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Small and dangerous? Potential toxicity mechanisms of common exposure particles and nanoparticles.

Authors: Rachel E. Hewitt[†], Helen F. Chappell[‡] and Jonathan J. Powell[†]

[†]Biomineral Research Group, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK.

[‡]School of Food Science and Nutrition, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, UK

Corresponding author: Jonathan Powell. jjp37@cam.ac.uk

Short title: *Toxicity mechanisms of common exposure particles*

Abstract

We are continuously exposed to large numbers of non-biological, persistent particulates through dermal, oral and inhalation routes. At sizes perfect for cell interactions, such modern particle exposures are derived from human engineering either purposefully (e.g. additives/excipients) or inadvertently (e.g. pollution). Whether oral or dermal exposure to common particles has significantly adverse effects is not yet known. However, relationships between increased morbidity or mortality and airborne particle exposure are well established. Large nanoparticles and microparticles adsorb environmental molecules, including antigens and allergens, and deliver them to cells potentially with an adjuvant effect. Smaller nanoparticles may have enhanced redox activity due to increased surface areas or band gap effects. Under some circumstances, ultrasmall nanoparticles can ligate cellular receptors or interact with other cell machinery and drive distinct cell signalling. These, as well as the potential for inflammasome activation, are discussed as feasible pathways to understanding or de-bunking particle toxicity.

Keywords: Environment, Nanoparticles, Microparticles, Toxicity, Exposure, Mechanisms

Introduction

It is well established that, even away from occupational hazards, the human population is still consistently exposed to bio-persistent and absorbable particles via the lungs, skin, and oral route [1,2,3]. In the UK, for example, around 10^{13} micro- and nano- particulate food additives and excipients, which are non-digestible, are ingested per adult per day [4]. Many modern sun screens are formulated with notably high concentrations of titanium dioxide and zinc oxide particles, resulting in sizable daily dermal exposures of up to 150 mg/kg body weight for adults due to repeated application of sunscreens, with even higher estimated per-Kg exposure levels for infants [5]. Definitive negative impacts on population health have not yet been shown for dermal or intestinal exposure to such particles. In contrast, for inhalation, it is now very clear that significant exposure to airborne particulate matter is associated with significantly enhanced morbidity and mortality, especially in terms of cardiovascular and lung disease [1,6]. The questions now are why are these exposures impacting population health (what mechanisms) and what is the true population risk from persistent particle exposure through all routes? Here we consider the 'why?'. Known particles and fibres with marked and specific toxicity profiles (such as asbestos, erionite, α-quartz, carbon nanotubes etc.) have been reviewed elsewhere [7,8,9,10]. Instead we consider the general properties that *could* make common exposure particles cell active.

What is a (nano) particle?

A fashion has emerged for labelling particles as 'nano' regardless of their size, presumably fuelled by the notion that this carries greater impact. The field is further confused through the rather arbitrary and loose definition of nano-dimensionality (typically stated as one dimension of the material being < 100 nm). Whether a 100 nm cut-off makes sense, is applicable to nanostructured or only dispersed materials, what fraction needs to have one

dimension < 100 nm and what constitutes a ‘particle’, are some of the debated issues, generally with much heat but little light. It is, however, worth reminding ourselves of why size might matter when it comes to particles, for which there are five potential avenues of exploration, visually summarized in figure 1 and discussed here in further detail.

