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Role of the Alveolar Macrophage in Lung Injury: Studies with Ultrafine Particles

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We conducted a series of experiments with ultrafine particles (~ 20 nm) and larger particles (< 200 nm) of "nuisance" dusts to evaluate the involvement of alveolar macrophages (AM) in particle-induced lung injury and particle translocation in rats. After intratracheal instillation of both ultrafine particles and larger particles of TiO_2, we found a highly increased interstitial access of the ultrafine particles combined with a large acute inflammatory reaction as determined by lung lavage parameters. An additional experiment revealed that intratracheal instillation of phagocytized ultrafine TiO_2 particles (inside AM) prevented both the pulmonary inflammatory reaction and the interstitial access of the ultrafine particles. Another experiment showed that the influx of polymorphonuclear cells (PMN) into the alveolar space unexpectedly decreased with higher doses of ultrafine particles, wheras alveolar epithelial permeability (protein leakage) increased. The divergence between PMN influx into the alveolar space and changes in alveolar epithelial permeability implies that they are separate events. Pulmonary inflammatory parameters determined by lung lavage analysis correlated best with the surface area of the retained particles rather than with their mass, volume, or numbers. Because higher doses resulted in an increased interstitialized fraction of particles, we suggest that inflammatory events induced by particles in the interstitial space can modify the inflammation in the alveolar space detectable by lung lavage. Our results demonstrate the dual role of AM for modifying particle-induced lung injury, i.e., both preventing such injury and contributing to it. We conclude that the increased pulmonary toxicity of ultrafine particles is related to their larger surface area and to their increased interstitial access. Further, we suggest that the interstitialization of particles is important for induction of pulmonary fibrotic reactions and that ultrafine particles of nuisance dusts should have different threshold limit values for occupational exposure because of their increased pulmonary toxicity.

Introduction

The primary role of alveolar macrophages (AM), to protect the lung from inhaled substances, may be reversed in situations when high particulate burdens are deposited and accumulate in the lung. In such cases, referred to as particle "overload," it is conceivable that activated AM release excessive amounts of mediators such as oxygen radicals, proteases, and growth-regulating proteins that are subsequently involved in the pathogenesis of acute and chronic lung injury. These injuries include fibrosis and even lung tumors, which were observed in several long-term inhalation studies at high exposure concentrations, even with so-called nuisance particles (*1*). One characteristic finding in such studies is a serious retardation of AM-mediated particle clearance with a concomitant increase of particles in the interstitial space of the lung.

Such interstitialization is likely to depend on the size of the particles and on the particulate lung burden, as can be deduced from results reported in the literature. The above-mentioned studies on particle overload reviewed by Morrow (*1*) included particles ranging in size from nanometer (carbon black particles, ~ 20 nm) to micrometer (polymer particles, ~ 4 μm). Once a critical particulate lung burden was reached, all of these different particles showed increased interstitialization as evidenced by an increased particulate burden in the lymph nodes. However, in addition to the total particulate lung burden, particle size seems to be a critical determinant for interstitialization. For example, Ferin and Feldstein (*2*) reported that 0.2-μm TiO_2 particles cross the alveolar barrier to an increasing degree when the deposited lung burden in the rat approaches 1 mg; at lower doses they found the TiO_2 particles predominantly in AM. On the other hand, Takenaka et al. (*3*) observed that 20-nm TiO_2 particles (ultrafine particles) appeared readily in the pulmonary interstitial space and in interstitial macrophages after long-term inhalation by rats. (In this paper, we will refer to particle sizes below 50 nm as "ultrafine" particles.) Another nuisance particle, Ga_2O_3, was found to be of low pulmonary toxicity and to behave like inert dusts if administered to rats as large particles (>6 μm) (*4*). In contrast, Wolff et al. (*5*) reported that Ga_2O_3 particles of about 14 nm diameter (ultrafine particles) were highly toxic and fibrogenic when inhaled by rats; their fibrogenic potential was comparable to that of quartz.

Based on these studies we formulated the following hypotheses: *a*) ultrafine particles enter the interstitium more readily than larger-sized particles of the same chemical composition and thereby cause increased pulmonary toxicity. *b*) Phagocytosis of such ultrafine particles by AM prevents the interstitialization

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of these particles and their effects. *c*) Increasing doses of ultrafine particles administered to the lung will lead to an overload situation and thereby elicit even greater effects due to a larger interstitialized fraction. We performed three different types of studies to test these hypotheses: a basic instillation study to compare the kinetics and effects of ultrafine and larger-sized nuisance particles; a reinstillation study to compare the kinetics and effects of phagocytized and free ultrafine particles; and a dose–response study with different types of particles.

