have attracted much attention owing to their unique structure. A recent study reported that Au-NRs had lower toxicity than the spherical ones.[17] Hence, we asked whether the CTAB- and PEG-coated Au-NRs would cause any adverse effects in the human immune system, in particular the induction of allergies.

In our previous study,[18] we characterized our CTAB- and PEG-coated Au-NRs. Briefly, the Au-NRs show excellent shape and size uniformity (diameter: length = 15 ± 1 nm: 64 ± 5 nm). The extinction peaks of the CTAB- and PEG-coated Au-NRs are 790 and 800 nm, respectively (Fig. S1†). No fluorescence is emitted from the Au-NRs under excitation.

We were curious to know whether the Au-NRs had any effects to allergic reactions in human basophils. Two recent studies investigated the effect of NPs in human dendritic cells[19] and mice.[20] Yet, the effect of Au-NRs on the degranulation of allergic mediators in basophils has not been clearly elucidated. Therefore, we performed the histamine- and β-hexosaminidase-release assay to study allergic degranulation in KU812 cells after treatment with Au-NRs (Fig. 1).

Histamine and β-hexosaminidase are the two best-known mediators of allergic reactions, and are stored in the acidic granules in basophils. Upon specific binding of allergens to the corresponding IgE molecules on the Fc receptors of basophils, the allergic mediators will be released for IgE-mediated allergic reactions.[21,22] Also, recent findings indicate that many environmental pollutants, such as bisphenol A, are able to trigger allergic reactions in the absence of IgE production. In the light of these findings, we hypothesized that the CTAB-coated Au-NRs would be a potent secretagogue to release allergic mediators in the absence of IgE, while the PEG-coated Au-NRs show minimal effect. In this connection, we used ELISA to measure the histamine released from KU812, a common human basophil model,[23] and enzymatic assay to quantify the amount of β-hexosaminidase in the buffer after degranulation.

As shown in Fig. 1, the CTAB-coated Au-NRs induced the release of histamine and β-hexosaminidase readily in a dose-dependent manner (20–100% of total release). Notably, fMLP (1 μM), a strong chemoattractant that elicits degranulation,[24] only released ~50 and 40% of the total histamine and β-hexosaminidase respectively from the cell lysate. Apart from the Au-NRs, we also tested the toxicity of the supernatant of the Au-NRs, which contains free CTAB or PEG molecules as a result of desorption.[18] The supernatant also showed a positive effect, but the degree of release was much less than that of

[a]*Programme of Biochemistry, School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong (CUHK), Shatin, NT, Hong Kong. E-mail: kalun2004@cuhk.edu.hk; skkong@cuhk.edu.hk*
[b]*Department of Physics, Faculty of Science, CUHK, Shatin, NT, Hong Kong. E-mail: hjchen@phy.cuhk.edu.hk; jfwang@phy.cuhk.edu.hk*
[c]*Department of Electronic Engineering, Faculty of Engineering, CUHK, Shatin, NT, Hong Kong. E-mail: orchid720@gmail.com; hpho@ee.cuhk.edu.hk*
[d]*Department of Chemical Pathology, CUHK, Prince of Wales Hospital, Shatin, NT, Hong Kong. E-mail: ck-wong@cuhk.edu.hk*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2nr30435j

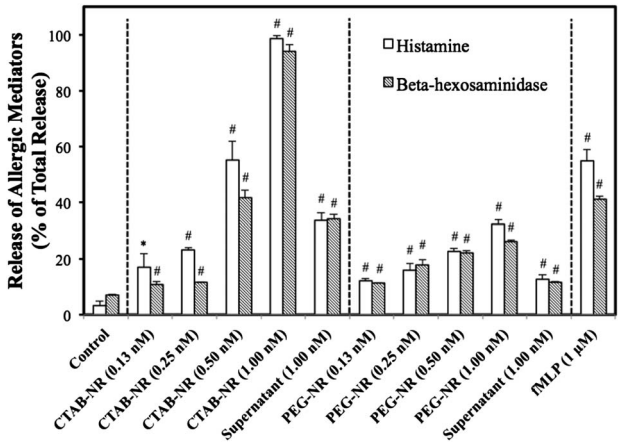

Fig. 1 Effects of the CTAB-, PEG-coated Au-NRs and their supernatants on the release of histamine and β-hexosaminidase in KU812 cells. Basophils (1×10^6 ml^{-1}) were treated with medium alone, fMLP (1 μM), the CTAB- or PEG-coated Au-NRs at the concentration as indicated or the supernatant from the 1.00 nM CTAB- or PEG-coated Au-NRs at 37 °C and 5% CO_2 for 20 min. After treatment, cell supernatants were submitted to the histamine release or β-hexosaminidase release assay. One hundred% total release was obtained from the cell lysate with 0.1% (v/v) Triton X-100. Results are mean ± SD ($n = 3$), * $p < 0.05$; # $p < 0.01$ compared to control.