1. The Band Gap

The initial driver for the ‘nano categorisation’ is the fact that as a material gets smaller and smaller, as a single unit, its inherent properties may change. Notably, smaller particles can have an increased band gap in their electronic configuration. For example, a semi-conducting bulk material may not be a conductor at all as a nanoparticle because the energy required to promote an electron to the conduction band has become too large. In a bulk solid the density of states is determined from an overlapping combination of the electronic configurations of all interacting atoms but, in a nanoparticle, the number of atoms is so small that the energy levels become more discrete, meaning greater jumps between energy states and, potentially, an opening up of the electronic band gap. This can impact any property determined by the electronic configuration of the particle, including conductivity and absorption spectra in physical measures, and alterations to cellular redox capacity in the intracellular environment [11]. As such, where there is overlap of the nanoparticle conduction band with the redox potential range of innate cellular reactions (estimated to be between -4.12 eV and -4.84 eV [12]), there is potential for electron transfer between the cellular material and the nanoparticle surface. This then has the capacity to initiate a chain of uncontrolled and undesirable oxidation-reduction reactions within the cell. Burello and Worth [11] have shown that in oxide nanoparticles in the range of 20-30 nm, this overlap of the conduction band with the cell’s own redox potential brings many more compounds into the potentially toxic range than would be expected from an analysis of bulk material properties [11].

2. Surface Area

It is also worth recalling that the relationship between diameter and particle number (volume) is cubic, such that one million 10 nm diameter spherical particles are mass equivalent to a single one micron diameter particle of the same material. However, the surface area of those one million particles is 100 times larger than the single particle, thereby vastly increasing the size of the reactive surface.

Collectively for (1) and (2), enhanced reactivity caused by small size (in contrast to the same mass of corresponding larger particles) *may*, within the cell, manifest in inflammasome activation and in 'nuisance dust' properties driven by generation of reactive oxygen species. The latter has been well discussed elsewhere [13] but the former merits further scrutiny. Caution must be exercised in attributing inflammatory properties to particles via inflammasome-activation, in spite of the trend for doing so. For example, *in vivo,* it is rare for cells to gorge on exogenously-derived particles as, usually, they migrate following uptake and freshly recruited cells continue in their place. In cell culture this is not possible and per cell particle dosing is often enormous. Abnormal gorging leads to lysosome rupture, itself a trigger for the inflammasome, and potentially to cell death with related pro-inflammatory signalling. Cell culture techniques may also, inadvertently, activate cells in a way that does not mimic the *in vivo* situation. These artefact concerns have been well described by Pele *et al* [14].

Notwithstanding, there are clear examples of where particles within the nano range (and larger), *can* activate the inflammasome - whether that leads to inflammatory outcomes is another matter. The inflammasome is an innate immune reaction that may occur in response to cellular damage and stress. It is an intracellular platform of sensor molecules and the CARD-containing apoptosis-associated speck-like protein (ASC) [15]. When brought

together through a danger molecule trigger, the enzyme caspase 1 is activated. If there has been a prior or concomitant 'priming signal' to drive the transcription of intracellular pro-IL-1β and/or pro-IL-18, then these pro-molecules will be cleaved by caspase 1 to allow secretion of the active cytokines: i.e. mature IL-1β and IL-18. In simplified terms these cytokines are pro-inflammatory: they promote the secretion of additional cytokines and, with those, are capable of inducing T helper type 1 (Th1) T cell differentiation and Th17 T helper cell responses [16]. Specifically, it is the NLRP3 inflammasome that may be activated by (nano) particles of diverse chemical composition [17,18] and most commonly so in professional phagocytes. The particle mechanism could be direct, or indirect through lysosomal rupture but, for a pro-inflammatory outcome, the separate priming signal is still required. This signal would activate the transcription factor NF-κB to transcribe pro–IL-1β and pro–IL-18, as well as NLRP3 itself. Typically it is cell exposure to microbial fragments that engage TLR or NOD receptors that enables this priming [14]. How a cell could be exposed *concomitantly* to particles and microbial fragments is most easily proposed by the 'corona' concept whereby a particle surface may be loaded with organic molecules that have strongly adsorbed from the environment prior to cell uptake. The gut lumen, for example, is an environment that is awash with microbial fragments from the endogenous microbiome. Whether such 'dual exposure' *actually* happens, and what the consequences are, for the regular population, has not yet been shown although it has been proven in principle in an animal model of colitis with oral exposure to nano-sized titanium dioxide [19].