Methods

For the basic instillation study, four male Fischer 344 rats each (body weight ~ 220 g) were intratracheally instilled under halothane anesthesia with 500 μg of either TiO_2-F (diameter ~ 250 nm) or TiO_2-D (diameter ~ 20 nm). Control rats received 0.2 mL saline, the vehicle in which the other particles had been suspended. Twenty-four hours later, the rats were exsanguinated under pentobarbital anesthesia, and the lungs were excised and extensively lavaged 10 times with 5 mL saline each aided by gently massaging the lungs. Cells and cell differential and total protein content in the lavage were determined; the amount of Ti in the lavagate, the lavaged lung, and the tracheobronchial lymph nodes was determined by direct current plasma atomic emission spectroscopy after low-temperature tissue ashing.

Because the amount of material remaining in the lavaged lung is not equivalent to the amount of particles retained in the interstitium (the alveolar space cannot completely be lavaged) the amount remaining in the alveolar space had to be determined to quantify the interstitially translocated fraction. In a separate pilot lavagability study, ^{85}Sr-labeled polystyrene particles (~ 6 μg, ~ 4 μm diameter) were intratracheally instilled together with 500 μg TiO_2-F or 500 and 1000 μg TiO_2-D. The assumption was made that the relatively large ^{85}Sr-labeled polystyrene particles will not penetrate into the interstitial space within 24 hr and thus their quantification in the lavagate and lavaged lung will be a good indicator of lavagability of particles from the alveolar space after 10 lavages. The result showed that 81% of the polystyrene particles, even under the condition of inflammation induced by the co-instilled ultrafine particles, were lavagable 24 hr after instillation and that, therefore, 19% remained in the alveolar space. Thus, the total particulate amount retained in the alveolar space ($Dose_{alv}$) can be determined as: $Dose_{alv} = 1.23 \times$ lavaged amount. For the particulate amount that is retained in the tissue (tissue dose) it follows: $Dose_{tissue} = Dose_{total\ lung} - Dose_{alv}$. The fraction labeled as "$Dose_{tissue}$" consists of particles in the epithelial cells as well as those in the interstitium, which cannot be separated by this method. The particle content of the thoracic lymph nodes determined separately is also part of the tissue dose. No polystyrene particles as measured by ^{85}Sr activity were detected in the lymph nodes.

The re-instillation study involved *in vivo* phagocytosis of ultrafine TiO_2-D by AM after the particles were intratracheally instilled into donor rats. This was followed by lavage of the AM containing the ultrafine particles 24 hr later. These cells with the phagocytized particles were re-instilled into recipient rats. The effect was then compared with that of the same amount instilled as free, nonphagocytized particles (Fig. 1). Because the initial instillation of TiO_2-D into the donor rats elicited an inflammatory reaction, we included also groups of rats in this study that were

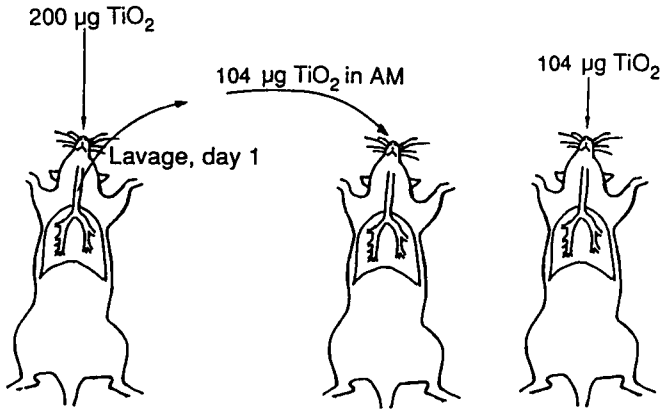

FIGURE 1. Lavaged alveolar macrophages (AM) of donor-rat that contain phagocytized, ultrafine TiO_2 particles 1 day after their intratracheal instillation are instilled into recipient rat. The pulmonary response is compared to that of instilled "free" (nonphagocytized) ultrafine TiO_2 particles.

instilled with either AM only (lavaged from untreated control rats) and with polymorphonuclear neutrophils (PMN) only (separated from the peripheral blood of untreated control rats) to separate a particle effect from a cell effect. Moreover, since it is conceivable that simply coating the ultrafine particles may affect and alter their kinetics and toxicity, similar to phagocytized particles, a group of rats was additionally instilled with serum-coated TiO_2-D particles that had been incubated with rat serum (Gibco BRL, Gaithersburg, MD) for 1 hr and had then been washed twice. Thus, the six groups listed in Table 1 were included in the reinstillation study.

