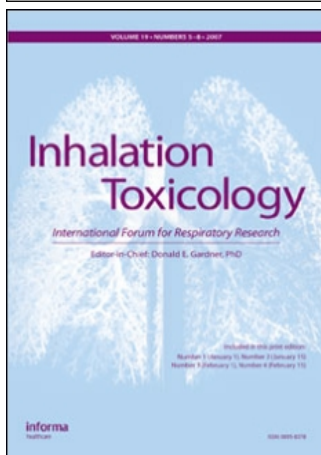


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Total and Regional Deposition of Ultrafine Particles in a Mouse Model of Allergic Inflammation of the Lung

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Total and Regional Deposition of Ultrafine Particles in a Mouse Model of Allergic Inflammation of the Lung

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Epidemiological studies have shown an association between ambient particle inhalation and adverse respiratory health effects. Inhalation of ultrafine particles (UFP, diameter <100 nm) has been suggested to contribute to exacerbation of allergic airway inflammation. Here we analyze the potential effects of allergen sensitization and challenge on total and regional deposition of UFP in the lung. Ovalbumin (OVA)-sensitized and nonsensitized mice were exposed for 1 h to ultrafine iridium particles radiolabeled with ^{192}Ir (UF-Ir) (0.2 mg m^{-3}) at 2 different time points either before or after allergen (OVA) challenge. Additional sensitized and nonsensitized mice were exposed to UF-Ir without allergen challenge. Lung total and regional UF-Ir deposition were calculated according to the distribution of radioactivity in the body and in the excreta during 3 days following UF-Ir inhalation. OVA-sensitized mice showed a 21% relative increase of total UF-Ir deposited fraction compared to nonsensitized mice. When UF-Ir inhalation was performed after allergen challenge, no difference in total UF-Ir deposited fraction between

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sensitized and nonsensitized mice was detectable. Furthermore, no differences in extrathoracic deposition or in regional particle deposition were detected between all experimental groups. This study indicates that allergen sensitization alone can affect UFP deposition in the lungs. Whether higher UFP deposition in sensitized individuals compared to nonsensitized individuals or whether other factors, like alterations in long-term clearance kinetics, contribute substantially to the susceptibility of allergic individuals to particle exposure has yet to be elucidated.

A number of epidemiological studies have shown an association between increased ambient air particle concentrations and adverse respiratory and cardiovascular health effects (Schwartz et al., 1993; Peters et al., 2004; Pope et al., 1995; Dockery & Brunekreef, 1996). There is evidence that the number concentration of ultrafine particles (UFP, $<0.1 \mu\text{m}$ in thermodynamic diameter) is at least stagnant if not increasing in the last decades (Cyrys et al., 2003; Kreyling et al., 2003). UFP, characterized by a high number concentration, low mass concentration, and a large surface area, may contribute to the health effects of ambient particulate matter (Oberdorster, 1995). Due to their small size, they have a higher deposition rate in the peripheral lung compared to fine particles ($<2.5 \mu\text{m}$ in aerodynamic diameter), and they have been shown to cross the pulmonary epithelium and reach the interstitium and then translocate to other organs (Oberdorster, 2001; Kreyling et al., 2002). Asthma is a disease characterized by periodic airflow limitation due to airway inflammation and airway hyperresponsiveness (AHR). Evidence for the effects of particulate air pollution and, in particular, for particles in the ultrafine range on asthma exacerbation and hospital admissions is increasing (Schwartz et al., 1993; Lipsett et al., 1997; Atkinson et al., 2001; Hiltermann et al., 1998; Timonen & Pekkanen, 1997; Tolbert et al., 2000; von Klot et al., 2002; Peters et al., 1997). People with allergic asthma display a higher susceptibility to fine and ultrafine particle exposures (Pope, 2000). There are few human studies on UFP deposition during rest and exercise in healthy individuals and following various pulmonary pathologic conditions (Chalupa et al., 2004; Brown et al., 2002; Frampton et al., 2004; Daigle et al., 2003; Svartengren et al., 1999). These studies show a relatively high pulmonary deposition rate of UFP, which increases inversely to particle size within the ultrafine range and with exercise. Asthmatic subjects as well as subjects affected by moderate to severe airway obstruction are characterized by higher UFP deposition compared to healthy subjects when breathing at rest. To our knowledge, there is no study evaluating either particle deposition patterns in allergen-sensitized versus nonsensitized individuals and particle clearance pathways during the elicitation phase of the allergic response. We used a well-established mouse model of ovalbumin (OVA)-induced allergic inflammation of the lung (Alessandrini et al., 2006) in order to address the questions as to whether UFP deposition in the lung may be altered by allergen sensitization and whether lung regional deposition of UFP may be modified during an allergic

inflammation of the lung. Ultrafine iridium particles radiolabeled with ^{192}Ir (UF-Ir) have been successfully used in order to evaluate UFP regional deposition in the airways and translocation to other organs in a rat model (Kreyling et al., 2002). We used a similar approach to evaluate total UFP pulmonary deposition in OVA-sensitized and nonsensitized mice, assessing regional UFP deposition pathways at different time points during the allergic inflammation of the lung, before and after allergen challenge.

MATERIAL AND METHODS

Animals and Materials

Female BALB/c mice (5–7 wk old, body weight 21.6 ± 1.3 , mean $\pm$ SD) were obtained from Charles River, Sulzfeld, Germany, and housed in an animal facility in isolated ventilated cages (VentiRack, cage type CU-31, Biozone, Margate, UK). They received a standard pellet diet and water ad libitum. The study was conducted under the guidelines of Helsinki convention for the use and care of laboratory animals and was approved by the Government of the District of Upper Bavaria (approval number 209.1/211-2531-82/03) and the Animal Care and Use Committee of the GSF Research Center. All chemicals were purchased from Sigma-Aldrich Chemie, Deisenhofen, Germany, unless otherwise specified.