Finally, *in extremis,* with (nano)particle uptake, cell death may ensue and has long been intricately linked to caspase-1/inflammasome activation [20], as this may promote pyroptotic death. Recently, however, Ken Rock and colleagues showed that particle-induced (*sic*) cell death occurred independently of NLRP3/caspase-1 and was non-pyroptotic. In fact, it depended upon activation of multiple intracellular cathepsins. Moreover, through this

mechanism, particle-induced cell death may, itself, release danger signals and even pro-IL-1β [21]. So this is another cautionary example of the potential for artefact when trying to relate *in vitro* pro-inflammatory measures to particle exposures of relevance. Occupational exposure to overtly toxic particles (e.g. α-quartz), or endogenous exposure to large numbers of localized ectopic particles (e.g urate crystals), may break the threshold required for this to occur. For the regular population, however, any 'exogenous (nano)particle -inflammasome case' would need to be built around frequent exposure, known uptake, targeting of specific sites/cell types, cell accumulation (bio-persistence) and effector demonstration of the inflammasome (e.g. locally elevated IL-1β). One test case has been pigment cell formation in the intestine [22], due to continued large scale population exposure to inorganic microparticles and nanoparticles (food and excipient grade silicates, including aluminosilicates, and titanium dioxide) [4]. The findings, in humans, satisfy many of the criteria for risk concern but effector function has not been demonstrated as these cells appear to be of very low immunological and metabolic activity [23], presumably to protect the host against the potential sequelea we have described following particle loading of cells. It is also likely that the known adverse effects of ambient airborne particle exposure is mostly related to 'nuisance dust' activity [13], and/or the adjuvanticity of particles as discussed in section (5), rather than specific inflammasome effects. Certainly it was reported recently that inhibition of caspase-1 did not prevent particulate matter-induced lung immunosuppression in a murine model [24].

In conclusion of this section, regular particle exposure to the population is deleterious at least for air-borne particulates. The effects are chronic and are likely related to particles' nuisance dust (redox) and/or adjuvant properties (see section (5)): which of these two mechanisms is dominant for any given exposure situation should relate to the precise conditions because nuisance dust properties are driven by small nano-domain sizes whilst adjuvant effects are

characteristic of larger particles (figure 1). The trend for focusing on ‘nano’ as the sole offender deserves further scrutiny in terms of real world human exposures. Inflammasome activity, cell death and pro-inflammatory signaling, which, for example, is observed for the overtly toxic particle, α-quartz, is less likely as a ‘real life’ mechanism of action for common exposure ambient particles. How this extends or differs for effects of common particles following dermal or oral exposure requires further work.

3. Differential cellular processing

One hundred nanometres is, approximately, the diameter limit of the cellular vesicles involved in endocytosis (either caveolin-mediated, or non–clatherin, non-caveolin-mediated), and receptor-mediated (also known as clatherin-mediated) endocytosis [25]. Above this size specialist phagocytic processes, notably phagocytosis or micropinocytosis, are required to engulf larger materials. In other words, small particles and large particles will experience different cell uptake and processing. Moreover the latter will have restricted access to specialist cells only (the major phagocytes, including monocytes, macrophages, dendritic cells and neutrophils), but the former access broader cell types (notably epithelial cells). Once in the cell smaller particles may also access areas not available to the larger particles. For example, with correct surface characteristics, and bearing a surface bound nuclear import carrier molecule (such as importin), particles < 40 nm appear, remarkably, to be able to access the nucleus via nuclear pore complexes [26]. Precisely how different cell types process common exposure particles, of varying size and surface characteristics, and how the host responds, requires further study. The approximate 100 nm size switch, in terms of different cell uptake mechanisms, helps to provide some rationale for the common definition that differentiates ‘nano’ from ‘micro’.