the corresponding CTAB Au-NR preparation (1.00 nM) (35% *vs.* 100% of the total histamine). This result indicates that CTAB in the supernatant is not the primary source for histamine and β-hexosaminidase release. The PEG-coated Au-NRs also gave a similar trend of release but to a lesser extent (10–35% of total release) (Fig. 1). Consistent with this observation, the supernatant from the PEG Au-NRs also showed a minimal effect (~10%). Of particular note here is that incubation of KU812 cells with the Au-NRs for 20 min was sufficient to trigger allergic degranulation.

Next, we examined the cytotoxicity in KU812 cells by the alamar blue assay, an assay monitoring the metabolic activity through redox reactions.[25] As shown in Fig. 2a, the PEG-coated Au-NRs did not show any immediate or long-term toxic effects. More than 70% of the cells remained viable after challenge (Fig. 2a). Under the same conditions, the CTAB-coated Au-NRs exhibited a strong cytotoxicity with an IC_{50} around 0.15 nM in both the 4 and 24 h treatment. Also, a strong toxicity was observed in KU812 cells when they were incubated with the supernatant of the CTAB-coated Au-NRs (1.00 nM) while the supernatant from the PEG Au-NRs (1.00 nM) had no toxicity when both were compared to the apoptogenic positive control staurosporine (STS) (1 μM) (Fig. 2b). These observations agreed well with our previous findings in human erythrocytes[18] that the free CTAB is one of the sources of toxicity. The presence (0, 5, 10, 20% (v/v)) of fetal bovine serum (FBS) in the culture medium had no effect on the cytotoxicity of both Au-NRs (Fig. S2†). Furthermore, incubation of cells with CTAB- or PEG-coated Au-NRs up to 1.00 nM for 20 min did not elicit any cell death (viability >85%, data not shown) indicating that the release of allergic mediators by the Au-NRs is not a result of cell lysis.

In searching for the mechanism, we examined the toxic effect of the Au-NRs in KU812 cells using two assays to determine the membrane integrity and asymmetry. In the calcein leakage assay, cells were loaded with calcein-AM (2 μM) for 10 min in the dark, which allowed non-polar calcein-AM to be converted into polar calcein inside the cells. After washing, the cells were treated with different concentrations of the Au-NRs. The cells were expected to show some degree of calcein leakage as a result of the loss of plasma membrane integrity in the late phase of apoptosis.[26] Fig. S3a† shows the flow cytometric histogram of the calcein fluorescence from KU812. In the control, only 16.7% of the cell population fell in the selected M1 region, indicating that most of the cells retained calcein. On the contrary, 58.5% of the cells were found in M1 after the 24 h treatment with STS (1 μM) and 27.2% in the 4 h treated group (Fig. S3a†). For the CTAB-coated NRs, a dose-dependent leakage of calcein was observed in the 4 and 24 h groups (Fig. 3a), whereas the leakage was not significant, comparable to the control, in the group with an incubation time of 20 min (data not shown). Obviously, the longer the incubation time, the higher the toxicity. On the other hand, the PEG-coated NRs did not show any significant effect in the short- or long-term treatment (Fig. 3a). The supernatants of both types of NRs did not cause any leakage of calcein too (Fig. S3a†).

The second assay to study the membrane integrity is the propidium iodide (PI) staining assay. PI passes only through the leaky

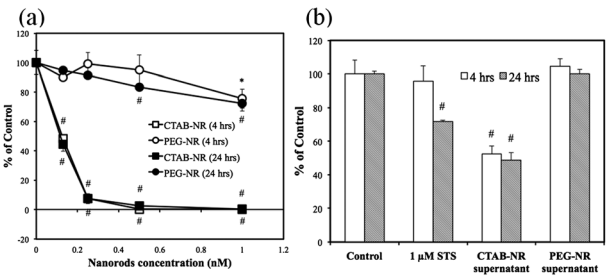

Fig. 2 Effects of the CTAB, PEG-coated Au-NRs, and their supernatants on the cytotoxicity in KU812 cells. Basophils (1×10^6 ml^{-1}) were treated with the CTAB- and PEG-coated Au-NRs at the concentrations as indicated at 37 °C and 5% CO_2 for 4 or 24 h. The cell viability was then determined by alamar blue assay as described (opened square: CTAB Au-NR, 4 h; closed square: CTAB Au-NR, 24 h; opened circle: PEG Au-NR, 4 h; closed circle: PEG Au-NR, 24 h) (a). The toxicity of the supernatants from the CTAB- and PEG-Au-NR supernatants (1.00 nM) was compared to the control and positive control (1 μM STS) (b). Results are mean ± SD ($n = 3$). * $p < 0.05$; # $p < 0.01$ compared to control.

