Journal of Autoimmunity 34 (2010) J226–J233

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract

Jonathan J. Powell*, Nuno Faria, Emma Thomas-McKay, Laetitia C. Pele

Elsie Widdowson Laboratory, MRC-HNR, Fulbourn Road, Cambridge CB1 9NL, UK

Keywords:
Microparticles
Nanoparticles
Gastrointestinal tract

A B S T R A C T

Humans have evolved with oral exposure to dietary microparticles and nanoparticles as a normal occurrence but the ever-growing exploitation of nanotechnology is likely to increase exposure further, both qualitatively and quantitatively. Moreover, unlike the situation with respirable particles, relatively little is known about gastrointestinal intake and handling of nanoparticles. With a long term interest in gut exposure and responses to dietary microparticles, our group is now applying its expertise to nanoparticles in the gastrointestinal tract. Here we aim to address (i) the current challenges associated with the characterisation of particle–host or particle–cell interactions, (ii) the origin and mechanisms of uptake of particles in the gastrointestinal tract, especially via the Peyer's patch and (iii) potential cellular effects of nanoparticles in the generation of reactive oxygen species and inflammasome activation, or microparticles in their adjuvant activity in pro-inflammatory signalling and immune responsiveness.

1. Introduction

Nanoscience is an exciting and fast expanding field of research which aims to generate new materials and devices with wide-ranging applications (e.g. in nutrition, medicine, electronics, energy production, etc.). The Woodrow Wilson International Center for Scholars reported a near tripling in numbers of consumer products that contained nanoparticles between early March 2006 and late February 2008 [1] and, by the end of August 2009, more than one thousand commercial nanomaterial-containing products had been identified [2]. This growth is mirrored by commercial value in the sector which was estimated at $150 billion for 2007 but predicted to rise to $3.1 trillion by 2015 [3].

Human exposure to novel nanoparticles, or, existing materials that have now been nano-engineered, is inevitable. For example, in the food sector alone, there are multiple potential applications in agriculture (e.g. nanosensors for the detection of animal and plant pathogens), food processing (e.g. nanocapsules for flavour/taste enhancement), food packaging (e.g. nanoclays and nanofilms as barrier materials to prevent spoilage and oxygen absorption) and dietary supplements (e.g. nanoencapsulation of nutrients and nutraceuticals for better absorption, stability or targeted delivery) [4]. Table 1 shows 'nanofoods' that are currently available in the USA and registered on a database from "The Project on Emerging Nanotechnologies". Other purposeful exposure may include dermal application (sun-creams, cosmetics, therapeutics, etc.) or inhalation, nasal and gastrointestinal delivery of particle-based therapeutics while inadvertent exposure also occurs through environmental and industrial means. The 'Nano Revolution' and fears that we have a tiger by the tail have captured scientific and public imagination: why?

2. The Nano Revolution

The essence of nano-engineering is that when a material is produced at a small enough scale, its physico-chemical properties differ to that of the same material with larger particle sizes [5]. An arbitrary cut off of <100 nm in diameter has often been used as a simple definition for 'nanoparticulate',[1] and, biologically, may

* Corresponding author. Tel.: +44 1223 426 356; fax: +44 1223 437 515.
E-mail address: jonathan.powell@mrc-hnr.cam.ac.uk (J.J. Powell).

0896-8411/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jaut.2009.11.006

[1] The words 'nanoparticle' or 'nanoparticulate' have been the subject of a number of definitions but, clearly, simply having a particle diameter or molecular diameter of <100 nm is not sufficient as this will invoke all soluble molecules and even water itself (~0.3 nm molecular diameter)! We propose that a dominant characteristic of the material must be its behaviour as a 'particle' in the environment of study: e.g. in biological systems, we would suggest that a nanoparticle is non-biological and able to be imaged as a physical, solid-phase structure. Further characteristics to consider may be aggregation or agglomeration versus mono-dispersity; sedimentation versus suspension and Brownian motion; surface interactions with soluble molecules, etc. In the presence of cells, the material will be subjected to some form of endocytic uptake and, as implied in the text, we have argued that the uptake mechanism may be a useful determinant of the 'micro' (active recognition, cytoskeletal re-arrangement) versus 'nano' (constitutive, fluid-phase) designation.

Table 1
Registered Nanofoods in the USA as of October 2009.

Product	Canola Active Oil®	Nanotea™	Nanoceuticals™ Slim Shake Chocolate	Maternal Water	Nanosupplements
Details	Nanomicelles with phytosterols to inhibit cholesterol absorption	Nanograde ball-milling technology for tenfold release of phytonutrients and selenium	Nanoencapsulated cocoa that offers enhanced flavour without the need for excess sugar	Nanocolloidal silver	51 different nanosupplements, e.g. nanominerals, vitamin B12 nanospray, nanoencapsulated omega-3, prebiotics, etc.
Company	Shemen Industries Ltd.	Shenzhen Become Industry & Trade Co., Ltd.	RBC Life Sciences®, Inc	La Posta del Aguila®,	Various
Manufacturing country	Israel	China	USA	Argentina	Mostly USA

Data taken from The Project on Emerging Nanotechnologies which was established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts. Source: www.nanotechproject.org/inventories/consumer (accessed on 29/09/09).

have some value (see below) but, recently, Auffan et al. [6] have argued that particles larger than ~30 nm diameter do not generally show properties that deserve regulatory scrutiny above and beyond those of their larger counterparts. Either way, the fact that nano-sized materials can adopt novel physico-chemical properties has led to a surge in activities around novel material structures and an accompanying surge in concern that these different properties may translate to different biological responses (i.e. toxicity). The issue is compounded by a couple of other factors. First, above a particle size of 100–200 nm diameter, non-biological particles appear to activate cell-membrane ruffling, cytoskeletal re-arrangement and access phagocytic cells through classical endocytic mechanisms such as phagocytosis or macropinocytosis [7]. Recently, Nel et al. [8] have, correctly, cautioned against over-interpretation of such observations. Data are limited and may vary for particle types while cell systems in particle research have often been poorly characterised with respect to particle behaviour in the cell culture medium (e.g. aggregation and agglomeration versus mono-dispersity, or, particle interactions with other components of the media). Nonetheless, it is most likely that for any given material, different particle sizes can be achieved that result in different mechanisms of cellular uptake, and, therefore, a different intracellular fate. Secondly, as particle size is altered, surface chemistry alters, leading to different surface interactions in the particle's environment. For example, it is well known that the α-quartz form of silica is pro-inflammatory *in vivo* in the lungs and *in vitro* in varying cell cultures. Primarily, it appears to activate secretion of the cytokine interleukin-1β (IL-1β) [9] because of lysosomal rupture following cellular uptake. The cytosolic NOD-like receptor, NALP3 (also termed cryopyrin), senses lysosomal damage and recruits the adapter molecule ASC such that the pro-enzyme, pro-caspase-1, can be engaged. This complex, which is one form of the inflammasome, leads to autocatalytic processing of procaspase-1, and the active caspase-1 then cleaves pro-IL-1β leading to secretion of the mature, pro-inflammatory cytokine, IL-1β. This may activate the transcription factor NFκB, leading to a cascade of pro-inflammatory signalling. In contrast, amorphous silica particles fail to induce significant pro-inflammatory signalling, presumably because they fail to induce lysosomal rupture following uptake. But, what happens when the amorphous silica is nano-sized? Effects on surface chemistry and determinants of cell–membrane interactions are complex [9] but we have often observed that a variety of nanoparticles, following cellular uptake, are located to the intra-lysosomal membrane surface (e.g. Fig. 1).