4. Ultrasmall Nanoparticles and Biomolecule Mimicry

Our group, and others, are starting to demonstrate how very small non-biological particles, that are in the size range of biological macromolecules and molecular complexes of cells (typically $\leq$ 10 nm), can interlope cell machinery. Particles of this size are generally now referred to as 'ultrasmall nanoparticles'. Size and surface charge compatibility, coupled with the inherent entropic favourability that particles have for surface interactions, mean that some ultrasmall nanoparticles will bind effectively to certain cell structures and trigger signalling or activation in a way that biology intended for itself. A recent example of this is ultrasmall nanosilica particles, residing within a very specific size range which allows them to bind directly to T Cell receptor (TCR) complexes. The particle binding most likely occurs at the CD3 flanking regions and triggers the 'signal 1' signalling cascade for T cell activation [27,28]. Further examples are likely to emerge for the ligation of additional receptors by ultrasmall nanoparticles. Current knowledge of inadvertent exposure to non-biological particles in this size range is very limited and significant further work is required.

5. Particles as Cell Adjuvants

For particles that are large enough to adsorb functional levels of immuno-active biomolecules from the environment, their most feasible cellular impact is not through the generation of reactive oxygen species but through adjuvant activity (Figure 1). The potential for particle adsorption of bacterial fragments (NLR/TLR ligands) has been described in section (2), and implications for innate immune responses, described previously [3,29]. Airborne particulates, interacting with, and acting in synergy with, biological airborne allergens is also likely [30] and proof of principal for this has long been demonstrated in animal models [e.g. 31]. However, neither mechanisms nor particle sizes that drive such enhanced humoral responses associated with micron sized particles are well worked out [30, 31, 32]. Better studied are

particle effects on T cell immunity. This manifests as a change in and/or enhancement of antigen presentation, by the recipient phagocyte, for the particle-carried antigen versus native (soluble) antigen alone. Class switching, from MHC(II) to MHC (I), is typical with macrophage acquisition of particulate antigen and, in turn, the resulting T cell response not only focuses to the $CD8^+$ population, as opposed to the more usual $CD4^+$ for exogenous antigen, but the response (T cell proliferation) is greatly amplified. The classical description of this was, again, initially reported by Ken Rock's group back in 1993 [33], and microparticles were particularly effective, although Song and Harding indicated that adjuvanticity was retained for particles down to 50 nm [34]. However, these studies pre-dated routine sizing of particle distributions, or measurements of their dispersion in cell culture medium, and how particle size relates to precise antigen adjuvant effects remains a point of discussion [35]. In addition, the extent to which gorging ('phagocyte indigestion') of particle-antigen constructs contributes to such dramatic effects *in vitro, versus* real-life cell exposure to particles, also deserves renewed attention [36]. Certainly, the adjuvant properties of particles have long been shown and investigated for commercial exploitation *in vivo* by vaccine development scientists [37]: Environment driven conjugation (corona formation) of environmental antigens to particle surfaces is most likely, especially given that ingested or inhaled particles bathe in gut *succus entericus* or lung lining fluid, respectively, which are rich soups of antigenic material. As protein antigen and particle preparations have become more refined and less prone to contamination with bacterial ligands, it has become apparent that the adjuvant effect on T cell immunity is further enhanced when particles deliver both antigen *and* a biological adjuvant [38]. Again, such a triple-complex could well be mimicked through environmental interactions before cell uptake, especially in the gut [3,29].

So, how do particles influence adaptive immune responses by causing alterations in antigen presentation pathways? Antigen presentation of material originating from within cells (i.e

resident within the cell cytoplasm) occurs within the context of the MHC Class I pathway, expressed by all cell types. Presentation of exogenous antigen on the other hand (i.e. material phagocytosed by macrophages or other professional antigen presenting cells) uses the MHC Class II pathway, normally expressed only by professional antigen presenting cells. These presentation pathways dictate the type of response mounted by the immune system upon antigen recognition. Responses to 'altered self'- cancerous or virally infected cells for example - rely upon antigen presented on the MHC Class I pathway and result in $CD8^+$ (cytotoxic) T lymphocyte cellular immune responses, as opposed to $CD4^+$ ('helper') T cells that engage MHC Class II. It has been found that phagocytosed particles with any antigenic motifs that they carry can either rupture or otherwise escape endosome and lysosomal structures within cells and enter the cell cytosol. This enhances the processing and presentation of any antigen that they bare for MHC Class I presentation. This attribute, first described as cross presentation, is being exploited for cancer treatment and vaccine development [39,40,41]. On the other hand, uncontrolled, inadvertent promotion of $CD8^+$ T cell responses to environmental antigens because of particle intervention would, in the normal population, fuel unwanted T cell responses.