For the dose response study, different TiO_2 types and carbon black particles were intratracheally instilled into four rats each. With regard to TiO_2, both anatase and rutile were used, which are different crystalline forms of TiO_2. The ultrafine TiO_2-rutile (TiO_2-S) was received as a gift from Dr. Siegal at Argonne National Laboratory (Argonne, IL) where its physical characteristics are studied for use in new technology ceramic materials. Its particle size of only 12 nm, approaching almost molecular size, gives it unique ductile properties for use in highly resistant ceramic composites (*6*). Carbon black was used in an additional group because it consists also of ultrafine particles yet has a much higher potency than TiO_2 to induce *in vitro* chemotactic serum factors (*7*). These factors may be potentially important *in vivo* to facilitate AM particle encounter (*8*). The dose–response study consisted of the groups and intratracheal doses listed in Table 2.

Table 1. Groups in the reinstillation study.

Groups	Treatment	*n*
Control (saline)	0.2 mL saline	5
Free particles, TiO_2-D	104 ± 8 μg	10
Phagocytized particles	104 ± 10 μg + 9.5 × 10^6 alveolar macrophages + 3.9 × 10^6 polmorphonuclear neutrophils	5
Alveolar macrophages (from untreated lung)	6.8 × 10^6	4
Polymorphonuclear neutrophils (from peripheral blood)	2.2 × 10^6	4
Coated particles (rat serum)	100 μg	4

Table 2. Groups in the dose-response study.

Group	Dose, μg
TiO_2-F (anatase, ~ 250 nm)	500; 1000
TiO_2-D (anatase, ~ 20 nm)	65; 107; 200; 500; 1000
TiO_2-R (rutile, ~ 220 nm)	500
TiO_2-S (rutile, ~ 12 nm)	500
Carbon black (Cabot, 660 R, ~ 20 nm)	500

Twenty-four hours after instillation, extensive lung lavages were performed to determine inflammatory parameters (cell differential, protein) and dosimetry (dose in alveolar space and tissue) as described for the basic instillation study.

Results

The results of the basic instillation study are given in Table 3. About 15% of the total retained amount 24 hr after instillation of TiO_2-D was retained as "tissue dose," i.e., either in the interstitium or epithelial cells, which was significantly more than after instillation of TiO_2-F. Indicators of an acute inflammatory response were significantly increased as well in the TiO_2-D group, i.e., total lavagable cells, PMNs (50.5% of total cells) and total protein content, whereas cell viability was not affected. These results show that ultrafine TiO_2 particles have a significantly greater pulmonary inflammatory potency than larger TiO_2 particles and that this response is accompanied by an increased interstitial access of these particles.

Results of the reinstillation study are presented in Figure 2. The instillation of free particles and serum-coated particles gave the same results for all measured parameters, i.e., coating the ultrafine particles with rat serum did not prevent the inflammatory response. On the other hand, instillation of the phagocytized ultrafine TiO_2 particles did not cause a significant inflammatory response. An increase in total cell counts and in PMN in this group can be explained by the instilled cells themselves, i.e., 9.5×10^6 AM plus 3.9×10^6 PMN together with the particles (see "Methods"). Likewise, instillation of AMs and PMNs alone did not result in a significant inflammatory reaction either; protein leakage and PMN increase did not occur. With regard to dosimetry it was found that the rats instilled with free particles retained more particles in the extensively lavaged lung than rats instilled with phagocytized particles ($20.4 \pm 0.6\%$ versus $16.9 \pm 1.4\%$ of the total particulate lung burden; $p < 0.05$). Thus, it appears that both the interstitial access of the particles as well as the acute inflammatory reaction did not occur or were significantly reduced when the instilled

Table 3. Results of basic instillation study.[a]

Group	Total retained dose, μg	Tissue dose[b] μg	Tissue dose[b] % of total	Lavage parameters: Total cells, $\times 10^{-7}$	% AM	% PMN	% Viability	Protein, mg/mL
Control	—	—	—	1.15 ± 0.14	98.3 ± 0.5	1.6 ± 0.5	95.5 ± 0.8	0.196 ± 0.023
TiO_2-F	348 ± 37	2.8	0.8	1.48 ± 0.13	90.6 ± 2.1	8.9 ± 2.2	95.8 ± 1.0	0.180 ± 0.019
TiO_2-D	337 ± 41	51.8*	15.4*	4.68 ± 0.73*	48.9 ± 6.6*	50.5 ± 6.2*	96.5 ± 1.5	0.284 ± 0.028*

Abbreviations: AM, alveolar macrophage; PMN, polymorphonuclear neutrophil.
[a]Mean values ± SD, $n = 4$; 24 hr after instillation of 500 μg.
[b]Tissue dose as defined in "Methods".
*Significantly different from TiO_2-F, $p < 0.05$ (*t*-test).