Whether this would be sufficient to perturb lysosomal structure is unclear but certainly Gerloff et al. have preliminary evidence indicating that nano-sized (~14 nm diameter) amorphous silica invokes some toxicity in epithelial cells [10]. Such considerations are important in light of nanoparticulate, amorphous silica being introduced to the USA diet [11] that the company claims to be sized at 4–6 nm diameter [12].

3. Nanoparticles in the gut: lessons from microparticles

To summarise the above section, nanoparticles may have different physico-chemical properties to their larger counterparts, have different surface properties, and access cells via different pathways. Surprisingly, however, we have been exposed to nanoparticles throughout evolution and, at least in the gut, there is even emerging evidence that we have generated strategies to utilise nanoparticles for dietary and physiological benefit (see below). Thus, nanoparticulate structures are neither inherently toxic nor inherently safe: like all molecules these decisions will rest upon molecular structure, biological environment, degree of exposure and host susceptibility. What is clear, however, is that recent advances in physics and chemistry have led to new methodologies for the synthesis and imaging of nanotechnology and, therefore, its characterisation and application. It is on this background that our group, with a long term interest in how the gut handles mineral particles (especially 'microparticles') [13–16], has become involved in applying our expertise to the gastrointestinal handling of nanoparticles. It is essential to recognise, however, that the majority of literature surrounding nanoparticles in human-relevant biological systems, concerns isolated cell systems or lung biology. There is little current literature that exists for the gastrointestinal tract and some of that is contradictory. Our aims here, therefore, are

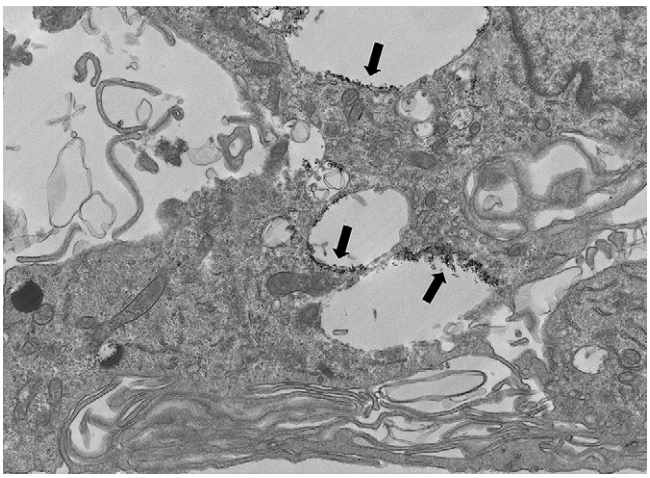

Fig. 1. Scanning Electron Micrograph of human monocyte-derived macrophages incubated with 100 μg/ml iron hydroxide nanoparticles. Black arrows indicate nanoparticle clustering at the intra-lysosomal membrane surface.

to deliver broad concepts in the field rather than to review, critique and distill the total literature on microparticles and the gut or the much lesser literature on nanoparticles and the gut.

4. Methodological considerations

Recently and rightly there has been marked emphasis on methodological issues that surround the study of particle–cell interactions (e.g. [8,17–20]) while, in considering applications to the gastrointestinal tract, there are further specific caveats that need to be noted.

Briefly, particles may readily agglomerate[2] (a relatively loosely bound collection of particles with a surface area similar to the sum of the individuals) or aggregate[2] (relatively strongly bonded or even fused particles where the surface area is reduced compared to the sum of the individuals) when changing environments. Correctly-used static (angle-diffraction) or dynamic (Brownian motion) light scattering techniques can help determine such events. Even then, many technical issues need to be considered such as sedimentation (dynamic), excessive obscuration that leads to secondary scattering (static), particle concentrations that are undetectable (both), masking of a lot of small particles by a few large particles (both, especially dynamic), incorrect refractive indices (both, especially static), sample fluorescence (both) and the presence of particle-like structures in the medium (dynamic typically). Many published data have not characterised, or have only poorly characterised, systems and, typically, this means that cell culture experiments are not studying true 'nanoparticle' uptake and effects but collections that behave as microparticles – at least until some further cell processing occurs. Whether this is then relevant to the *in vivo* situation is also not easy to elucidate: particles may coalesce in the gut lumen or remain disperse. The preparation of samples for microscopy has to be carefully considered to avoid artefacts (i.e. induction of aggregation, agglomeration, dissolution or even dispersion) that are not representative of the native environment.

A second issue concerns particle interactions with soluble molecules. Recently described for protein coating of nanoparticle surfaces, and referred to as a 'corona' [23], this phenomenon has been known for decades and will inevitably happen in the particle's native environment. In the gastrointestinal tract it is likely that the acidic pH of the stomach, which mainly is maintained even post-prandially, and the presence of gastrointestinal enzymes, will serve to denude ingested particles of their surface-adsorbed molecules but then re-adsorption of novel entities will occur in the less acidic small bowel lumen (where there are high concentrations of luminal proteins and glycoproteins). In addition to the physical reasons described by Nel et al. for such adsorptive phenomenon [8], entropy of the system is likely to be a strong driving force and even where repulsive ionic forces exist, soluble ions may act as 'sandwich filling' elements that allow two equally charged species to interact. Our group and others have shown this, for example, for the binding of bacterial lipopolysaccharide (endotoxin) to titanium dioxide particles, a phenomenon that is greatly promoted by the presence of calcium ions (Ca^{2+}) at typical gut luminal concentrations [24,25]. Complicatingly, the addition of calcium ions to certain cell culture media leads to precipitation of an evolving calcium phosphate phase and that also can become part of the titanium dioxide–LPS conjugate, significantly shifting particle size and cellular reactivity [25]. Thus, understanding the *in vivo* particle 'corona' and re-capitulating it *in vitro* is a significant challenge.

