Inflamm. res. 56 (2007) 353–361
1023-3830/07/090353-9
DOI 10.1007/s00011-007-7068-4

Inflammation Research

Dietary microparticles implicated in Crohn's disease can impair macrophage phagocytic activity and act as adjuvants in the presence of bacterial stimuli

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Received 26 January 2007; returned for revision 27 March 2007; accepted by I. Ahnfelt-Rønne 19 April 2007

Abstract. *Objective and design:* Western diets regularly expose the gastrointestinal tract (GI) to large quantities (>10^{12}/day) of man-made, submicron-sized, particles derived from food additives and excipients. These are taken up by M cells, accumulate in gut macrophages, and may influence the aetiology of inflammatory bowel diseases (IBD).
Materials: We investigated the effects of common dietary microparticles on the function of macrophages from healthy donors or active Crohn's disease (CD) patients.
Methods: Macrophages were incubated for 24 h with microparticles before being assayed for cytokine production and phagocytic activity.
Results: Microparticles alone were non-stimulatory but, in the presence of bacterial antigens such as LPS, they could act as adjuvants to induce potent cytokine responses. Uptake of high concentrations of microparticles also impaired macrophage phagocytic capacity – but not their ability – to take up 2μM fluorescent beads.
Conclusions: While dietary microparticles alone have limited effects on basic macrophage functions, their ability to act as adjuvants could aggravate ongoing inflammatory responses towards bacterial antigens in the GI tract.

Key words: Inflammatory bowel disease – Crohn's disease – Macrophage – Phagocytosis – Diet

Introduction

Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the gut involving genetic and environmental components and are believed to be the result of an aberrant immune response towards common commensal microorganisms. While much progress has been made in identifying IBD-associated genetic polymorphisms, the incidence of Crohn's disease has also been linked to environmental factors such as diet. Indeed, the spread of diets rich in processed foods (such as the modern Western or 'urban' diet) closely parallels the epidemiology of Crohn's disease [1–4]. In addition to refined sugars, 'westernised' diets contain large quantities of inorganic, bacteria-sized, microparticles (typically 0.1–0.7 μm diameter) mainly derived from foods and pharmaceuticals as additives or contaminants [5–10]. Two of the most common ingredients are aluminosilicates (AlSi) – used as anti-caking agents in the food industry (typically as kaolinite; E559) and titanium dioxide (TiO_2) – a common food brightner or whitener (typically as anastase; E171) [6]. The presence of these agents in the diet has been linked to the development of gastrointestinal "pigment cells" – often detected at the base of Peyer's patches and present in virtually all western Europeans after early childhood [7, 8]. These cells are frequently observed in areas where the first signs of Crohn's disease manifest themselves [6] and various authors have questioned their contribution to the development of IBD [7–9]. Phenotypic analysis has shown them to be $CD68^+$ macrophages while the pigment itself consists of aluminium, silicon and titanium [9, 10].

Dietary microparticles can enter the body by crossing the intestinal epithelium via endocytic M cells in the dome of the Peyer's patch before reaching mature macrophages at the Peyer's patch base [9, 11, 12]. Cellular processes are apparently unable to degrade the material and an accumulation of particles can be observed in phagocytic vacuoles [11, 13, 14]. While these agents are believed to be 'immunologically inert' in the gut, similar particles can cause granulomatous diseases in other organs [15, 16] and recent research suggests dietary microparticles could be potent initiators and drivers of inflammation under the appropriate conditions [6, 17, 18].

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Indeed, Powell *et al.* found that food-grade TiO_2 microparticles could act as adjuvants in the presence of lipopolysaccharide (LPS), significantly enhancing IL-1 β responses in peripheral blood mononuclear cells (PBMCs), intestinal explants, and lamina propria mononuclear cells (LPMCs) [18, 19]. These effects resulted from adsorption of LPS, in the presence of excess calcium ions (4.5 mM), to the charged surfaces of TiO_2 microparticles, leading to uptake of particulate, rather than soluble LPS [18, 19]. It is currently unclear whether, in susceptible individuals, these particulate complexes could overcome tolerogenic processes and induce inflammation.

Under normal conditions macrophages in the gastrointestinal tract play a crucial role in maintaining homeostasis-phagocytosing apoptotic cells and producing anti-inflammatory cytokines such as TGF β and IL-10 [20]. However, upon stimulation, they can act as potent mediators of inflammation – releasing inflammatory cytokines, lipid mediators, nitric oxide, and reactive oxygen species that mediate cellular cytoxicity [21, 22]. Since macrophages avidly scavenge dietary microparticles, we have investigated the effects of microparticle uptake on macrophage function with particular focus on cytokine responses and phagocytic activity. In addition, we have addressed the hypothesis that microparticles could play a role in the development of Crohn's disease.

Materials and methods

Generation of monocyte-derived macrophages

Heparinised blood (20 ml) was obtained from healthy volunteers (n = 8), or Crohn's disease patients with active disease but no current treatment (n = 6). Peripheral blood was separated by centrifugation on a ficoll-hypaque (Nycomed, UK) density gradient (according to the manufacturer's instructions). $CD14^+$ monocytes were isolated from the PBMC layer by positive selection using MACS beads (Miltenyi Biotech, Germany) as per the manufacturer's instructions and were routinely found to be >95 % pure $CD14^+$ cells by flow cytometry. Macrophages were differentiated in X-VIVO 15 serum-free medium (Biowhittaker, USA) containing 10 % AB human serum (Sigma Chemicals, Poole, UK) and 25 ng/ml M-CSF (First Link Ltd, Birmingham, UK) in 6 well tissue culture plates at a concentration of 3–5 × 10^5 cells per ml. Cells were incubated for 6 days at 37 °C in an atmosphere of 5 % CO_2/95 % air. Day 6 cells showed a distinct macrophage morphology and were $CD14^{low}$, MHC class II^+, $CD68^+$, $CD206^+$ (macrophage mannose receptor) – data not shown.