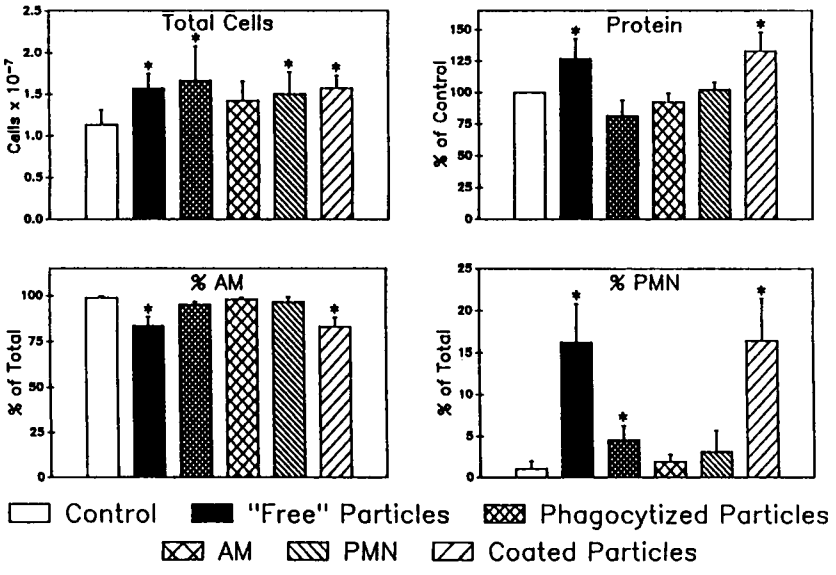

FIGURE 2. Lung lavage parameters from rats instilled with ultrafine TiO_2 particles either as untreated "free" particles, as phagocytized particles, or as serum-coated particles and from rats instilled with inflammatory cells (alveolar macrophages, AM, and polymorphonuclear cells, PMN). (*) Significantly different from control (saline-instilled) rats, $p < 0.05$, two-tailed *t*-test.

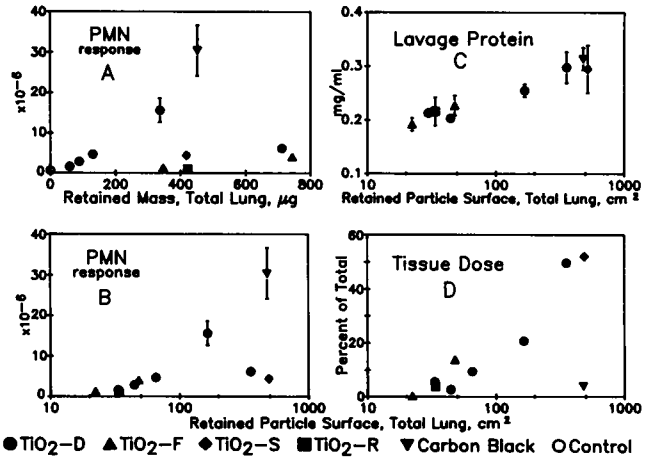

FIGURE 3. Inflammatory response and retained particulate dose 1 day after intratracheal instillation of different doses and types of nuisance particles (mean ± SD). (*A*) Lavaged polymorphonuclear neutrophils (PMN) versus retained mass in total lung compartment. (Total lung compartment = lavagable + nonlavagable particles. The open circle refers to saline-instilled control animals.) (*B*) Lavaged PMN versus surface area of retained particles in total lung compartment. (*C*) Lung lavage protein versus surface area of retained particles in total lung compartment. (*D*) Percentage of retained particles that was interstitialized (tissue dose) versus surface area of retained particles in total lung compartment.

ultrafine particles were phagocytized, whereas both events did occur when these particles were free or coated with serum.

The results of the dose-response study are given in Figure 3. With regard to an inflammatory cell response (absolute numbers of PMN in lavage), it can be seen that an increasing retained mass in the total lung compartment (i.e., lavagable plus nonlavagable amount) of the different particle types did not correlate well with the inflammatory cell influx. Even if the PMN response of only one particle type, TiO_2-D is examined, only instillation of doses from 65 to 500 μg caused a dose-dependent response, whereas instillation of a larger dose, 1000 μg (720 μg retained after 24 hr; Fig. 3*A*) led to a significantly lower response than 500 μg instilled (350 μg retained). Indeed, this lower PMN response is not significantly different from that induced by instillation of 1000 μg TiO_2-F, the larger-sized TiO_2. A similarly low PMN response is caused after instillation of 500 μg of the even finer rutile TiO_2-S, the PMN response being close to the 500 μg larger-sized rutile TiO_2-R. Carbon black, on the other hand, showed the greatest PMN response in the lavage fluid at an administered dose of 500 μg.

Because the most prominent difference of the differently sized particle types lies in the surface area and numbers of particles per given mass, we compared the inflammatory cell response to both of these parameters. The result for the surface area of the retained particles is shown in Figure 3*B*. The PMN response correlated quite well with the retained surface area except for the high dose of TiO_2-D and for TiO_2-S. In contrast, lavage protein, when plotted against the retained surface area, correlated well with all doses and particle types (Fig. 3*C*). Thus, in agreement with our third hypothesis described in the Introduction, the higher doses of ultrafine particles elicited also a higher protein leakage as one sign of inflammation; on the other hand, the cellular PMN influx into the alveolar space, another sign of inflammation, decreased with higher doses of ultrafine particles (Fig. 3*B*), seemingly in contrast to the third hypothesis.