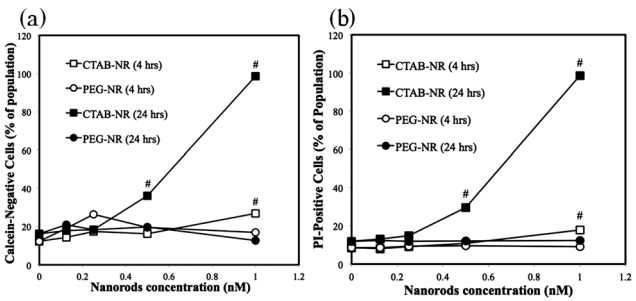

Fig. 3 Membrane permeabilization induced by the CTAB-, but not PEG-coated Au-NRs in KU812 cells. Basophils (1×10^6 ml^{-1}) were loaded with calcein-AM (2 μM) and treated with the CTAB- and PEG-coated Au-NRs in the calcein leakage assay (a), or stained with PI (2 μg ml^{-1}) after treatments in the PI staining assay (b) at 37 °C, 5% CO_2 for either 4 or 24 h as indicated. Data represent % of the total cell population in the selective region M1 (a) or M2 (b) in Fig. S3a and S3b†. Results are mean ± SD ($n = 3$) (Error bars are too small to be seen). # $p < 0.01$ compared to control.

membrane of dying cells, stains their nuclear DNA and emits fluorescence.[27] Any red fluorescence from PI is an indicator of the late apoptosis phase where cells lose membrane integrity.[27] As can be seen in the flow cytometric histogram in Fig. S3b†, incubation of cells with STS (1 μM) for 24 h generated a second peak with strong PI fluorescence when compared to that of the control. Similar to the results in the calcein leakage assay, the CTAB-coated Au-NRs showed a dose-dependent increase in the PI fluorescence after 24 h treatment (Fig. 3b). Almost all the cells were PI positive after the incubation with 1 nM of the CTAB Au-NRs (Fig. 3b and S3b†). On the contrary, all concentrations of PEG-coated NRs and supernatants of both types of NRs did not show any significant effect on membrane permeabilization (Fig. 3b and S3b†). Furthermore, externalization of phosphatidylserine (PS) from the plasma membrane was observed in the cells treated with the CTAB-, but not PEG-coated Au-NRs (Fig. S4†). Increase in the cytosolic free Ca^{2+} ion level in basophils was induced by the CTAB-, but not PEG-coated Au-NRs after 24 h incubation (Fig. S5†). No significant increase in the Ca^{2+} level was observed in the 4 h incubation groups (Fig. S5†). Taken together, these results show that the CTAB Au-NRs are likely the primary source that causes the membrane damage in basophils and the free CTAB in the supernatant desorbed from the Au-NRs only contributes a small part. These observations regarding cytotoxicity of the NPs using CTAB and PEG coating agreed with the studies performed by other groups.[28–31] Also, increase in the cytosolic Ca^{2+} level seems to be an important regulator for the degranulation and induction of apoptosis in the basophils.

Conclusion

In conclusion, our data indicate that the CTAB-coated Au-NRs exhibited a stronger cytotoxicity in terms of the metabolic activity (alamar blue assay) and apoptosis induction (calcein, PI and annexin-V binding assays) than the PEG-coated Au-NRs. Similar observations were obtained in the release of allergic mediators. Remarkably, the toxicity of the CTAB Au-NRs in basophils was not solely due to the CTAB residues desorbed from the NRs or incomplete purification, which is different from our previous observation in human erythrocytes.[18] The materials on the surface coating seem to be more important for the allergic reactions. Taken together, our study reveals a safety concern of these useful Au-NPs in allergy. As more and more knowledge is accumulated, we have confidence that proper attention will be given to the surface modification and dosage of Au-NRs so that their full potential can be developed.