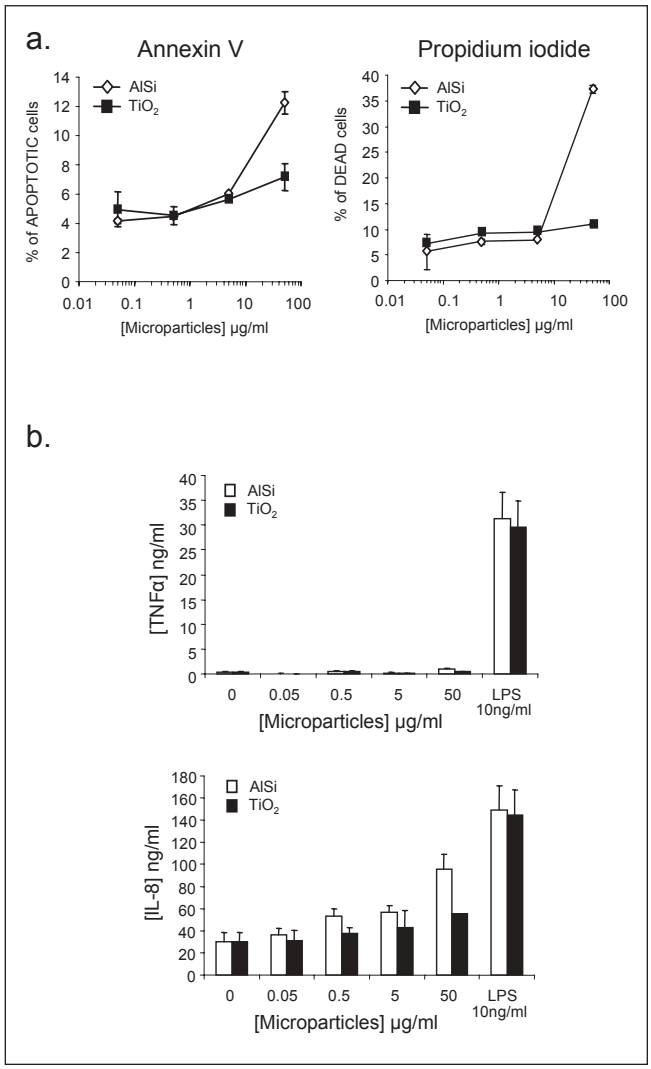

Fig. 1. Microparticle toxicity. (a) Macrophages were analysed for viability by annexin-V and propidium iodide staining. Graphs show mean percentage of annexin V-positive (apoptotic) cells and propidium iodide-positive (necrotic) cells following microparticle incubation for 24 h ± standard deviations (SD) for triplicate samples. (b) Levels of IL-8 and TNF α in supernatants. Cells stimulated with 10 ng/ml LPS were used as positve controls. Data are representative of five experiments.

Microparticle uptake assay

The aluminosilicate (AlSi; kaolinite, BDH, Germany) and food-grade titanium dioxide (TiO_2 Anatase, Special AHR, Tioxide UK Ltd, Cleveland, UK) were added to cells over a range of doses (0.05–50 µg/ml) and incubated for 24 h. Cells were analysed by flow cytometry (FACScalibur, BD Biosciences) set to measure size (forward scatter; FSC) and granularity (side scatter; SSC) as originally described by Kobsik et al. [23]. Results were plotted as percentage increases in granularity (SSC) to enable direct comparisons between cells from healthy controls and patients with Crohn's disease.

Macrophage viability

Macrophages were assessed for viability by flow cytometric-observed staining with annexin-V-APC (BD Biosciences) and propidium iodide (Sigma) as per the manufacturer's instructions. Results are presented as the percentage of cells in a population undergoing apoptosis (annexin-V staining) or necrosis (propidium iodide uptake). Macrophage cell culture supernatants were also analysed for inflammatory cytokine (IL-8 and TNF) secretion following treatment with microparticles.

Cytokine ELISA

Following culture with dietary microparticles and/or 10 ng/ml LPS (from *Escherichia coli* type 055: B5; Sigma), supernatants (from 5 × 10^5 macrophages in 500 µl medium) were harvested and spun to remove cells and debris. IL-8 and TNF α were detected by sandwich ELISA using paired antibodies (BD Biosciences) and avidin-horseradish peroxidase (Biosource International Inc., California, USA), added at 0.1 µg/ml