When the fraction of the retained particles that was translocated to interstitium or epithelial cells (here referred to as "tissue dose") was correlated with the surface area of the retained particles, it became apparent that about 50% of the high TiO_2-D dose and of the TiO_2-S dose had become "interstitialized" (Fig. 3*D*). The tissue dose for carbon black, on the other hand, was only minimal (~ 4%). As the two groups with the highest tissue dose (~ 50% of retained TiO_2-D when 1000 μg were administered, and of retained TiO_2-S when 500 μg were administered) are those with a very low PMN response (Fig. 3*B*), it is tempting to propose a causative relationship between the interstitialized dose and the PMN response in the lavage, as discussed below.

Discussion

Our starting hypothesis, that ultrafine particles (~ 20 nm) cause increased pulmonary toxicity because they enter the interstitium of the lung to a greater extent than larger-sized particles, seems to be confirmed by results of the basic instillation study and of the reinstillation study. The lavage characteristics of the larger-sized TiO_2-F particles retained 24 hr after instillation implied that essentially all of these particles had been retained in the alveolar space, whereas the result for the ultrafine TiO_2-D particles was consistent with the interpretation that a significant fraction of these particles was interstitialized, i.e., had either been taken up by epithelial cells or was already in the interstitial space. This increased interstitial access of the ultrafine particles was accompanied by a high inflammatory response as determined from inflammation-specific lavage parameters. Once phagocytized by AM, both the inflammatory response to the instilled ultrafine particles (Fig. 2) and their interstitialization no longer occurred. However, it cannot be concluded from the result of the re-instillation study that the process of particle penetration into the interstitium per se caused the observed inflammatory reaction because the acute phase of particle–cell encounter leading to phagocytosis, including all of the accompanying biochemical events, did not occur in the recipient rats. Therefore, it is as likely that the process of phagocytosis by AM with subsequent release of inflammatory mediators from these cells is the cause of the acute inflammation. This process did occur in the donor rats and was already completed when the phagocytized particles were instilled into the recipient rats. Thus, it can only be concluded from the results of the reinstillation study that either the interstitial access of the ultrafine particles or cellular/molecular events connected with the acute phase of their phagocytosis by AM are responsible for the observed inflammatory response.

Examination of the results of the dose–response study (Fig. 3) suggests that, indeed, the second explanation is more plausible. These results of the dose–response study show that the inflammatory cellular response, as measured by lavage parameters, can decrease with increasing doses of instilled ultrafine particles despite a very large fraction (~ 50%) of the retained dose being "interstitialized." On the other hand, if ultrafine particles are not interstitialized to a significant degree (as was the case with carbon black) a highly increased inflammatory response was ob-

served (Fig. 3). Thus, an increased access of ultrafine particles (50% of TiO_2-D and TiO_2-S) per se did not cause an increased cellular inflammatory response in the alveolar space. These results demonstrate once more the dual role of AM for preventing lung injury on the one hand (results of reinstillation study) and for inducing lung injury on the other (instillation of carbon black particles).

Both carbon black and ultrafine TiO_2 particles elicited an acute inflammatory response (Fig. 3*B*); yet, in contrast to ultrafine TiO_2, only a small portion of carbon black particles in the lung could be attributed to the interstitialized fraction (Fig. 3*D*). We offer the following explanation for the different behavior of carbon black and ultrafine TiO_2 particles: The well-documented ability for different types of particles to induce chemotactic serum factors (probably C5a from the complement cascade) (*8*) is highly effective for carbon black, but only poorly existent for TiO_2 (*7*). Thus, TiO_2 particles depositing in the lung will presumably be present at the site of deposition for a longer period of time as "free" particles, i.e., not phagocytized by AM. These free, ultrafine TiO_2 particles are, therefore, more likely to be taken up by type I epithelial cells and thus gain access into the interstitium. On the other hand, carton black particles induce local chemotactic factors much more effectively (*7*) to attract AM, which effectively phagocytize these particles, thereby preventing the particles from gaining access into the interstitium. In addition, it is conceivable that ultrafine TiO_2 particles disagglomerate faster upon deposition than carbon black particles of the same primary particle size. This disagglomeration may increase the time to be "detected" and subsequently phagocytized by AM and may, therefore, increase the uptake by epithelial cells of these ultrafine TiO_2 particles compared to agglomerates of carbon black particles.