A third issue concerns the potential for particles to interfere with assays that are used to examine cellular outcomes. This may be direct interference with physical assays such as light scattering or epi-fluorescence or indirect through adsorption of the analyte such as cytokines in ELISA assays. Carefully conducted control experiments, such as incubating a cytokine-enriched media with the particles in an acellular environment, can help to control for these effects. A further point to consider is that particles used in cellular experiments may well have trace contamination of pathogen associated molecular patterns (PAMPs) such as PGPS, LPS, etc. and care must be taken to attribute effects to the particle or the particle–PAMP combination.

A fourth issue concerns particle dose. Not only is it arguable as to how best dose should be expressed (i.e. concentration, or, mass per surface area [17]) but unrealistic dosing will lead to unrealistic outcomes. Cells have numerous endogenous defences and, additionally, may well deal efficiently with particle exposure *in vivo* through a series of physical barriers and intercellular signals that are largely absent *in vitro*. Those interested in alcohol and health will know that health benefits may ensue from a drink a day [26] while toxicity will ensue from seven drinks taken on just one day of the week. Results from one situation do not inform upon the other. Ironically, many of the issues noted above may determine the concentration of particles required in the cellular system, such that the system is characterizable and particle dispersity is appropriate, but these concentrations may not represent the *in vivo* situation. By over-whelming cellular defences with an inappropriately high dose, there is little information to be gained about more chronic exposure to lower doses. Awareness of how the *in vitro* exposure maps onto potential *in vivo* exposure is, therefore, important.

Regarding the gut and gut cells there are a number of specific issues that are well known in the field and will just be mentioned here. Whole animal experiments may suffer from abnormal transit times, induced by stress during gavage for example, or abnormal perfusion times or even ileus induction (paralysis of the bowel) during perfusion experiments [27]. In both cases, it is vital to check for the adsorption (i.e. loss) of particles on either the perfusion apparatus or the gavage tube/syringe so that an accurate estimate of dosing can be made [27]. Feeding experiments are more physiological but balance experiments may be difficult in estimating exposure (accounting for non-ingested losses), excretion and whole animal retention. Where particles are radiolabelled, or of a material that is not found endogenously, then whole tissue analysis is a straightforward method that allows insight into compartmentalisation of particles at any given time [28]. However, true absorption and kinetic profiles may still be difficult to dis-entangle from such data because of the multiple 're-circulation' phenomena that are in operation at any given time. For example, particles that access mononuclear cells below the gut epithelial barrier may migrate, with the cell, to mesenteric lymph nodes and back to the intestinal mucosa. Secondly, even in metabolic cages, rats engage in coprophagia while, thirdly, intestinal cells and those of the reticuloendothelial system may be shed back into the lumen at various times carrying their cargo that has not been 'further absorbed'. Biliary clearance of hydrophobic particles is surprisingly dominant, with an efficiency that is inversely related to particle size [29] and, recently, biliary clearance has even been shown for non-hydrophobic particles [30]. Overall, for *in vivo* systems, (i) the delivery of the particles to the animal must, as with *in vitro* systems, be representative of the situation being mimicked (dose, dispersity, etc.) (ii) balance studies from the dosing equipment through to retention and

[2] The definitions of 'agglomerate' and 'aggregate' have been the source of much discussion as reviewed by Nichols et al. [21] and we will not, here, address this issue further. Instead, we have used the classification which accords to the British Standards in 2007 [22], and was recently adopted by Nel et al. [8] and Stone et al. [17] for example.

losses are vital and (iii) kinetic interpretation is complex and would require a 'multi-variate' analytical approach.

Imaging is, of course, a powerful tool in examining the fate of particles. These may be labelled (fluorescent for example) or be of a material that is easily detected by microscopy – such as reflectance for light (dark field)/laser microscopies or elementally heavy for imaging and micro-analysis with electron microscopy [31]. The issue of sampling becomes important with imaging studies. Tissue sections represent a minute fraction of the overall organ, so multiple samples are required, and also it is relatively easy for one's eye to be drawn to small areas of high particle concentration (for example, the Peyer's patches) versus large areas of low particle concentration (for example, the regular mucosa) and make assumptions that may not be borne out by more painstaking quantitative analysis. For example, and as discussed below, intestinal lymphoid aggregates have an extraordinary ability to take up nanoparticles and small microparticles but, using 60 nm polystyrene spheres and a total analysis approach, Hillery et al. [32] still showed that some 40% of particles in the small bowel were actually in non-Peyers's patch locations. Microscopy would have been hard pressed to identify this distribution. Ideally, whole or dissected organ analyses and imaging approaches are combined. Finally, it should be noted that although particles much below 0.5–1 μm diameter are not directly visible by light microscopy they are often indirectly visible, either because their reflectance/fluorescence acts to amplify their presence or because they collect in certain cellular compartments (e.g. lysosomes) where they can be observed *en masse*. The confocal microscope remains, therefore, an extremely useful tool for the initial tracking of particles in the gastrointestinal tract with both fluorescent and reflected light capabilities. Moreover, it can be used to optically section the image and confirm that particles are within the plane of the tissue [31]. Higher resolution analyses will require, mainly, electron microscopic techniques but, again, in our experience, these are most useful when used in conjunction with light microscopy imaging to develop the picture from the whole organ level through to the sub-cellular level [31].

Ex-vivo tissue cultures are effectively dieing preparations, of limited surface area, that may have excessive mucus production and long term become hyper-permeable and are not recommended for particle uptake studies.