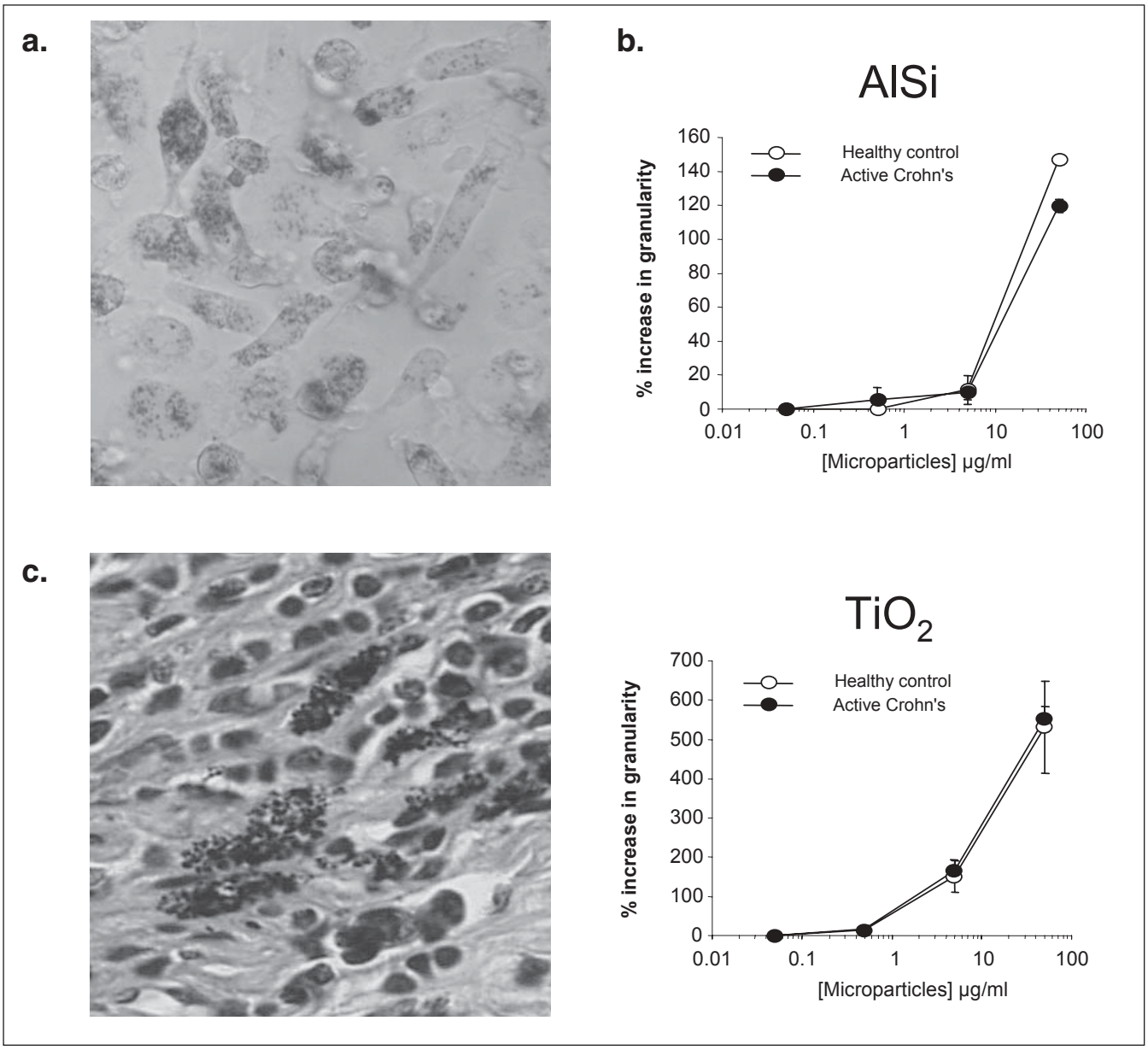

Fig. 2. Uptake of dietary microparticles. Macrophages from healthy controls or active Crohn's patients were incubated with dietary microparticles (5 µg/ml) for 24 h then analysed by (a) light microscopy or (b) flow cytometry. (c) H+E stain for "pigment cells" at base of human peyer's patch. Large increases in cell granularity reflected microparticle uptake. Results show mean percentage increases in granularity ±SD of macrophages from 8 healthy controls and 6 active Crohn's patients.

for 30 min. TMB (Zymed, San Francisco, USA) was used as a substrate. IL-10 and TGF β -1 were detected using duoset kits (R&D Systems, Minneapolis, USA) as per the manufacturer's instructions. Colour development was stopped by the addition of 0.5 M H_2SO_4 and plates were read for optical density at 450 nm (ELISA plate reader; Titertek Mulitskan – Flow laboratories, Maryland, USA). Samples were analysed in triplicate and data are presented as 'box and whisker' plots to show spread of data (plotted using GraphPad Software, San Diego, California, USA). Data were analysed by Wilcoxon signed rank test (**$p < 0.005$; *$p < 0.05$). Comparisons between healthy control and Crohn's patient responses were analysed by Mann-Whitney U test (**$p < 0.005$; *$p < 0.05$).

Phagocytosis assay

Cells were incubated for 1 h with 500 µL of fresh medium containing green fluorescent 2 µM, carboxlyate-modified, Fluospheres® (Molecular Probes; Invitrogen, California, USA) at a ratio of 5 beads to 1 cell. After incubation, cells were washed 3 times before harvesting with Trypsin-EDTA (Sigma). Cells phagocytosing fluorescent beads could be clearly distinguished by flow cytometry using a histogram plot. Peaks corresponding with cells taking up 1, 2, 3, or 4+ beads were gated and used to provide data. Data were analysed by Wilcoxon signed rank test (**$p < 0.005$; *$p < 0.05$).