Thus, not all types of ultrafine particles penetrate readily into the pulmonary interstitial space as evidenced by the difference between ultrafine TiO_2 and carbon black particles (Fig. 3*D*). There is further evidence in the literature that not all types of ultrafine particles penetrate to the same degree into the interstitial space: When Patrick and Stirling (*9*) administered colloidal gold particles (15–30 nm) into the alveolar space of rats they found that 5 hr after administration nearly all particles had been taken up by AM. On the other hand, we found that ultrafine Al_2O_3 particles (20 nm) behaved in the same way as ultrafine TiO_2 particles when instilled into the lungs (*10,11*). They elicited a highly inflammatory response and showed an increased interstitial access as compared to larger-sized Al_2O_3 particles.

We observed a decreased inflammatory cell response in the alveolar space in spite of an increased administered dose of the ultrafine TiO_2 particles (Fig. 3*B*). To explain this phenomenon, we propose the following hypothesis: The interstitialization of particles and their subsequent uptake by and activation of interstitial macrophages creates a chemotactic gradient in the lungs that shifts the inflammatory cell influx to the interstitium and thereby decreases respective signs of inflammation in the alveolar space. Figure 4 illustrates this hypothesis by depicting schematically the chemotactically directed movements of PMN from the blood compartment into the interstitial and alveolar compartment. Case A (Fig. 4) reflects a situation in which all administered particles are in the alveolar space (e.g., 24 hr after instillation of 500 μg TiO_2-F) where they will be phagocytized by AM. These in turn are activated to release a host of mediators, including chemotactic factors like LTB_4 (*12*). The resulting chemotactic gradient causes PMN to be attracted into the alveolar space, either via the interstitium or directly across the blood–air barrier. In case B some of the particles have gained access into the interstitium (e.g., 24 hr after instillation of 500 μg TiO_2-D) resulting in a weakening of the chemotactic gradient between the alveolar space and the interstitium. This still causes a high influx of PMN into the alveolus, yet more PMN will also be attracted into the interstitium due to chemotactic factors generated there by particle–cell (e.g., interstitial macrophages) interactions. Case C depicts a situation where many of the particles have reached the interstitium (e.g., 24 hr after instillation of 1000 μg TiO_2-D), thus creating in the interstitium a strong chemotactic stimulus which may even reverse the chemotactic gradient between the alveolus and the interstitium. The result would be a lower number of PMN in the alveolar space than seen after administration of a lower dose of the particles, exactly the finding which we observed in our dose–response study (Fig. 3*A*, *B*). Obviously, this hypothetical chemotactically based behavior of PMN needs to be confirmed in further studies, e.g., by showing PMN accumulation in the interstitial spaces and by assessing the chemotactic activity of the alveolar lavage fluid versus that of interstitial space fluid.

An alternative explanation for the observed effects might be that interstitialized particles are taken up by PMN, which subsequently renders these PMN less capable of migrating through the alveolar epithelium toward a chemotactic gradient. However,

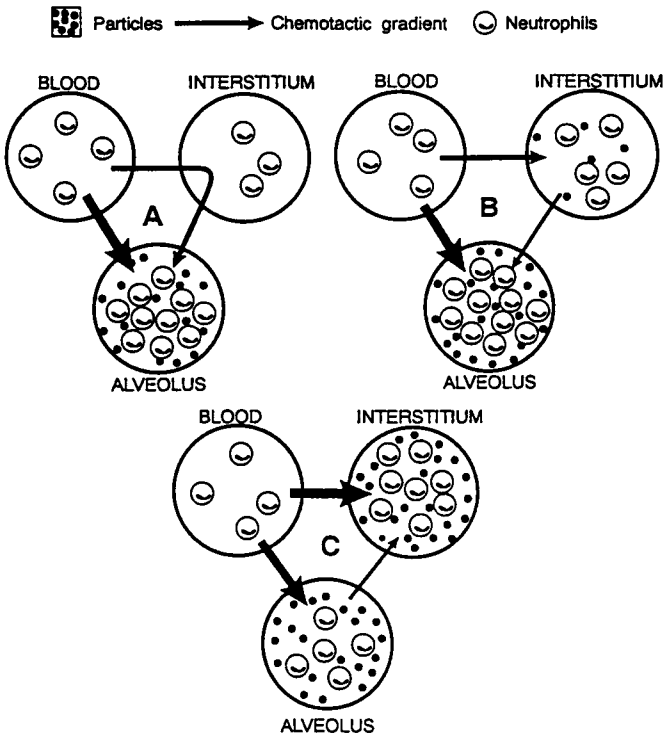

FIGURE 4. Scheme of hypothetical movement of polymorphonuclear neutrophils from blood compartment to pulmonary interstitium and alveoli in response to different particle load. The interstitialization of particles in the lung creates chemotactic gradients that control the cellular inflammatory response in the lung.

additional PMN should in this case emigrate from the blood compartment, which would not be burdened with particles and which should show up in the alveolus.