In summary to this section, it has long been demonstrated that otherwise innocuous particles in a large range of sizes (probably > 50 nm diameter to micron sized particles) can trap and deliver antigen [42,43]. The ability of particles to act as antigen depots together with the phagocyte's natural nepotism for particles of various sizes (especially those greater > 100 nm in diameter), coupled with the potential for MHC pathway switch and T cell amplification responses, makes (non-nano) particles plausible potent adjuvants. Real life evidence is now required.

Conclusion

Whilst particle size certainly can influence particle behaviour in biological systems, attempts to capture this complexity with basic definitions of dimensionality, as some regulation seeks to achieve, is fraught with issues. Nonetheless, the nano-obsession has at least shone a light on the importance that physical form has in defining a material's properties in addition to the much more frequently considered variable of chemical structure. How bio-clinical scientists build on the above knowledge to probe, intelligently, systems of relevance is key to understanding common particle-cell interactions in humans and animals. Real life environments (which include realistic particle-modifying factors), gene polymorphisms within susceptible populations and physiological exposures should all be considered in experimental studies going forward.

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Conflict of Interests statement

The authors Rachel E. Hewitt, Helen F. Chappell and Jonathan J. Powell declare they have no competing interests. JJP is a guest editor of this series but he has had no role or input into the editorial handling or independent review of this manuscript.

Figure Legend

Figure 1. Particle Size Influences Cellular Interactions and Activity.

As an approximate rule, particles under 100 nm in diameter are taken up by cells through various endocytotic mechanisms unlike larger particles which are taken up by professional phagocytic mechanisms. Ultrasmall nanoparticles have the capacity to interact with cellular

receptors and other bio-molecular machinery. Slightly larger, but still small nanoparticles tend to exhibit greater redox activity than their larger counterparts not only due to more surface area on a per mass basis but through band gap effects. In contrast, the larger particles are adept at carrying on their surfaces, and delivering to cells, antigenic or other bio-active molecules and, under these circumstances, the particle may also act an adjuvant in cell responsiveness to its surface cargo.

References

1. Künzli N, Kaiser R, Medina S, Studnicka M, Chanel O, Filliger P, Herry M, Horak F Jr, Puybonnieux-Texier V, Quénel P, Schneider J, Seethaler R, Vergnaud JC, Sommer H. *Public-health impact of outdoor and traffic-related air pollution: a European assessment.* Lancet. 2000 Sep 2;356(9232):795-801.
2. Cuddy MF, Poda AR, Moser RD, Weiss CA, Cairns C, Steevens JA. *A weight-of-evidence approach to identify nanomaterials in consumer products: a case study of nanoparticles in commercial sunscreens.* J Expo Sci Environ Epidemiol. 2016 Jan-Feb;26(1):26-34. doi: 10.1038/jes.2015.51.
3. Powell JJ, Faria N, Thomas-McKay E, Pele LC. *Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract.* J Autoimmun. 2010 May;34(3):J226-33. doi: 10.1016/j.jaut.2009.11.006.
4. Lomer MC, Hutchinson C, Volkert S, Greenfield SM, Catterall A, Thompson RP, Powell JJ. *Dietary sources of inorganic microparticles and their intake in healthy subjects and patients with Crohn's disease.* Br J Nutr. 2004 Dec;92(6):947-55. doi: 10.1079/BJN20041276.
5. Larsen, P. B., F. Christensen, K. A. Jensen, A. Brinch & S. H. Mikkelsen. *Exposure assessment of nanomaterials in consumer products.* Danish Environmental Protection