There is an evolving interest in studying particle interactions with cells in culture – especially in cell lines such as the colonic carcinoma cell line, CaCo-2 [33–36], which has differentiated features consistent with small intestinal enterocytes. While many of the caveats and considerations that are required in such work have been outlined in preceding sections of this article, or are well known for cell lines in general, two further points should be noted about gut cells. First, although the enterocyte (gut epithelial cell) is at the interface of the gut lumen and gut tissue, it is quite clear that underlying mononuclear cells are also exposed to dietary particles and appear to be the preferential targets for microparticles and, probably, the larger nanoparticles (see below and [14,15]). Thus there should be considerable interest in how these respond to particle exposure as well as the epithelial cells. Secondly, and most importantly, gut cells are hypo-responsive to many stimuli [37] and it must NOT be assumed that results observed with peripheral or other non-gut cells will be re-capitulated with their gastrointestinal-derived counterparts. This is especially true for responses via the innate immune system and is a common mistake in gastrointestinal science *per se* where signalling effects of gut-dominant molecules, such as bacterial LPS or moieties of PGPS, are often inferred from results on non-gut cells but are seldom shown to be translatable to the gastrointestinal tract. We first showed this in the Journal of Autoimmunity [24] for particulate structures with the titanium dioxide–LPS–calcium phosphate conjugate described above. Using explanted gut biopsies, we identified IL-1β signalling to be upregulated just a few fold compared to several orders of magnitude with peripheral blood cells. Because of the concerns over tissue explants, as noted above, we compared gut cells to peripheral cells directly in subsequent work and confirmed this hyporesponsiveness of the former versus the latter [16,25].

In summary, therefore, interpretation of the effects of nano- or micro-particulate structures of the gut requires careful consideration of particle behaviour and gut physiology to reflect, accurately, *in vivo* responses.

5. Gastrointestinal particles

It is likely that the gastrointestinal tract has been exposed to nano- and micro-particles throughout evolution. For example, ferritin is a naturally-occurring nanoparticulate structure (circa 12 nm diameter with the protein shell) and containing an iron oxide core of 6–8 nm diameter that, in itself, is made up of 2–3 nm subunits [38]. This is widely ingested in both meat and plants-based foods and appears only partially digested in the gastric environment. Several groups have now described that nanoparticulate ferritin may be endocytosed and utilised by gut epithelial cells as a source of dietary iron [39]. Thus not only *can* nanoparticulate uptake occur in the gut but there may be some *beneficial* roles in doing so (i.e. nutrient capture). Whether this extends to microparticles is not clear but, certainly, inadvertent exposure through dust and contaminants of the diet is inevitable and, being mainly solid-phase silicates, such particles will not be degraded in the gastric environment and will be exposed to the intestinal epithelium. Again there are potential pathways for uptake (see below) but, first, it is worth considering the major sources of microparticle exposure to the gut in the Western world.

5.1. Sources

Typically, the gut is exposed to two types of inorganic microparticles which we have classified as 'exogenous' and 'endogenous', depending upon their origin. In this section, we will just briefly describe the common sources of exogenous particles and the proposed process of endogenous particle formation, as these have been previously reviewed by our group [13,40,41].

Exogenous inorganic particles are man-made particles comprising titanium dioxide or silicates/aluminosilicates. Titanium dioxide (designated E171 in Europe) is used for whitening and brightening foods, especially for confectionary, white sauces and dressings, and certain powdered foods [40,42]. It is also used in the pharmaceutical industry as an opacity agent. Titanium dioxide is typically found in gut tissue in the anatase polymorphic form and is a 100–200 nm diameter spherical particle that is resistant to gastrointestinal degradation [40]. Particulate silicates and aluminosilicates (E554, E556 and E559 in Europe) are used in the food industry as anti-caking agents and to allow the flow of powders, and some are present in cheeses, sugars and powdered milks [40]. In the UK, the major five food sources of particulate silicates are salt, drinking powders, chewing gum, instant pot savoury snacks and icing sugar. Pharmaceuticals or nutraceuticals and toothpaste are also major sources of particulate silicate and aluminosilicate intake [40]. Overall, intake of dietary inorganic microparticles in the UK has been estimated to be about 40 mg/person/day (~35 mg for the silicates and ~5 mg for titanium dioxide) which equates to a staggering daily exposure of 10^{12-14} particles/person.

Unlike their exogenous counterparts, endogenous inorganic microparticles are naturally occurring particles of the gut lumen that form *de novo*. Dietary calcium and phosphorous are solubilised in the gastric environment and then absorbed in the small

intestine. What is less well known, is that these ions are also partly re-secreted in the mid-distal aspect of the small intestine, which is generally termed the 'endogenous losses', where they to co-precipitate to form calcium phosphate microparticles. Their physiological relevance, if any, is still unknown but the subject of active research in our group. Thus, quite clearly, there is marked exposure of the gastrointestinal tract to nano- and micro-particles of non-biological form and next we consider how these can cross the intestinal barrier.

5.2. Translocation in the gastrointestinal tract

Following ingestion, translocation of particles into and across the gastrointestinal mucosa can occur via four different pathways as summarised in Fig. 2. Although the main function of the regular epithelial cells (enterocytes) is to absorb and transport nutrients for systemic dissemination and optimal cell function, there are some data showing that they can also take up material in the nano-range via endocytosis [39], as noted above for ferritin uptake. However, the most documented and common route of uptake for both nano- and micro-particles is via the M-cell rich layer of Peyer's Patches (small intestinal lymphoid aggregates). *In vitro*, des Rieux et al. reported about a thousand fold increase in the transport of particles (200 nm and 500 nm diameter) when a gut epithelial cell line was co-cultured with cells that had been differentiated to achieve M-cell like features [43]. M-cells are specialised, differentiated epithelial cells, that have an avid appetite for the transcytosis of macromolecules and particles [44–48] and are able to pass intact material from the lumen to abutting/interlocking mononuclear cells. These cells are at anatomical locations that are believed to be important immune-inductive sites and to represent a constitutive mechanism for the continued surveillance of luminal antigens and pathogens. A third possible route for translocation of particles, which has been widely described by Volkheimer, is persorption. Volkheimer described how enterocytes shed from the villous tip and into the gut lumen, which leaves a 'hole' in the epithelium, and allows the translocation of even large particles such as starch and pollen [49–51]. Since then, one study has demonstrated this phenomenon to be also true for nanoparticles (colloidal gold nanoparticles) [52]. However, its relevance on quantitative grounds remains unclear as it must be very inefficient compared to the active uptake of particles by M-cells. Jepson et al. for example, indicated that one lymphoid follicle dome of the rabbit Peyer's patch could transport $\sim 10^5$ microparticles (460 nm diameter) in 45 min [53] and, for smaller particles (perhaps < 250 nm diameter), this would be considerably more efficient. In contrast, according to Hussain et al. [54] Freedman calculated that, in humans, 100 g of ingested starch would lead to a total systemic exposure of 12,000 starch particles [55]. Hence, persorption is unlikely to be highly efficient although comparison still needs to be made with like-for-like particle sizes.