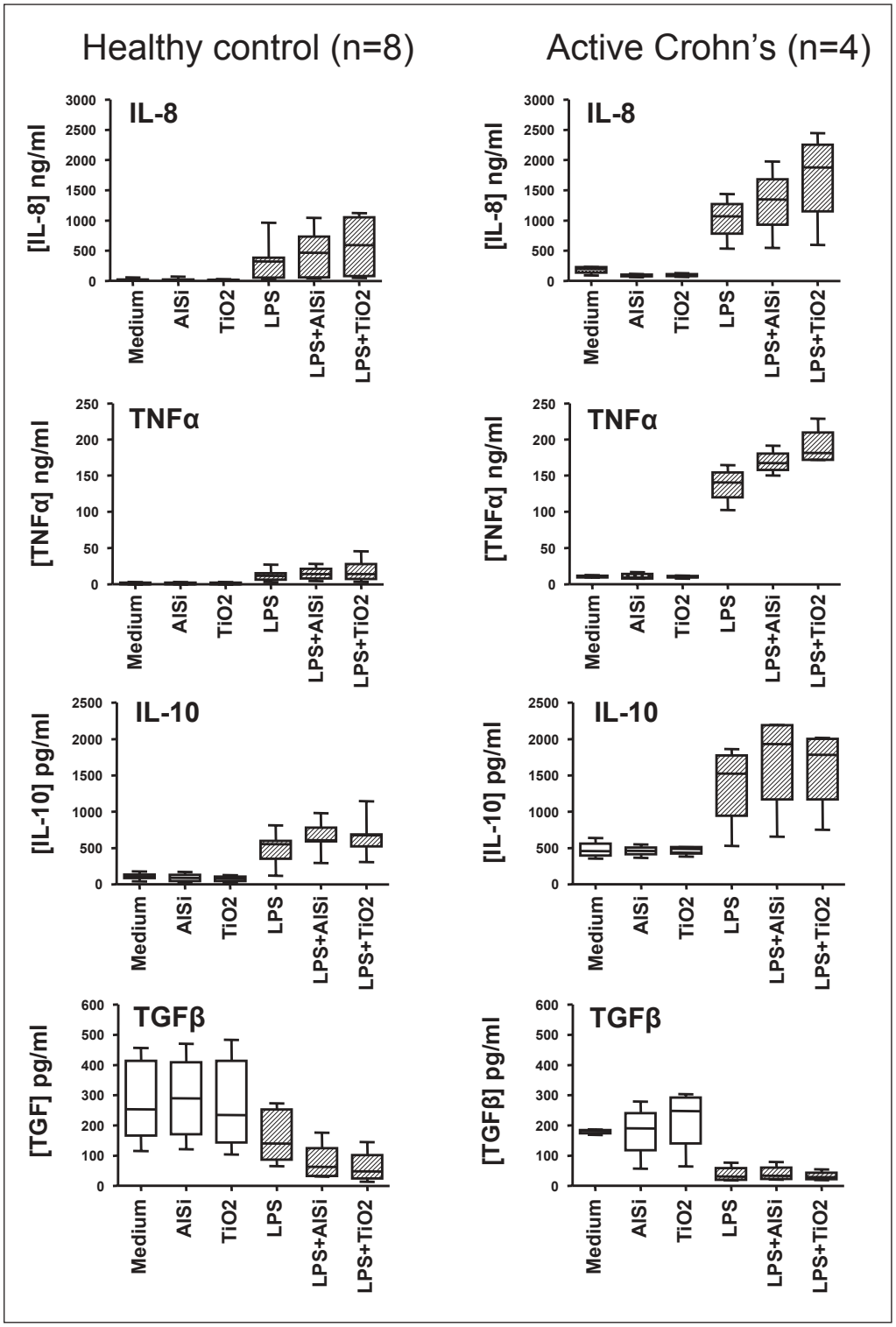

Fig. 3. Cytokine responses of macrophages following treatment with dietary microparticles. Macrophages from healthy controls were treated with 5 µg/ml of dietary microparticles ±10 ng/ml LPS for 24 h. IL-8, TNF α, IL-10, and TGF β production were not significantly affected by incubation with either AlSi or TiO2 alone. However, upon activation with 10 ng/ml LPS, the presence of dietary microparticles had an adjuvant effect. Box and whisker plots show spread of data from multiple donors (n = 8 for healthy controls; n = 6 active Crohn's patients).

Results

Toxic effects of dietary microparticles at high concentrations

We first investigated the effects of microparticle incubation on macrophage viability and cytokine secretion. Macrophages from healthy controls and Crohn's disease patients were not significantly affected by incubation with microparticles at concentrations at, or below, 5 µg/ml as assessed by annexin-V and propidium iodide staining (Fig. 1a). However, when treated with 50 µg/ml of microparticles, small increases in apoptosis and necrosis were detected in all samples. This was most apparent when using AlSi particles although a sim-

Cytokines (response to LPS)	Healthy control (n = 8)			Active Crohn's (n = 6)		
	Medium	AlSi	TiO_2	Medium	AlSi	TiO_2
IL-8 (ng/ml)	292.3*	423.3 (+44%) **	544.7 (+86%) **	1030.7	1262.3 (+23%)*	1656.3 (+61%)
TNFα (ng/ml)	12.5	14.3 (+18%) *	19.3 (+59%) *	128.4	161.9 (+26%) *	188.3 (+47%) *
IL-10 (pg/ml)	473.4	614.3 (+30%) **	685.2 (+45%) NS	1269.8	1605.8 (+27%) *	1687.1 (+33%)*
TGF β NS (pg/ml)	206.5	121.5 (-41%) *	103.4 (-50%) *	54.0	49.7 (-8%) NS	38.9 (-28%)

** $p < 0.005$ * $p < 0.05$ NS = not significant (Wilcoxon)

Table 1. Accumulated data (n = 8 for healthy controls; n = 6 active Crohn's patients) from Figure 3 showing mean cytokine responses to LPS ± microparticles. Figures in brackets are percentage increases/decreases based on cells treated with LPS only. Effect of microparticles was analysed by Wilcoxon signed ranks test **$p < 0.005$; *$p < 0.05$.

ilar trend could also be detected following TiO_2 treatment. These changes were reflected in the cytokine responses (Fig. 1b). Elevated levels of IL-8 were detected from cells treated with 50 µg/ml of both microparticle types although this never reached the concentration produced by cells following treatment with 10 ng/ml LPS (used as a positve control). TNF α levels were not significantly affected in these experiments. For subsequent experiments a dose of 5 µg/ml of microparticles – reflecting the maximal, non-toxic, dose – was generally used unless otherwise stated.

Microparticles are efficiently taken up by macrophages from healthy control subjects and patients with Crohn's disease

Macrophages were next examined for their ability to capture and internalise dietary microparticles (Fig. 2a). Virtually all cells (>95 %) were found to rapidly take up microparticles which were clearly visible and diversly spread within the cell cytoplasm after 24 h. The morphology of these cells also matched that of $CD68^+$ 'pigment cells' detected by haematoxylin and eosin staining at the base of Peyer's patches in patients with Crohn's disease (Fig. 2b). Cells treated in the same way were also analysed by flow cytometery set to measure cell size (FSC) and granularity (SSC). Microparticle-treated cells showed increases in granularity following incubation with a range of microparticle concentrations (Fig. 2b). This analysis suggested both AlSi and TiO_2 microparticles were taken up with similar kinetics by cells from active Crohn's disease patients and healthy controls alike.

Dietary microparticles alone have no significant effect on macrophage cytokine responses but act as mild adjuvants in the presence of lipopolysaccharide

Cytokine responses of macrophages incubated for 24 h with 5 µg/ml AlSi or TiO_2 were next analysed. Macrophage production of the inflammatory cytokines TNF α and IL-8, and the anti-inflammatory cytokines TGF β and IL-10, showed no difference following incubation with 5 µg/ml AlSi or TiO_2 alone (Fig. 3). This was true for macrophages from healthy controls or active Crohn's patients although macrophages from active Crohn's patients showed elevated background levels of IL-8, TNF α and IL-10, and decreased background levels of TGF β, as compared to macrophages from healthy controls. Taken together these data suggested that microparticles alone did not deliver strong inflammatory or anti-inflammatory signals and that macrophages from active Crohn's patients had an altered baseline cytokine response in general.