Agency Environmental Project 1636 (2015). Accessed online 05/11/2019 https://www2.mst.dk/Udgiv/publications/2015/01/978-87-93283-57-2.pdf

6. Steiner S, Bisig C, Petri-Fink A, Rothen-Rutishauser B. *Diesel exhaust: current knowledge of adverse effects and underlying cellular mechanisms.* Arch Toxicol. 2016 Jul;90(7):1541-53. doi: 10.1007/s00204-016-1736-5.
7. Kawasaki H. *A mechanistic review of silica-induced inhalation toxicity.* Inhal Toxicol. 2015;27(8):363-77. doi: 10.3109/08958378.2015.
8. Mossman BT. *Mechanistic in vitro studies: What they have told us about carcinogenic properties of elongated mineral particles (EMPs).* Toxicol Appl Pharmacol. 2018 Dec 15;361:62-67. doi: 10.1016/j.taap.2018.07.018.
9. Donaldson K, Poland CA, Murphy FA, MacFarlane M, Chernova T, Schinwald A. *Pulmonary toxicity of carbon nanotubes and asbestos - similarities and differences.* Adv Drug Deliv Rev. 2013 Dec;65(15):2078-86. doi: 10.1016/j.addr.2013.07.014.
10. Harik VM. *Geometry of carbon nanotubes and mechanisms of phagocytosis and toxic effects.* Toxicol Lett. 2017 May 5;273:69-85. doi: 10.1016/j.toxlet.2017.03.016.
11. Burello E, Worth AP. *A theoretical framework for predicting the oxidative stress potential of oxide nanoparticles.* Nanotoxicology. 2011 Jun;5(2):228-35. doi: 10.3109/17435390.2010.502980.
12. Auffan M, Rose J, Wiesner MR, Bottero JY. *Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro.* Environ Pollut. 2009 Apr;157(4):1127-33. doi: 10.1016/j.envpol.2008.10.002.
13. Nel A, Xia T, Mädler L, Li N. *Toxic potential of materials at the nanolevel.* Science. 2006 Feb 3;311(5761):622-7. doi: 10.1126/science.1114397
14. Pele L, Haas CT, Hewitt R, Faria N, Brown A, Powell J. *Artefactual nanoparticle activation of the inflammasome platform: in vitro evidence with a nano-formed*

calcium phosphate. Nanomedicine (Lond). 2015 May;10(9):1379-90. doi: 10.2217/nnm.14.58.

15. Latz E, Xiao TS, Stutz A. *Activation and regulation of the inflammasomes.* Nat Rev Immunol. 2013 Jun;13(6):397-411. doi: 10.1038/nri3452.
16. Dinarello CA. *Overview of the IL-1 family in innate inflammation and acquired immunity*. Immunol Rev. 2018 Jan;281(1):8-27. doi: 10.1111/imr.12621.
17. Schroder K, Tschopp J. *The inflammasomes*. Cell. 2010 Mar 19;140(6):821-32. doi: 10.1016/j.cell.2010.01.040. Review.
18. Sun B, Wang X, Ji Z, Li R, Xia T. *NLRP3 inflammasome activation induced by engineered nanomaterials.* Small. 2013 May 27;9(9-10):1595-607. doi: 10.1002/smll.201201962
19. Ruiz PA, Morón B, Becker HM, Lang S, Atrott K, Spalinger MR, Scharl M, Wojtal KA, Fischbeck-Terhalle A, Frey-Wagner I, Hausmann M, Kraemer T, Rogler G. *Titanium dioxide nanoparticles exacerbate DSS-induced colitis: role of the NLRP3 inflammasome.* Gut. 2017 Jul;66(7):1216-1224. doi: 10.1136/gutjnl-2015-310297. ***In this work, although nano-TiO_2 rather than pigment grade was used for the murine model, the point is made that particles, in numbers, can activate the inflammasome *in vivo* and enhance inflammation in the intestine, where TLR and NLR ligands are abundant.**
20. Iyer R, Holian A. *Involvement of the ICE family of proteases in silica-induced apoptosis in human alveolar macrophages.* Am J Physiol. 1997 Oct;273(4):L760-7. doi: 10.1152/ajplung.1997.273.4.L760.
21. Orlowski GM, Sharma S, Colbert JD, Bogyo M, Robertson SA, Kataoka H, Chan FK, Rock KL. *Frontline Science: Multiple cathepsins promote inflammasome-independent, particle-induced cell death during NLRP3-dependent IL-1β activation.* J