Finally, it is possible that under certain conditions, very small nanoparticles can gain access to the gastrointestinal tissue via paracellular transcytosis across tight junctions of the epithelial cell layer. This remains a theoretical route as tight junctions are remarkably efficient at preventing paracellular permeation although their integrity can be affected by disease and by epithelial cell metabolism (e.g. glucose), calcium chelators (e.g. citrate) [56] and even particle endocytosis [34].

Two key questions concern the determinants of particle uptake and the efficiency of particle uptake. Unfortunately these are not easily addressed because of marked differences in methodological approaches and particle types as well as variable characterisation of the experimental models reported in the literature. However, some general rules can be proposed. First, the surface chemistry of the particle will affect efficiency of uptake. des Rieux et al. showed in their *in vitro* cell culture system, which mimicked the Peyer's patch epithelium, that sub-micron sized aminated particles were much more efficiently taken up than carboxylated particles [43]. Similarly, hydrophobic particles appear to be much better taken up than hydrophilic particles as reviewed by Hussain et al. and such effects may relate to transport through the mucus layer [54]. Hussain et al. also showed that by coating 500 nm particles with tomato lectin they could not only switch the dominant site of absorption away from the lymphoid aggregates and towards the villous tissue (ratio 1:15), but they could also greatly enhance uptake, to 23% of the gavaged dose, compared to $<0.5\%$ when lectin binding was blocked [57]. This issue of overall particle uptake in the gastrointestinal tract is rarely addressed and not easily quantified for the reasons outlined earlier. Retention of particles can, however, be measured, and the extensive studies from Sandy (AT) Florence's group in London have shown that, generally, small particles are better taken up than large ones with, perhaps, an optimal size of around 50 nm diameter [58]. From the same group, Hillery et al. showed that about 10% of the administered dose was recovered from the total gastrointestinal tract using 60 nm polystyrene particles and that a much greater percentage was taken up by lymphoid tissue compared to non-lymphoid tissue, with 60% of uptake in the small

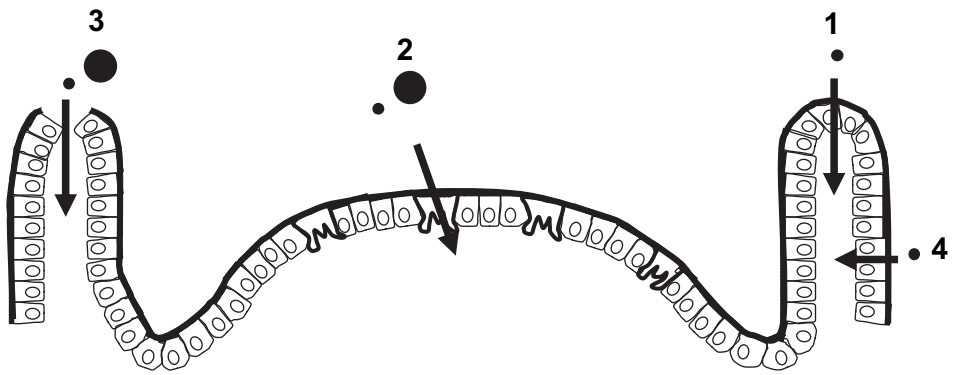

Fig. 2. Summary of particles translocation across the gastrointestinal tract 1. Endocytosis through 'regular' epithelial cells. Very small particles tentatively generally <50–100 nm in diameter. 2. M-cell-uptake (transcytosis) at the surface of intestinal lymphoid aggregates. This is the quintessential pathway for gut particle uptake and is very well described, especially for large nanoparticles (20–100 nm) and small microparticles (100–500 nm). 3. Persorption. Volkheimer's concept of passage through 'gaps' at the villous tip following loss of enterocyte(s) to the gut lumen. Small and large nanoparticles potentially access this route, but, quantitatively, it is unlikely to be efficient. 4. Putative paracellular uptake. Generally junctional complexes are unlikely to allow even the smallest of nanoparticles to permeate but certain drugs and/or dietary situations, and especially diseases, may alter this situation allowing influx of very small nanoparticles.

intestine occurring via Peyer's patches [32]. In contrast, the extensive studies from Kate (KE) Carr's group in Belfast, and now Oxford, have shown using 2 μm diameter latex particles that only 0.1–0.3% of the administered dose is taken up and almost exclusively, this is via non-lymphoid tissue (i.e. the villous route) [34,59,60]. Together such findings concur with cellular models showing that there is a marked fall off in the efficiency of M-cell-uptake of particles at somewhere between 200 and 500 nm diameter [43]. Interestingly, using 2.5 nm diameter dendrimers, Florence et al. have suggested that uptake was less than for larger polystyrene particles (≥50 nm diameter) and while surface characteristics clearly differed for the different particles, as well as size, Florence indicated that there may well be an optimum size for gut uptake. As noted above, tentatively this is around 50 nm diameter, with perhaps a range of 20–250 nm diameter, and chiefly occurring through the follicle-associated epithelium, but further studies are needed.

5.3. Immune responses

Overall, there are very few data on particle uptake and immunological or cellular responsiveness of the gastrointestinal tract. As a general rule in non-gut systems, nanoparticles appear to enhance the cellular generation of reactive oxygen species (ROS) and exert their, currently understood, 'toxic' effects through this pathway [61]. Whether there are realistic situations of nanoparticle exposure that lead to significantly abnormal ROS responses *in vivo* in the gut remains to be established. Additionally, as noted above, it will be of interest, both *in vitro* and *in vivo*, to see if certain nanostructures can trigger the inflammasome, and thus IL-1β and IL-18 secretion, in the gut. In contrast, for larger particles (>100 nm diameter) their likely cellular impact is not through ROS generation but through adjuvant activity (i.e. enhancement) of an existing immune response to surface-adsorbed molecules. T cell proliferation in response to APC-presented antigen is well known to be enhanced if the antigen was originally conjugated to a microparticle, especially where macrophages are acting as the APC [7,62,63]. MHC class switching may also be induced such that exogenous antigen is presented in the context of Class 1 MHC [7,64,65], although whether this is simply a function of non-physiological particle gorging needs to be resolved. The adjuvant properties of particles have long been investigated for commercial exploitation by vaccine development scientists – for example, for many years by Derek O'Hagan and colleagues (e.g. [66]) – and the gastrointestinal tract (i.e. orally) has been one of the major focuses for delivery. However, what has not been investigated is whether particle-antigen constructs impact locally leading to altered immune responses of intestinal T cells or, if as amply demonstrated, the effects are restricted to B and T cells of the peripheral compartments. While this is partly the focus of our current research activities, our previous studies have investigated the impact of microparticle–LPS conjugates on the innate immune response and whether such responses may differ in cells from patients with Crohn's disease [13,16,24,25,41,67,68].