Notes and references

1 A. M. Alkilany, P. K. Nagaria, C. R. Hexel, T. J. Shaw, C. J. Murphy and M. D. Murphy, *Small*, 2009, **5**, 701.
2 E. I. Barsumian, C. Isersky, M. G. Petrino and R. P. Siraganian, *Eur. J. Immunol.*, 1981, **11**, 317.
3 F. Blank, P. Gerber, B. Rothen-Rutishauser, U. Sakulkhu, J. Salaklang, K. De Peyer, P. Gehr, L. P. Nicod, H. Hofmann, T. Geiser, A. Petri-Fink and C. Von Garnier, *Nanotoxicology*, 2011, **5**, 606.
4 A. C. Bonoiu, S. D. Mahajan, H. Ding, I. Roy, K. Yong, R. Kumar, R. Hu, E. J. Bergey, S. A. Schwartz and P. N. Prasad, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 5546.
5 D. Bratosin, L. Mitrofan, C. Palii, J. Estaquier and J. Montreuil, *Cytometry, Part A*, 2005, **66**, 78.
6 C. Chen, Y. Liu, C. Wu, C. Yeh, M. Su and Y. Wu, *Adv. Mater.*, 2005, **17**, 404.
7 J. Y. Cheung, R. C. Ong, Y. Suen, V. Ooi, H. N. Wong, T. C. Mak, K. Fung, B. Yu and S. Kong, *Cancer Lett.*, 2005, **217**, 203.
8 R. Clark, K. Olson, G. Fuh, M. Marian, D. Mortensen, G. Teshima, S. Chang, H. Chu, V. Mukku, E. Canova-Davis, T. Somers, M. Cronin, M. Winkler and J. A. Wells, *J. Biol. Chem.*, 1996, **271**, 21969.
9 E. E. Connor, J. Mwamuka, A. Gole, C. J. Murphy and M. D. Wyatt, *Small*, 2005, **1**, 325.
10 W. H. De Jong and P. J. Borm, *Int. J. Nanomed.*, 2008, **3**, 133.
11 A. M. Dvorak, D. W. MacFlashan, Jr, E. S. Morgan and L. M. Lichtenstein, *Blood*, 1995, **86**, 3560.
12 S. Eustis and M. A. El-Sayad, *Chem. Soc. Rev.*, 2006, **35**, 209.
13 J. M. Harris, *Poly(Ethylene Glycol) Chemistry, Biotechnical and Biomedical Applications*, 1992.
14 T. S. Hauck, A. A. Ghazani and W. C. Chan, *Small*, 2008, **4**, 153.
15 A. S. Holpuch, G. J. Hummel, M. Tong, G. A. Seghi, P. Pei, P. Ma, R. J. Mumper and S. R. Mallery, *Pharm. Res.*, 2010, **27**, 1224.
16 K. Kishi, *Leuk. Res.*, 1985, **9**, 381.
17 A. Kulamarva, J. Bhathena, M. Malhotra, S. Sebak, O. Nalamsu, P. Ajayan and S. Prakash, *Nanotoxicology*, 2008, **2**, 184.
18 I. P. Lau, H. Chen, J. Wang, H. C. Ong, K. C. Leung, H. P. Ho and S. K. Kong, *Nanotoxicology*, 2011, DOI: 10.3109/17435390.2011.625132.
19 J. H. Lee, H. B. Lee and J. D. Andrade, *Prog. Polym. Sci.*, 1995, **20**, 1043.
20 M. Lee, J. Y. Chan, S. Kong, B. Yu, V. E. Ooi, H. N. Wong, T. C. Mak and K. Fung, *Cancer Biol. Ther.*, 2005, **4**, 1248.
21 H. M. Mansour, Y. Rhee and X. Wu, *Int. J. Nanomed.*, 2009, **4**, 299.
22 J. Meng, M. Yang, F. Jia, Z. Xu, H. Kong and H. Xu, *Nanotoxicology*, 2011, **5**, 583.
23 T. Niidome, M. Yamagata, Y. Okamoto, Y. Akiyama, H. Takahashi, T. Kawano, Y. Katayama and Y. Niidome, *J. Controlled Release*, 2006, **114**, 343.
24 A. A. Norma, E. S. Aydil and J. N. Israelachvili, *J. Biomed. Mater. Res.*, 2000, **51**, 343.
25 M. Ogris, G. Walker, T. Blessing, R. Kircheis, M. Wolschek and E. Wagner, *J. Controlled Release*, 2001, **72**, 65.
26 D. Pissuwan, S. M. Valenzuela and M. B. Cortie, *Biotechnol. Genet. Eng. Rev.*, 2008, **25**, 93.
27 M. E. Samberg, P. E. Orndorff and N. A. Monterio-Riviere, *Nanotoxicology*, 2011, **5**, 244.
28 L. B. Schwartz, K. F. Austen and S. I. Wasserman, *J. Immunol.*, 1979, **123**, 1445.
29 S. Shahangian, K. O. Ash and D. E. Rollins, *J. Anal. Toxicol.*, 1984, **8**, 273.
30 H. Takahashi, T. Niidome, T. Kawano, S. Yamada and Y. Niidome, *J. Nanopart. Res.*, 2008, **10**, 221.
31 M. Tarantola, A. Pietuch, D. Schneider, J. Rother, E. Sunnick and C. Rosman, *Nanotoxicology*, 2011, **5**, 254.