Leukoc Biol. 2017 Jul;102(1):7-17. doi: 10.1189/jlb.3HI0316-152R. ** **Describes the mechanisms of particle-induced, sterile inflammation and cell death independent of NLRP3 inflammasome induction.**

22. Powell JJ, Ainley CC, Harvey RS, Mason IM, Kendall MD, Sankey EA, Dhillon AP, Thompson RP. *Characterisation of inorganic microparticles in pigment cells of human gut associated lymphoid tissue.* Gut. 1996 Mar;38(3):390-5.

23. Thoree V, Skepper J, Deere H, Pele LC, Thompson RP, Powell JJ. *Phenotype of exogenous microparticle-containing pigment cells of the human Peyer's patch in inflamed and normal ileum.* Inflamm Res. 2008 Aug;57(8):374-8. doi: 10.1007/s00011-007-7216-x.

24. Colarusso C, De Falco G, Terlizzi M, Roviezzo F, Cerqua I, Sirignano M, Cirino G, Aquino RP, Pinto A, D'Anna A, Sorrentino R. *The Inhibition of Caspase-1- Does Not Revert Particulate Matter (PM)-Induced Lung Immunesuppression in Mice.* Front Immunol. 2019 Jun 21;10:1329. doi: 10.3389/fimmu.2019.01329. ** **This study demonstrates PM exposure leads to an immunosuppressive environment in the lung which is caspase-1-independent, giving insight into the *in vivo* situation and mechanisms which contribute to some respiratory disorders associated with exposure to air pollution.**

25. Rauch J, Kolch W, Laurent S, Mahmoudi M. *Big signals from small particles: regulation of cell signaling pathways by nanoparticles.* Chem Rev. 2013 May 8;113(5):3391-406. doi: 10.1021/cr3002627.

26. Panté N, Kann M. *Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm.* Mol Biol Cell. 2002 Feb;13(2):425-34. doi: 10.1091/mbc.01-06-0308.

27. Vis B, Hewitt RE, Faria N, Bastos C, Chappell H, Pele L, Jugdaohsingh R, Kinrade SD, Powell JJ. *Non-Functionalized Ultrasmall Silica Nanoparticles Directly and Size-Selectively Activate T Cells.* ACS Nano. 2018 Nov 27;12(11):10843-10854. doi: 10.1021/acsnano.8b03363. **** One of the first direct examples of ultrasmall unfunctionalised nano-particles directly mimicking biological interactions by binding protein receptors.**

28. Vis B, Hewitt RE, Monie TP, Fairbairn C, Turner SD, Kinrade SD, Powell JJ. *Ultrasmall Silica Nanoparticles Directly Ligate the T Cell Receptor Complex.* PNAS In press. ***Following on from reference 27, this paper identifies the mechanism by which ultrasmall nanosilica activates T lymphocytes.**

29. Ashwood P, Thompson RP, Powell JJ. *Fine particles that adsorb lipopolysaccharide via bridging calcium cations may mimic bacterial pathogenicity towards cells.* Exp Biol Med (Maywood). 2007 Jan;232(1):107-17.