As noted earlier, due to the ingestion of dust, soil, food additives, toothpaste and excipients for pharmaceuticals or nutraceuticals, the average person's gut is, in the Western world, exposed to many billions of sub-micron sized mineral particles on a daily basis. Most resist gastrointestinal and cellular degradation, are picked up by M-cells overlying intestinal lymphoid aggregates and passed to underlying phagocytes where they can be identified in vast numbers in basal 'pigmented cells' of the Peyer's patch. These pigment cells are so described because, under the microscope, the lysosomally-accumulated particles completely prevent the transmission of photons or electrons and thus the cells appear contrast-dense or 'pigmented' [14,15]. These cells are either mature or maturing macrophages, with the large majority being strongly positive for the CD68 antigen, and, histologically, do not differ in diseased states, including Crohn's disease [15]. They are, indeed, a normal occurrence of the basal intestinal lymphoid tissue throughout the population of, at least, the Western world. It is likely that these pigment cells are of low metabolic and immunological activity and, rather like tattoos, act as sinks for the safe storage of such non-degradable, non-biological particles. However, this tells us little of the prior signalling events with the initial particle–cell interaction. To investigate these effects, we have used cell culture and tried to mimic different *in vivo* scenarios. As discussed earlier, it is most likely that non-biological particles will adsorb luminal molecules to their surface and carry them into mucosal cells as a particle–biomolecule construct.

Our work has focussed on LPS, which is abundant in the gut lumen. In standard cell culture media, food-additive titanium dioxide particles (TiO_2) can enhance peripheral cell responses to LPS in terms of pro-inflammatory signalling (IL-8 and TNFα release with a reduction in TGF-β1 secretion) [67] but this effect and other pro-inflammatory signalling, is much more marked when the cell culture medium is enriched with calcium [16,25]. This is not only because Ca^{2+} ions facilitate TiO_2–LPS conjugation but because the additional calcium ions can saturate the cell culture medium with respect to calcium phosphate precipitation and these particles encourage LPS–TiO_2–calcium phosphate aggregation [16,25]. How this relates to the *in vivo* situation is unclear, although, as noted earlier, intestinal cells are considerably less responsive to this conjugate than peripheral cells. In either case, however, the secretion of IL-1β is especially noteworthy. The LPS is able to trigger pro-IL-1β production while the calcium phosphate alone or the calcium phosphate–TiO_2 aggregate promotes caspase 1 maturation and, therefore, cleavage of Pro-IL-1β to mature IL-1β [16,25]. The effects on IL-1β stimulation are, therefore, genuinely synergistic between the components of the conjugate [16,25]. Further work needs to determine if such IL-1β promoting effects are also observed in any *in vivo* modelling, and, if so, if this can impact on the acquired immune system by breaking immune-tolerance, for example. However, it is important to note that such particle–biomolecule synergies are far from theoretical. Not only is this pathway being exploited for particle-based therapeutic benefit, but, for example, oral transmissibility of prion disease is greatly enhanced by the binding of the acid- and enzyme-resistant prion protein to ingested soil particles [69,70]. In one experiment, that aimed to mimic potential natural exposure, this enhanced the effective infectious titer by 680 fold compared to the unbound prion alone. Overall, the ability of ingested, undegradable microparticles to enhance gut infectivity or immuno-stimulatory properties of surface-adsorbed agents deserves further scrutiny. It may be that particle sizes of around 100–250 nm are optimal, as they not only get well absorbed but they also appear better adjuvants than small particles.

6. Genotypic relevance

A final note is needed on the potential impact of genetic polymorphisms or host responses to oral nano- or micro-particles. It is likely that, overtime, inter-individual variation will prove important in determining host responses to ingested particles and we end with two examples that have interested our group. NOD2 is involved in the recognition of muramyl dipeptide (MDP), an essential structure of peptidoglycan derived from both Gram-positive and Gram-negative bacteria [71,72]. To date three common mutations have been associated with Crohn's disease, namely, R702W, G908R and 3020insC and these result in a signalling defect

in response to MDP [71,72]. Interestingly these mutations seem to be most linked with ileal-specific disease [73,74] and, therefore, where the presence of Peyer's patches is predominant.

A recent study, using a new mouse model invalidated for NOD2, has shown that NOD2 deficiency is associated with defective Peyer's patch homeostasis [75]. This translated to an increased number of Peyer's patches as well as their characteristic M-cell population, an increased intestinal permeability, a heightened production of inflammatory cytokines (investigated under basal conditions) and increased susceptibility to TNBS-induced colitis [75]. An increase in the number of Peyer's patches and M-cells should lead to a corresponding increase in the uptake of dietary particles and their conjugates. This model may be an interesting target for the investigation of exogenous dietary microparticles (e.g. TiO_2) on mucosal IL-1β production and maintenance of mucosal immune-tolerance.

Another relevant Crohn's disease-associated mutation is Atg16L1 [76,77], which has been linked to impaired autophagy [78,79]. Autophagy is a conserved process which was originally demonstrated to occur in cells undergoing starvation, or for the recycling of organelles, or for the clearance of ubiquitinated proteins. Since then, autophagy has been recognised to play additional important roles, both in innate/adaptive immunity [80] and thymic T cell selection [81,82]. Of interest to us is the recent finding that small nanoparticles (i.e. non aggregated) may engage the autophagic machinery and that this represents a pathway for nanoparticle clearance by cells [83]. It is therefore tantalising to speculate that such a process would be greatly compromised in patients bearing Atg16L1 mutation leading to nanoparticle persistence and on-going inflammation.

Consequently, and in the light of the ideas above, our group is currently investigating the effects of dietary nano- and microparticles, both on the innate and acquired immune system and in the cells of Crohn's disease patients with and without NOD2/Ag16L1 mutations.