Macrophages were next incubated for 24 h with 5 µg/ml AlSi or TiO_2 in the presence of bacterial LPS (Fig. 3 and Table 1). Unlike similar investigations by other groups, no excess calcium ions were added [18, 19]. In macrophages from healthy controls, LPS alone induced increased IL-8, TNF α and IL-10 responses with a concomitant decrease in TGF β production. In the presence of microparticles these responses were clearly enhanced. Thus, in healthy control samples the presence of dietary microparticles increased IL-8 responses (+44 % for AlSi and +86 % for TiO_2 compared to untreated cells); increased TNF α responses (+18 % for AlSi and +59 % for TiO_2); increased IL-10 responses (+30 % for AlSi and +45 % for TiO_2); and decreased TGF- β responses (–41 % for AlSi and -50 % for TiO_2) following LPS treatment. TiO_2 particles appeared to be the more potent adjuvants although there was considerable variation between donors. In the case of macrophages derived from patients with active Crohn's disease, the baseline responses alone, or to LPS, were found to be significantly elevated compared to the cytokine responses of healthy control cells ($p < 0.05$ for all cytokines by Mann-Whitney U test), with concomitant decreased production of TGF β (Fig. 3 and Table 1). Despite this, microparticle adjuvant activity was also detected in these cells with increased IL-8 responses (+23 % for AlSi and +61 % for TiO_2); increased TNF α responses (+26 % for AlSi and +47 % for TiO_2), increased IL-10 responses (+27 % for AlSi and +33 % for TiO_2); and decreased TGF β (–8 % for AlSi and –28 % for TiO_2). Again, TiO_2 appeared to have a larger adjuvant effect than AlSi. Overall, microparticle adjuvant activity appeared to be greater in the healthy control macrophages compared to those from subjects with active Crohn's disease.

Dietary microparticles impair macrophage phagocytic capacity

We next investigated the effects of dietary microparticle uptake on phagocytosis. We used 2 µM fluospheres to analyse macrophage phagocytic activity over 60 min following 24 h incubation with the much smaller AlSi or TiO_2 micro-

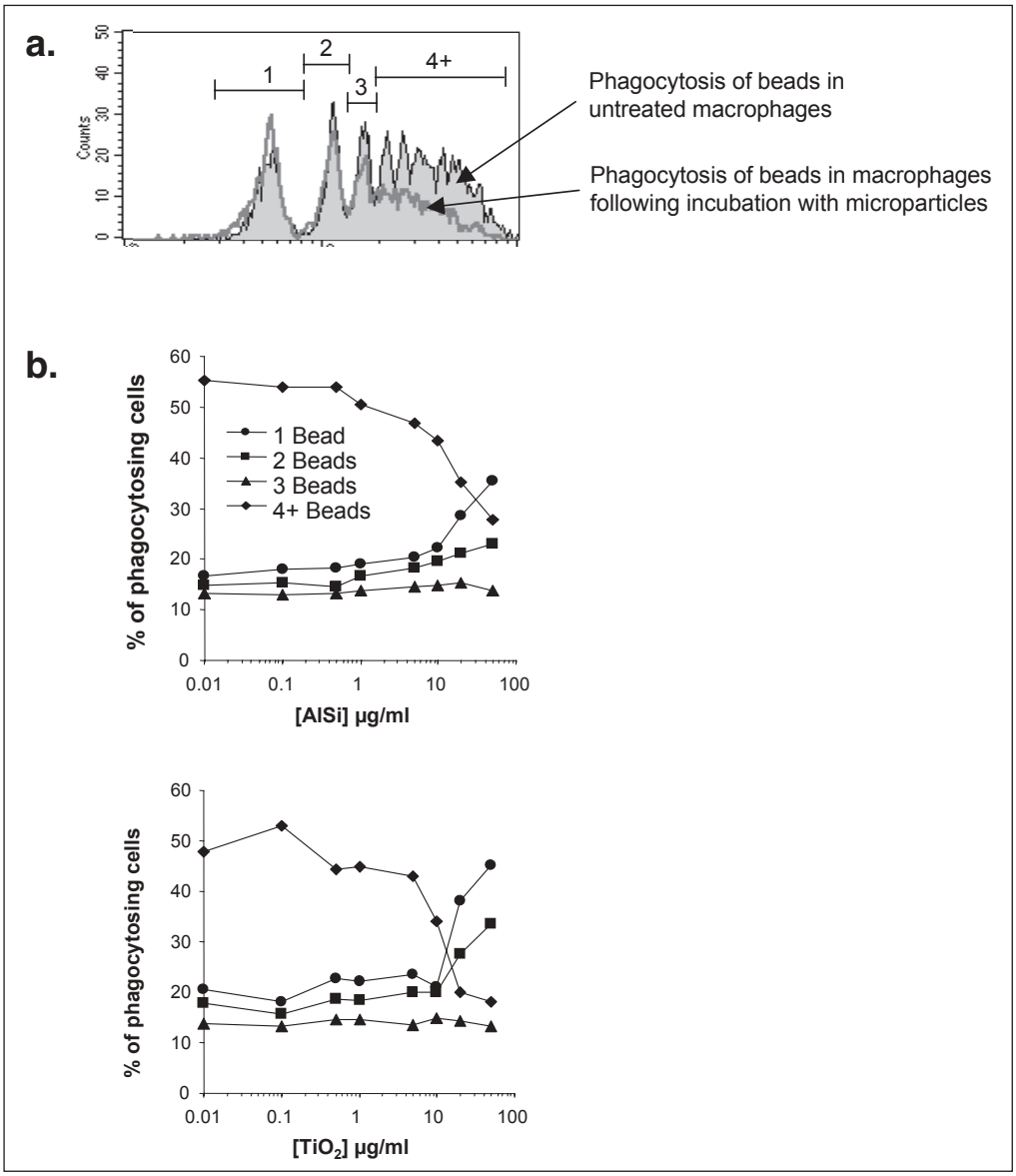

Fig. 4. Macrophage phagocytic activity following incubation with dietary microparticles. Macrophages from healthy controls or active Crohn's patients were incubated with dietary microparticles (5 µg/ml) for 24 h then analysed for phagocytic activity. (a) Flow cytometry by histogram allowed quantification of the number of beads taken up per cell. (b) Representative data showing phagocytosis of 1, 2, 3, or 4+ fluospheres following treatment with microparticles for 24 h.

particles. Fluorescence microscopy revealed bead uptake in >90 % of the macrophages from patients and healthy controls (not shown). Flow cytometric analysis (Fig. 4a) showed that increasing concentrations of microparticles reduced the proportion of macrophages able to accumulate large numbers of beads (4+) in a dose-dependent manner. Conversely, the number of cells taking up only 1 or 2 beads was unaffected or slightly increased at higher microparticle concentrations (Fig. 4b). This suggested that, while there was little effect on the phagocytic process itself, the phagocytic *capacity* of the macrophage was compromised following microparticle uptake.