Our hypothesis outlined in Figure 4 would also be consistent with the observation that protein leakage into the alveolar space, another inflammatory parameter, is not reversed with higher doses of administered ultrafine particles (Fig. 3*C*), unlike the PMN response: mediators, like oxidants and proteases, released from activated macrophages or PMN that potentially damage the epithelial integrity, will act on epithelial structures whether released in the alveolar or interstitial space; the resulting effect (leakiness of epithelium) should be proportional to the total dose retained in the alveolar space plus interstitium, consistent with our findings. This differential behavior of the cellular inflammatory reaction and of epithelial protein permeability implies that PMN influx and epithelial leakiness are separate events, epithelial permeability changes are not a consequence of the transepithelial PMN migration. This conclusion confirms results of earlier studies in our laboratory in which we showed that a huge PMN influx into the alveolar space caused by metallothionein was not accompanied by changes in epithelial permeability ("noninflammatory" PMN influx) and that, vice versa, the cellular influx of PMN was preceded by an increase in epithelial permeability after $CdCl_2$ administration (*13*).

The hypothesis shown in Figure 4 would also be consistent with results observed in chronic inhalation studies leading to particle overloading of the lungs. In such cases of chronic, high-lung burdens of particles, it has been found that an increasing fraction of the particulate lung burden becomes interstitialized. Bellmann et al. (*14*) reported recently that once an overload situation with nuisance particles was reached in the lung, the clearance of a small dose of test particles (a few micrograms) was severely retarded, even when the test particles were given 12 months after exposure to the overload-causing particles had ceased. The clearance retardation of the test particles occurred in spite of the fact that at this time point more than 80% of the AM were free of the overload-causing particles and, therefore, should be available for uptake and effective clearance of the small test-particle dose. An effective interstitially generated chemotactic gradient occurring in the overloaded lung, as shown in Figure 4 *C*, could, however, prevent AM from leaving the lung via the mucociliary escalator and would explain the findings of Bellmann et al. (*14*). Alternatively, as suggested by Bellmann et al. (*14*), sequestered AM in the alveolar space loaded with particles could generate a local chemotactic activity, thereby inhibiting effective clearance of newly deposited particles by other AM.

The surface area of the retained different particle types, rather than their mass, correlated best with the inflammatory response, except, as discussed above, when particle doses were given that led to a larger interstitial dose. This finding can be explained by the hypothesis that the induction of mediators (e.g., chemotactic factors, growth factors, enzymes) by macrophages is a function of the particle surface area that interacts with AM receptors. Other investigators have found likewise that the surface area of retained particles correlated best with a biological response, e.g., asbestos fibers and asbestosis (*15*). Underlying biochemical events responsible for the surface-cell interactions on a molecular basis need to be investigated in further studies.

Finally, we suggest that the interstitialization of particles is an important event that can lead to chronic lung injury, i.e., pulmonary fibrosis. The findings of Wolff et al. (*5*) with ultrafine Ga_2O_3 particles, mentioned in the Introduction, showing a highly fibrogenic potential of these otherwise innocuous particles, emphasize this suggestion. Additionally, results from studies with particle overload that resulted invariably in a high interstitialized particle fraction and subsequently in lung fibrosis are in line with this suggestion (*1*). Bowden et al. (*16*) and Adamson et al. (*17*) concluded likewise from their studies with combined irradiation and silica exposure and after PMN depletion that the interstitialized fraction of silica and its interaction with interstitial macrophages is most important for the fibrogenic potential of silica particles.

Furthermore, an important implication from our studies with ultrafine nuisance particles like TiO_2 and Al_2O_3 (*10,11*) is that ultrafine particles should not be considered as belonging to the category of nuisance dusts (now called PNOC, particles not otherwise classified) (*18*). This is also supported by the aforementioned inhalation studies with ultrafine Ga_2O_3 particles (*5*). The potentially greater health hazard of ultrafine particles requires classifying them separately, possibly even in the same category as quartz. Conceivably, there is a gradual transition to increasing toxicity with decreasing particle size. Although at present ultrafine particles may not be of major importance at the workplace, this may change with increasing future applications and use of new-technology compounds (*19*).

Conclusions

Ultrafine particles (below about 50 nm) of a compound of low *in vivo* solubility and low toxicity deposited in the alveoli of the lung enter the interstitium more readily than larger-sized particles of the same compound. Ultrafine particles elicit a greater inflammatory response in the alveolar space as compared to larger-sized particles, which is not caused by the process of interstitialization per se, but is more likely due to the interaction of the large surface area of the particles with alveolar macrophages and interstitial cells. Our results are consistent with the hypothesis that a high interstitialized particle fraction leads to an interstitial inflammatory response, which can modify inflammatory events in the alveolar space. Inflammatory events (PMN influx, epithelial damage) mediated by AM are effectively inhibited once the ultrafine particles are phagocytized, consistent with the possibility that AM release chemotactic and other factors mainly during the process of phagocytosis of the ultrafine particles. Our results demonstrate the dual role of AM for preventing particle-induced lung injury on one hand and contributing to such injury on the other. Finally, we suggest that occupational exposure limits for nuisance dusts should take into account the increased pulmonary toxicity of ultrafine particles.