References

[1] Service RF. Nanotechnology. Can high-speed tests sort out which nanomaterials are safe? Science 2008;321(5892):1036–7.
[2] The project on emerging Nano Technologies, http://www.nanotechproject.org/news/archive/8277/; 2009.
[3] Luxresearch. Lux research. Overhyped technology starts to reach potential: nanotech to impact $3.1 trillion in manufactured goods in 2015. July 22; http://www.luxresearchinc.com/press/RELEASE_Nano-SMR_7_22_08.pdf; 2009.
[4] Chaudhry Q, Scotter M, Blackburn J, Ross B, Boxall A, Castle L, et al. Applications and implications of nanotechnologies for the food sector. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008;25(3):241–58.
[5] Nanoscience and nanotechnologies: opportunities and uncertainties. The Royal Society & The Royal Academy of Engineering; July 2004.
[6] Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 2009;4(10):634–41.
[7] Harding CV, Song R. Phagocytic processing of exogenous particulate antigens by macrophages for presentation by class I MHC molecules. J Immunol 1994;153(11):4925–33.
[8] Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 2009;8(7):543–57.
[9] Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol 2008;9(8):847–56.
[10] Gerloff K, Albrecht C, Boots A, Forster I, Schins R. Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. Nanotoxicology 2009;3(4):1–10.
[11] The project on emerging NanoTechnologies, http://www.nanotechproject.org/inventories/consumer/browse/companies/4686/. Accessed 2009.
[12] BBC. Nanofoods, www.bbc.co.uk/radio4/science/frontiers_20081020.shtml; 2009.
[13] Powell JJ, Thoree V, Pele LC. Dietary microparticles and their impact on tolerance and immune responsiveness of the gastrointestinal tract. Br J Nutr 2007;98(Suppl. 1):S59–63.
[14] Powell JJ, Ainley CC, Harvey RS, Mason IM, Kendall MD, Sankey EA, et al. Characterisation of inorganic microparticles in pigment cells of human gut associated lymphoid tissue. Gut 1996;38(3):390–5.
[15] 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;57(8):374–8.
[16] Evans SM, Ashwood P, Warley A, Berisha F, Thompson RP, Powell JJ. The role of dietary microparticles and calcium in apoptosis and interleukin-1beta release of intestinal macrophages. Gastroenterology 2002;123(5):1543–53.
[17] Stone V, Johnston H, Schins RP. Development of in vitro systems for nanotoxicology: methodological considerations. Crit Rev Toxicol 2009;39(7):613–26.
[18] Dobrovolskaia MA, Germolec DR, Weaver JL. Evaluation of nanoparticle immunotoxicity. Nat Nanotechnol 2009;4(7):411–4.
[19] Bihari P, Vippola M, Schultes S, Praetner M, Khandoga AG, Reichel CA, et al. Optimized dispersion of nanoparticles for biological in vitro and in vivo studies. Part Fibre Toxicol 2008;5:14.
[20] Allouni ZE, Cimpan MR, Hol PJ, Skodvin T, Gjerdet NR. Agglomeration and sedimentation of TiO_2 nanoparticles in cell culture medium. Colloids Surf B Biointerfaces 2009;68(1):83–7.
[21] Nichols G, Byard S, Bloxham MJ, Botterill J, Dawson NJ, Dennis A, et al. A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization. J Pharm Sci 2002;91(10):2103–9.
[22] Institute BS. Terminology for nanoparticles, PAS 136. London: BSI; 2007.
[23] Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci USA 2008;105(38):14265–70.
[24] Powell JJ, Harvey RS, Ashwood P, Wolstencroft R, Gershwin ME, Thompson RP. Immune potentiation of ultrafine dietary particles in normal subjects and patients with inflammatory bowel disease. J Autoimmun 2000;14(1):99–105.
[25] 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;232(1):107–17.
[26] Jugdaohsingh R, O'Connell MA, Sripanyakorn S, Powell JJ. Moderate alcohol consumption and increased bone mineral density: potential ethanol and non-ethanol mechanisms. Proc Nutr Soc 2006;65(3):291–310.
[27] Powell JJ, Ainley CC, Evans R, Thompson RP. Intestinal perfusion of dietary levels of aluminium: association with the mucosa. Gut 1994;35(8):1053–7.
[28] Moller W, Felten K, Meyer G, Meyer P, Seitz J, Kreyling WG. Corrections in dose assessment of 99mTc radiolabeled aerosol particles targeted to central human airways using planar gamma camera imaging. J Aerosol Med Pulm Drug Deliv 2009;22(1):45–54.
[29] Jani PU, Nomura T, Yamashita F, Takakura Y, Florence AT, Hashida M. Biliary excretion of polystyrene microspheres with covalently linked FITC fluorescence after oral and parenteral administration to male Wistar rats. J Drug Target 1996;4(2):87–93.
[30] Cho M, Cho WS, Choi M, Kim SJ, Han BS, Kim SH, et al. The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. Toxicol Lett 2009;189(3):177–83.
[31] Powell JJ, Evans SM, Lomer MC, Thompson RPH. What are intestinal pigment cells? Inflamm Bowel Dis Mon 1999;1(3):71–4.
[32] Hillery AM, Jani PU, Florence AT. Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. J Drug Target 1994;2(2):151–6.
[33] McClean S, Prosser E, Meehan E, O'Malley D, Clarke N, Ramtoola Z, et al. Binding and uptake of biodegradable poly-DL-lactide micro- and nanoparticles in intestinal epithelia. Eur J Pharm Sci 1998;6(2):153–63.
[34] Moyes SM, Smyth SH, Shipman A, Long S, Morris JF, Carr KE. Parameters influencing intestinal epithelial permeability and microparticle uptake in vitro. Int J Pharm 2007;337(1–2):133–41.
[35] Gaumet M, Gurny R, Delie F. Localization and quantification of biodegradable particles in an intestinal cell model: the influence of particle size. Eur J Pharm Sci 2009;36(4–5):465–73.
[36] Cartiera MS, Johnson KM, Rajendran V, Caplan MJ, Saltzman WM. The uptake and intracellular fate of PLGA nanoparticles in epithelial cells. Biomaterials 2009;30(14):2790–8.
[37] Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest 2005;115(1):66–75.
[38] Pan YH, Sader K, Powell JJ, Bleloch A, Gass M, Trinick J, et al. 3D morphology of the human hepatic ferritin mineral core: new evidence for a subunit structure revealed by single particle analysis of HAADF-STEM images. J Struct Biol 2009;166(1):22–31.
[39] Kalgaonkar S, Lonnerdal B. Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. J Nutr Biochem 2009;20(4):304–11.
[40] Lomer MC, Thompson RP, Powell JJ. Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proc Nutr Soc 2002;61(1):123–30.
[41] Pele LC, Powell JJ. Microparticles: a link between modern life and inflammatory bowel disease? In: Colombel JF, Gasche C, Scholmerich J, Vucelic B, editors. IBD: translation from basic research to clinical practice. Kluwer Academic Publishers; 2004. p. 123–37.