To compare the phagocytic activity of macrophages from controls and patients we assayed the number of viable (PI negative) macrophages taking up 4+ beads following incubation with a range of microparticles in the presence or absence of LPS (Fig. 4c). High concentrations (50µg/ml) of AlSi inhibited phagocytic capacity of macrophages from both healthy controls (51.4 ± 17.3 % reduction) and from patients (29.5 ± 3.6 % reduction). Similar results were seen when the experiment was carried out in the presence of LPS – although the overall level of phagocytosis was reduced (Fig. 4c). High concentrations of TiO_2 also impaired phagocytic accumulation of beads in healthy control cells (48.5 ± 13.9 % reduction) and in patient's cells (11.5 ± 12.5 % reduction). Again, a similar effect was seen in the presence of LPS (Fig 4c).

Discussion

We have investigated the possibility that dietary microparticles have a role to play in the development of IBD via their effects on macrophage activity. To this end, we studied the functional responses of macrophages isolated from healthy controls and active Crohn's disease patients following exposure to two types of common dietary microparticles – AlSi and TiO_2. In addition, we investigated the effects of these particles in the presence of bacterial LPS since it has been

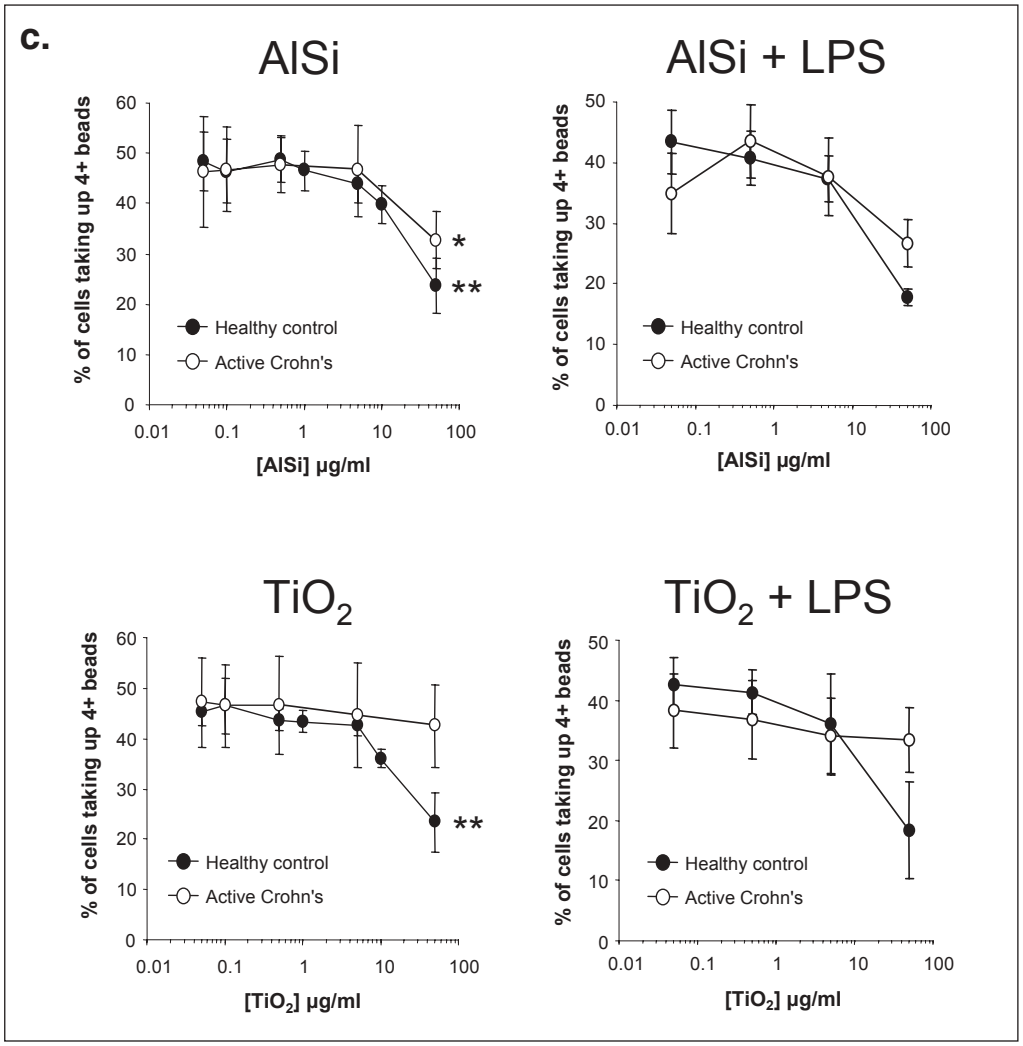

Fig. 4. (c) Accumulated data from 8 healthy control and 6 active Crohn's patient samples showing mean percentage of cells accumulating 4+ fluospheres following treatment with microparticles in the presence or absence of 10ng/ml. Error bars are ±SD. Wilcoxon signed ranks test $^{**}p < 0.005$; $^{*}p < 0.05$.

postulated that absorption of bacterial antigens to the surface of particles could overcome tolerising mechanisms and trigger inflammatory responses in the gut [6]. Although mildly toxic at high concentrations (>50 µg/ml), AlSi and TiO_2 were rapidly phagocytosed and accumulated in diffuse intracellular spaces. The morphology of the cells closely resembled that of $CD68^+$ macrophages which are frequently observed at the base of Peyer's patches in subjects consuming diets rich in processed foods [7, 8]. While incubation with microparticles alone had no effect on macrophage cytokine production, their presence following treatment with bacterial LPS appeared to enhance IL-8, TNF α, and IL-10 production (whilst simultaneously impairing the secretion of TGF β). These data suggested that both AlSi and TiO_2 particles possess adjuvant activity and corresponded with reports indicating AlSi and TiO_2 particles require surface modification before they become stimulatory [17]. Adjuvant activity was most potent when using TiO_2 microparticles and has been previously observed in the IL-1 β response of PBMCs and LPMCs to LPS in the presence of additional calcium cations [18, 19]. Indeed, TiO_2 microparticles have been shown to avidly bind LPS and other biomolecules [6, 18]. Interestingly, we were also able to detect adjuvant activity of TiO_2 microparticles in the presence of other TLR and NOD2 agonists, such as Pam3CSK4 (TLR2 agonist) and muramly-dipeptide (NOD2 agonist) (M. Butler, unpublished observations), implying dietary microparticles could act as adjuvants in the presence of a large number of bacterial antigens and lead to enhancement of inflammatory responses (IL-8, TNF α) and suppression of regulatory mechanisms (TGF β) *in vivo*.