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REFERENCES

1. Morrow, P. E. Possible mechanisms to explain dust overloading of the lungs. Fundam. Appl. Toxicol. 10: 369–384 (1988).

2. Ferin, J., and Feldstein, M. L. Pulmonary clearance and hilar lymph node content in rats after particle exposure. Environ. Res.16: 342–352 (1978).
3. Takenaka, S., Dornhöfer-Takenaka, H., and Muhle, H. Alveolar distribution of fly ash and of titanium dioxide after long-term inhalation by Wistar rats. J. Aerosol Sci. 17: 361–364 (1986).
4. Webb, D. R., Wilson, S. E., and Carter, D. E. Comparative pulmonary toxicity of gallium arsenide, gallium (III) oxide or arsenic (III) oxide intratracheally instilled into rats. Toxicol. Appl. Pharmacol. 82: 405–416 (1986).
5. Wolff, R. K., Henderson, R. F., Eidson, A. F., Pickrell, J. A., Rothenburg, S. J., and Hahn, F. F. Toxicity of gallium oxide particles following a 4-week inhalation exposure. J. Appl. Toxicol. 8: 191–199 (1988).
6. Siegel, R. W., Ramasamy, S., Hahn, H., Zongquan, L., and Ting, L. Synthesis, characterization and properties of nanophase TiO_2. J. Mater. Res. 3: 1367–1372 (1988).
7. Oberdörster, G., Ferin, J., Corson, N., Gavett, S., and Hemenway, D. Particle induced *in vitro* chemotaxis of alveolar macrophages (AM) and neutrophils and *in vivo* effects. In: NATO ASI Series, Vol. H30, Effects of Mineral Dusts on Cells (B. T. Mossman and R. O. Begin, Eds.), Springer-Verlag, Berlin, 1989, pp. 313–320.
8. Warheit, D. B., Overby, L. H., George, G., and Brody, A. R. Pulmonary macrophages are attracted to inhaled particles on alveolar surfaces. Exp. Lung Res. 14: 51–66 (1988).
9. Patrick, G., and Stirling, C. The clearance of particles of colloidal gold from subpleural alveoli. Ann. Occuc. Hyg. 32(suppl.): 1164–1166 (1988).
10. Ferin, J., Oberdörster, G., Soderholm, S. C., and Gelein, R. Pulmonary tissue access of ultrafine particles. J. Aerosol Medicine, 4: 57–68 (1991).
11. Oberdörster, G., Ferin, J., Finkelstein, J. N., Wade, P.,and Corson, N. Increased pulmonary toxicity of ultrafine particles? II. Lung lavage studies. J. Aerosol Sci. 21 : 384–387 (1990).
12. Martin, T. R., Rangi, G., Merritt, T. L., and Henderson, W. R. Relative contribution of leukotriene B_4 to the neutrophil chemotactic activity produced by the resident human alveolar macrophage. J. Clin. Invest. 80: 1114–1124 (1987).
13. Oberdörster, G., Guth, D. J., Lee, Y. H., and Mavis, R. D. Lung toxicity of cadmium: Importance of its chemical form. In: Heavy Metals in the Environment, Vol. 1 (T. D. Lekkas, Ed.), CEP Consultants Ltd., Edinburgh, 1985, pp. 571–574.
14. Bellman, B., Muhle, H., Creutzenberg, O., and Mermelstein, R. Irreversible pulmonary changes induced in rat lung by dust overload. Environ. Health Perspect. 97: 189–192 (1992).
15. Timbrell, V., Ashcroft, T., Goldstein, B., Heyworth. F., Meurman, L. O., Rendall, R. E. G., Reynolds, J. A., Shilkin, K. B., and Whitaker, D. Relationships between retained amphibole fibres and fibrosis in human lung tissue specimens. Ann. Occup. Hyg. 32(suppl.): 323–340 (1988).
16. Bowden, D. H., Hedgecock, C., and Adamson, I. Y. R. Silica induced pulmonary fibrosis involves the reaction of particles with interstitial rather than alveolar macrophages. J. Pathol. 158: 73–80 (1989).
17. Adamson, I. Y. R., Letourneau, H. L., and Bowden, D. H. Enhanced macrophage-fibroblast interactions in the pulmonary interstitium increases fibrosis after silica injection to monocyte-depleted mice. Am. J. Pathol. 124: 411–418 (1989).
18. ACGIH. 1990–1991 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. American Conference of Governmental Industrial Hygienists, Cincinnati, OH, 1990.
19. Amato, I. Making the right stuff. Sci. News 36: 108–110 (1989).