30. Baldacci S, Maio S, Cerrai S, Sarno G, Baïz N, Simoni M, Annesi-Maesano I, Viegi G; *HEALS Study. Allergy and asthma: Effects of the exposure to particulate matter and biological allergens.* Respir Med. 2015 Sep;109(9):1089-104. doi: 10.1016/j.rmed.2015.05.017.

31. Steerenberg PA, Withagen CE, Dormans JA, van Dalen WJ, van Loveren H, Casee FR. *Adjuvant activity of various diesel exhaust and ambient particles in two allergic models.* J Toxicol Environ Health A. 2003 Aug 8;66(15):1421-39.

32. Gutierro I, Hernández RM, Igartua M, Gascón AR, Pedraz JL. Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. Vaccine. 2002 Nov 22;21(1-2):67-77. https://doi.org/10.1016/S0264-410X(02)00435-8

33. Kovacsovics-Bankowski M, Clark K, Benacerraf B, Rock KL. *Efficient major histocompatibility complex class I presentation of exogenous antigen upon phagocytosis by macrophages.* Proc Natl Acad Sci U S A. 1993 Jun 1;90(11):4942-6. doi: 10.1073/pnas.90.11.4942.

34. Harding CV, Song R. *Phagocytic processing of exogenous particulate antigens by macrophages for presentation by class I MHC molecules.* J Immunol. 1994 Dec 1;153(11):4925-33.

35. Mant A, Chinnery F, Elliott T, Williams AP. *The pathway of cross-presentation is influenced by the particle size of phagocytosed antigen.* Immunology. 2012 Jun;136(2):163-75. doi: 10.1111/j.1365-2567.2012.03558.x

36. Reis e Sousa C, Germain RN. *Major histocompatibility complex class I presentation of peptides derived from soluble exogenous antigen by a subset of cells engaged in phagocytosis.* J Exp Med. 1995 Sep 1;182(3):841-51. doi: https://doi.org/10.1084/jem.182.3.841.

37. Singh M, Chakrapani A, O'Hagan D. *Nanoparticles and microparticles as vaccine-delivery systems.* Expert Rev Vaccines. 2007 Oct;6(5):797-808.

38. Schlosser E, Mueller M, Fischer S, Basta S, Busch DH, Gander B, Groettrup M. *TLR ligands and antigen need to be coencapsulated into the same biodegradable microsphere for the generation of potent cytotoxic T lymphocyte responses.* Vaccine. 2008 Mar 20;26(13):1626-37. doi: 10.1016/j.vaccine.2008.01.030.

39. Shen H, Ackerman AL, Cody V, Giodini A, Hinson ER, Cresswell P, Edelson RL, Saltzman WM, Hanlon DJ. *Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles.* Immunology. 2006 Jan;117(1):78-88.

40. Hirai T, Yoshioka Y, Takahashi H, Ichihashi K, Yoshida T, Tochigi S, Nagano K, Abe Y, Kamada H, Tsunoda S, Nabeshi H, Yoshikawa T, Tsutsumi Y. *Amorphous silica nanoparticles enhance cross-presentation in murine dendritic cells*. Biochem Biophys Res Commun. 2012 Oct 26;427(3):553-6. doi: 10.1016/j.bbrc.2012.09.095.

41. Molino NM, Anderson AK, Nelson EL, Wang SW. *Biomimetic protein nanoparticles facilitate enhanced dendritic cell activation and cross-presentation.* ACS Nano. 2013 Nov 26;7(11):9743-52. doi: 10.1021/nn403085w.

42. Singh M, Kazzaz J, Ugozzoli M, Malyala P, Chesko J, O'Hagan DT. *Polylactide-co-glycolide microparticles with surface adsorbed antigens as vaccine delivery systems.* Curr Drug Deliv. 2006 Jan;3(1):115-20.

43. Pele LC, Thompson SJ, Kirkham B, Thompson RP, Powell JJ. *Peripheral blood mononuclear cell proliferative responses to soluble and particulate heat shock protein 65 in health and inflammatory bowel disease.* Inflamm Res. 2007 Apr;56(4):143-8.