It is currently unclear how particulate delivery (vs soluble) affects LPS recognition leading to enhanced immune responses. Recognition of soluble LPS involves a receptor complex consisting of CD14, LPS binding protein, TLR4 and MD-2 – assembly of which might be enhanced by receptor cross-linking when particulate LPS is present [25]. In addition, particulate LPS is known to require recognition by phagocytic receptors such as the β 2-integrins (CD11b/CD18), scavenger receptors, and Fc receptors [26, 27] and activation of these phagocytic pathways could potentiate LPS recognition pathways. Indeed, TLR2 responses to bacterial lipoproteins are known to be enhanced following engagement of the endocytic C-type lectin, dectin-1 [28, 29]. Furthermore, recent reports have shown that TLRs are recruited to newly formed phagosomes even in the absence of activation [30, 31] which suggests that sampling by TLRs following internalisation of bacterial antigens might be more efficient than recognition at the cell surface. By utilising

such mechanisms to distinguish soluble LPS from particulate LPS, cells also have the potential to detect the break-down products of infection as well as the microbes themselves and to respond accordingly.

While the adjuvant effect of the microparticles was detectable in both groups, macrophages from patients with active Crohn's disease exhibited elevated baseline levels of IL-8, TNF α, and IL-10 and responded to LPS stimulation with larger increases in cytokine production overall compared to controls. This effect did not involve microparticles since it occurred in macrophages treated with LPS alone. PBMC from Crohn's disease patients have long been known to elicit enhanced inflammatory responses to various stimuli although the mechanisms behind this remain elusive [32–35]. One possibility is that bacterial antigens, such as LPS, gain access to the serum and maintain circulating mononuclear cells in a state of 'semi-activation' [36], thus reducing their threshold for stimulation.

In our phagocytosis experiments, macrophages rapidly took up 2 μm fluorospheres (as well as labelled apoptotic Jurkat T cells – unpublished observations) and accumulated them in intracellular compartments. Microparticles impaired the accumulation of phagocytosed beads, with a sharp decrease in the number of macrophages accumulating 4+ latex beads. These results suggested that dietary microparticle uptake decreased the phagocytic *capacity* of macrophages rather than affecting the process of phagocytosis itself and is consistent with the 'particle overload' hypothesis proposed by Morrow *et al.* to explain the toxicity of inhaled particles [37]. Studies using alveolar macrophages found that when the phagocytosed particle burden occupied ~6% of the internal cell volume, clearance mechanisms were impaired and completely blocked when the burden reached ~60% [38, 39]. Effects such as these could have important implications *in vivo*. Phagocytosis of apoptotic cells by macrophages has been shown to induce the production of anti-inflammatory cytokines such as TGF β [20] and results in the presentation of self-antigens via MHC molecules leading to the induction of tolerance [40, 41]. Conversely, uptake of necrotic cells promotes the upregulation of co-stimulatory molecules and leads to inflammatory responses [42, 43]. Accumulation of dietary microparticles inside intestinal macrophages could prevent the adequate clearance of apoptotic cells allowing the development of secondary necrosis which could contribute to inflammation [44]. Alternatively, uptake of non-degradable particles could also lead to granuloma formation. Granulomas are a 'foreign-body' immune response normally associated with persistent pathogens such as *Mycobacterium tuberculosis* [45]. However, granulomas can also be induced by inorganic particles such as silica and beryllium oxide in the lung, leading to diseases such as sarcoidosis and berylliosis [46, 47]. Significantly, granuloma formation is also a common clinical feature of Crohn's disease and their aetiology still requires adequate explanation [48].

Currently, clinical data on the role of dietary microparticles remains inconclusive. A small pilot study, in which Crohn's patients were fed a microparticle-low diet, showed improvements in disease symptoms [49]. However a more recent placebo controlled study, incorporating a larger cohort of active Crohn's disease patients, was unable to detect significant benefits of a reduced microparticle diet – although these patients were also treated with corticosteroids thus potentially masking subtle dietary effects [50]. Based on this recent clinical trial data, and the data presented here, it seems unlikely that low-level accumulation of dietary microparticles in macrophages is a significant driving factor for the pathogenesis of Crohn's disease. However, it is currently not known if dietary microparticles can affect the development of adaptive immune responses – perhaps via subtle effects on dendritic cells and skewing of Th1/Th2 responses, or if they have a role to play in NOD2-associated Crohn's disease. Furthermore, their ability to impair macrophage phagocytic activity and act as adjuvants in the presence of bacterial antigens (which are known to drive IBD) suggests that high concentrations of dietary microparticles should not be completely ruled out as a potential contributors to intestinal inflammation.

Acknowledgements. This work was funded by the Eli and Edythe L. Broad Foundation.

References

[1] Mayberry JF, Rhodes J, Allan R, Newcombe RG, Regan GM, Chamberlain LM et al. Diet in Crohn's disease two studies of current and previous habits in newly diagnosed patients. Dig Dis Sci 1981; 26: 444–8.
[2] Mahmud N, Weir DG. The urban diet and Crohn's disease: is there a relationship? Eur J Gastroenterol Hepatol 2001; 13: 93–5.
[3] Russel MG. Changes in the incidence of inflammatory bowel disease: what does it mean? Eur J Intern Med 2000; 11: 191–6.
[4] Martini GA, Brandes JW. Increased consumption of refined carbohydrates in patients with Crohn's disease. Klin Wochenschr 1976; 54: 367–71.
[5] Lomer MC, Thompson RP, Commisso J, Keen CL, Powell JJ. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. Analyst 2000; 125: 2339–43.
[6] 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: 123–30.
[7] Shepherd NA, Crocker PR, Smith AP, Levison DA. Exogenous pigment in Peyer's patches. Hum Pathol 1987; 18: 50–4.
[8] Urbanski SJ, Arsenault AL, Green FH, Haber G. Pigment resembling atmospheric dust in Peyer's patches. Mod Pathol 1989; 2: 222–6.
[9] 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: 390–5.
[10] 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: 947–55.
[11] Smith MW, Thomas NW, Jenkins PG, Miller NG, Cremaschi D, Porta C. Selective transport of microparticles across Peyer's patch follicle-associated M cells from mice and rats. Exp Physiol 1995; 80: 735–43.
[12] Neutra MR. Current concepts in mucosal immunity. V Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system. Am J Physiol 1998; 274: G785–91.
[13] Snipes MB. Long-term retention and clearance of particles inhaled by mammalian species. Crit Rev Toxicol 1989; 20: 175–211.
[14] Florence AT. The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. Pharm Res 1997; 14: 259–66.
[15] Evans EJ. Cell damage in vitro following direct contact with fine particles of titanium, titanium alloy and cobalt-chrome-molybdenum alloy. Biomaterials 1994; 15: 713–7.