[42] Lomer MC, Hutchinson C, Volkert S, Greenfield SM, Catterall A, Thompson RP, et al. Dietary sources of inorganic microparticles and their intake in healthy subjects and patients with Crohn's disease. Br J Nutr 2004;92(6):947–55.
[43] des Rieux A, Ragnarsson EG, Gullberg E, Preat V, Schneider YJ, Artursson P. Transport of nanoparticles across an in vitro model of the human intestinal follicle associated epithelium. Eur J Pharm Sci 2005;25(4–5):455–65.
[44] Seifert J, Sass W. Intestinal absorption of macromolecules and small particles. Dig Dis 1990;8(3):169–78.
[45] Sass W, Dreyer HP, Seifert J. Rapid insorption of small particles in the gut. Am J Gastroenterol 1990;85(3):255–60.
[46] Seifert J, Haraszti B, Sass W. The influence of age and particle number on absorption of polystyrene particles from the rat gut. J Anat 1996;189 (Pt 3):483–6.
[47] Gebert A, Rothkotter HJ, Pabst R. M cells in Peyer's patches of the intestine. Int Rev Cytol 1996;167:91–159.
[48] Beier R, Gebert A. Kinetics of particle uptake in the domes of Peyer's patches. Am J Physiol 1998;275(1 Pt 1):G130–7.
[49] Bockmann J, Lahl H, Eckert T, Unterhalt B. Blood titanium levels before and after oral administration titanium dioxide. Pharmazie 2000;55(2):140–3.
[50] Volkheimer G. Passage of particles through the wall of the gastrointestinal tract. Environ Health Perspect 1974;9:215–25.
[51] Volkheimer G. Persorption of microparticles. Pathologe 1993;14(5):247–52.
[52] Hillyer JF, Albrecht RM. Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. J Pharm Sci 2001;90(12): 1927–36.
[53] Jepson MA, Simmons NL, Savidge TC, James PS, Hirst BH. Selective binding and transcytosis of latex microspheres by rabbit intestinal M cells. Cell Tissue Res 1993;271(3):399–405.
[54] Hussain N, Jaitley V, Florence AT. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv Drug Deliv Rev 2001;50(1–2):107–42.
[55] Freedman BJ. Persorption of raw starch: a cause of senile dementia? Med Hypotheses 1991;35(2):85–7.
[56] Powell JJ, Whitehead MW, Lee S, Thompson RPH. Mechanisms of gastrointestinal absorption: dietary minerals and the influence of beverage ingestion. Food Chem 1994;51:381–8.
[57] Hussain N, Jani PU, Florence AT. Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat. Pharm Res 1997;14(5):613–8.
[58] Jani P, Halbert GW, Langridge J, Florence AT. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J Pharm Pharmacol 1990;42(12):821–6.
[59] Smyth SH, Feldhaus S, Schumacher U, Carr KE. Uptake of inert microparticles in normal and immune deficient mice. Int J Pharm 2008;346(1–2):109–18.
[60] Doyle-McCullough M, Smyth SH, Moyes SM, Carr KE. Factors influencing intestinal microparticle uptake in vivo. Int J Pharm 2007;335(1–2):79–89.
[61] Xia T, Li N, Nel AE. Potential health impact of nanoparticles. Annu Rev Public Health 2009;30:137–50.
[62] 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 USA 1993;90(11):4942–6.
[63] 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;56 (4):143–8.
[64] Song R, Harding CV. Roles of proteasomes, transporter for antigen presentation (TAP), and beta 2-microglobulin in the processing of bacterial or particulate antigens via an alternate class I MHC processing pathway. J Immunol 1996;156(11):4182–90.
[65] Shen H, Ackerman AL, Cody V, Giodini A, Hinson ER, Cresswell P, et al. Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. Immunology 2006;117(1):78–88.
[66] Singh M, Chakrapani A, O'Hagan D. Nanoparticles and microparticles as vaccine-delivery systems. Expert Rev Vaccines 2007;6(5):797–808.
[67] Butler M, Boyle JJ, Powell JJ, Playford RJ, Ghosh S. Dietary microparticles implicated in Crohn's disease can impair macrophage phagocytic activity and act as adjuvants in the presence of bacterial stimuli. Inflamm Res 2007;56 (9):353–61.
[68] Evans SM, Powell JJ, Ashwood P, Thompson RPH, Harvey RS. Ultrafine particles in the Aetiology of Crohn's disease. Inflamm Bowel Dis Mon 1999;1(2):39–45.
[69] Johnson CJ, Pedersen JA, Chappell RJ, McKenzie D, Aiken JM. Oral transmissibility of prion disease is enhanced by binding to soil particles. PLoS Pathog 2007;3(7):e93.
[70] Johnson CJ, Phillips KE, Schramm PT, McKenzie D, Aiken JM, Pedersen JA. Prions adhere to soil minerals and remain infectious. PLoS Pathog 2006; 2(4):e32.
[71] Bonen DK, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, et al. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 2003;124(1):140–6.
[72] Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, et al. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 2003;278(11):8869–72.
[73] Cuthbert AP, Fisher SA, Mirza MM, King K, Hampe J, Croucher PJ, et al. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. Gastroenterology 2002;122(4):867–74.
[74] Ahmad T, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, et al. The molecular classification of the clinical manifestations of Crohn's disease. Gastroenterology 2002;122(4):854–66.
[75] Barreau F, Meinzer U, Chareyre F, Berrebi D, Niwa-Kawakita M, Dussaillant M, et al. CARD15/NOD2 is required for Peyer's patches homeostasis in mice. PLoS One 2007;2:e523.
[76] Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 2007;39 (5):596–604.
[77] Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 2007;39(2):207–11.
[78] Xavier RJ, Huett A, Rioux JD. Autophagy as an important process in gut homeostasis and Crohn's disease pathogenesis. Gut 2008;57(6):717–20.
[79] Kuballa P, Huett A, Rioux JD, Daly MJ, Xavier RJ. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. PLoS One 2008;3(10):e3391.
[80] Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. Nat Rev Immunol 2007;7(10):767–77.
[81] Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. Nature 2008;455(7211):396–400.
[82] Nedjic J, Aichinger M, Klein L. A novel role for autophagy in T cell education. Autophagy 2008;4(8):1090–2.
[83] Zabirnyk O, Yezhelyev M, Seleverstov O. Nanoparticles as a novel class of autophagy activators. Autophagy 2007;3(3):278–81.