[16] Redline S, Barna BP, Tomashefski JF, Jr., Abraham JL. Granulomatous disease associated with pulmonary deposition of titanium. Br J Ind Med 1986; 43: 652–6.
[17] Lee JC, Halpern S, Lowe DG, Forbes A, Lennard-Jones JE. Absence of skin sensitivity to oxides of aluminium, silicon, titanium or zirconium in patients with Crohn's disease. Gut 1996; 39: 231–3.
[18] 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: 99–105.
[19] 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: 1543–53.
[20] Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 1998; 101: 890–8.
[21] Hamilton T. The Macrophage: Oxford University Press, 2002: 73–102.
[22] Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003; 3: 23–35.
[23] Kobzik L, Huang S, Paulauskis JD, Godleski JJ. Particle opsonization and lung macrophage cytokine response. In vitro and in vivo analysis. J Immunol 1993; 151: 2753–9.
[24] Hoves S, Krause SW, Schutz C, Halbritter D, Scholmerich J, Herfarth H et al. Monocyte-derived human macrophages mediate anergy in allogeneic T cells and induce regulatory T cells. J Immunol 2006; 177: 2691–8.
[25] Finberg RW, Re F, Popova L, Golenbock DT, Kurt-Jones EA. Cell activation by Toll-like receptors: role of LBP and CD14. J Endotoxin Res 2004; 10: 413–8.
[26] Flo TH, Ryan L, Kilaas L, Skjak-Braek G, Ingalls RR, Sundan A et al. Involvement of CD14 and beta2-integrins in activating cells with soluble and particulate lipopolysaccharides and mannuronic acid polymers. Infect Immun 2000; 68: 6770–6.
[27] Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001; 19: 275–90.
[28] Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med 2003; 197: 1119–24.
[29] Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med 2003; 197: 1107–17.
[30] Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 1999; 401: 811–5.
[31] Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. Annu Rev Immunol 2002; 20: 825–52.
[32] Grip O, Janciauskiene S, Lindgren S. Circulating monocytes and plasma inflammatory biomarkers in active Crohn's disease: elevated oxidized low-density lipoprotein and the anti-inflammatory effect of atorvastatin. Inflamm Bowel Dis 2004; 10: 193–200.
[33] Takakura R, Kiyohara T, Murayama Y, Miyazaki Y, Miyoshi Y, Shinomura Y et al. Enhanced macrophage responsiveness to lipopolysaccharide and CD40 stimulation in a murine model of inflammatory bowel disease: IL-10-deficient mice. Inflamm Res 2002; 51: 409–15.
[34] Sawada-Hase N, Kiyohara T, Miyagawa J, Ueyama H, Nishibayashi H, Murayama Y et al. An increased number of CD40-high monocytes in patients with Crohn's disease. Am J Gastroenterol 2000; 95: 1516–23.
[35] Liu ZX, Hiwatashi N, Noguchi M, Toyota T. Increased expression of costimulatory molecules on peripheral blood monocytes in patients with Crohn's disease. Scand J Gastroenterol 1997; 32: 1241–6.
[36] Baldassano RN, Schreiber S, Johnston RB, Jr., Fu RD, Muraki T, MacDermott RP. Crohn's disease monocytes are primed for accentuated release of toxic oxygen metabolites. Gastroenterology 1993; 105: 60–6.
[37] Morrow PE. Possible mechanisms to explain dust overloading of the lungs. Fundam Appl Toxicol 1988; 10: 369–84.
[38] Oberdorster G, Ferin J, Morrow PE. Volumetric loading of alveolar macrophages (AM): a possible basis for diminished AM-mediated particle clearance. Exp Lung Res 1992; 18: 87–104.
[39] Dorries AM, Valberg PA. Heterogeneity of phagocytosis for inhaled versus instilled material. Am Rev Respir Dis 1992; 146: 831–7.
[40] Larsson M, Fonteneau JF, Bhardwaj N. Dendritic cells resurrect antigens from dead cells. Trends Immunol 2001; 22: 141–8.
[41] Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000; 191: 411–6.
[42] Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Immunol 2001; 13: 114–9.
[43] Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J Exp Med 2000; 191: 423–34.
[44] Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2002; 2: 965–75.
[45] Ulrichs T, Kaufmann SH. New insights into the function of granulomas in human tuberculosis. J Pathol 2006; 208: 261–9.
[46] Agostini C, Adami F, Semenzato G. New pathogenetic insights into the sarcoid granuloma. Curr Opin Rheumatol 2000; 12: 71–6.
[47] Haley PJ. Mechanisms of granulomatous lung disease from inhaled beryllium: the role of antigenicity in granuloma formation. Toxicol Pathol 1991; 19: 514–25.
[48] Matsumoto T, Nakamura S, Jin-No Y, Sawa Y, Hara J, Oshitani N et al. Role of granuloma in the immunopathogenesis of Crohn's disease. Digestion 2001; 63 Suppl 1: 43–7.
[49] Lomer MC, Harvey RS, Evans SM, Thompson RP, Powell JJ. Efficacy and tolerability of a low microparticle diet in a double blind, randomized, pilot study in Crohn's disease. Eur J Gastroenterol Hepatol 2001; 13: 101–6.
[50] Lomer MC, Grainger SL, Ede R, Catterall AP, Greenfield SM, Cowan RE et al. Lack of efficacy of a reduced microparticle diet in a multi-centred trial of patients with active Crohn's disease. Eur J Gastroenterol Hepatol 2005; 17: 377–84.