Allergen Sensitization/Challenge Protocol

Mice were sensitized by repetitive intraperitoneal injections of $1 \mu\text{g}$ ovalbumin (OVA, grade VI)/alum (Pierce Chemical Co., 2.5 mg) in phosphate-buffered saline (PBS) on days 0, 14, 28, 49, and 70. Blood samples were taken before and after sensitization and the levels of OVA-specific immunoglobulin (Ig) E and IgG1 were measured in plasma samples by enzyme-linked immunosorbent assay (ELISA) as described by Herz et al. (1998). When compared to nonsensitized controls, OVA-sensitized mice (day 80) were characterized by high titers of OVA-specific IgE (790 ± 69 vs <2 arbitrary units) and OVA-specific IgG1 (1066 ± 108 vs $<0.1 \mu\text{g/ml}$). Subsequently, allergen challenge was performed with aerosolized OVA (1% in PBS) for 20 min (Figure 1a), or for 15 min on 2 consecutive days (Figure 1b), delivered by a Pari-Boy nebulizer (Pari, Starnberg, Germany) (Alessandrini et al., 2006).

Study Design of Particle Deposition Measurements

A schematic representation of the study protocol is shown in Figure 1. OVA-sensitized (S) and nonsensitized (NS) mice were exposed to ultrafine iridium particles radiolabeled with ^{192}Ir (UF-Ir) for 1 h. In Figure 1a, UF-Ir exposure was performed immediately or 24 h before $1 \times$ OVA challenge (NS and S/UF-Ir/OVA, NS and S/UF-Ir/OVA+24, respectively). In Figure 1b, UF-Ir exposure was performed 24 or 96 h after $2 \times$ OVA challenge (NS and S/ $2 \times$ OVA-24/UF-Ir, NS and S/ $2 \times$ OVA-96/UF-Ir, respectively). Before UF-Ir exposure, spontaneous breathing

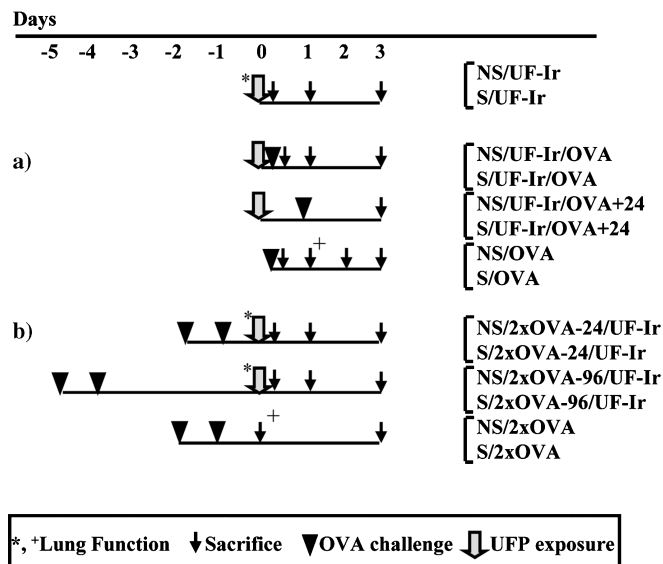


FIG. 1. Experimental design. Protocols of particle exposure, challenge, and sacrifice are depicted on the left. Large arrow: 1 h exposure to ultrafine ^{192}Ir radiolabeled iridium particles; OVA aerosol challenge (∇ , 20 min), ($\nabla\nabla$, 2×15 min), 1% aerosolized OVA; small arrow, time of sacrifice, and for NS/OVA/($2 \times$ OVA) and S/OVA/($2 \times$ OVA) also BAL and histology; asterisk indicates spontaneous breathing pattern measurements and plus sign indicates lung function tests following methacholine provocation performed by body plethysmography. The corresponding designation is displayed on the right. NS, nonsensitized; S, sensitized; OVA, challenged with aerosolized OVA; UF-Ir, exposure to ultrafine ^{192}Ir radiolabeled iridium particles for 1 h; OVA+24, $2 \times$ OVA-24, and $2 \times$ OVA-96 indicate time points when OVA challenge was completed in reference to UF-Ir exposure.

patterns were evaluated by whole-body plethysmography (asterisk, Figure 1). Both in Figure 1a and Figure 1b mice not exposed to UF-Ir served as controls for cellular infiltrate and lung function tests following methacholine provocation (plus sign, Figure 1; NS and S/OVA; NS and S/ $2 \times$ OVA, $n = 4$ /time point). All mice were sacrificed 0, 24, or 72 h after UF-Ir exposure ($n = 4$ /time point). In NS and S/UF-Ir/OVA+24 only, mice were sacrificed 72 h after UF-Ir exposure ($n = 4$).

Collection and Analysis of Bronchoalveolar Lavage (BAL) and Histopathology

In NS/OVA, S/OVA, NS/ $2 \times$ OVA, and S/ $2 \times$ OVA the airways were lavaged 5 times with 0.8 ml PBS. Total protein was measured in bronchoalveolar lavage (BAL) fluid using the Coomassie protein assay (Rockfort, IL). Total cell viability and yield were quantified by trypan blue exclusion and differential BAL cells count (400 cells/sample) was performed on cytopspins (600 rpm for 10 min) fixed and stained with Diff-Quick (Dade Behring, Marburg, Germany). After BAL, the lungs were removed and fixed by immersion in 10% buffered formalin. After

paraffin embedding, sections for microscopy were stained with hematoxylin–eosin or with periodic acid Schiff (PAS).

Lung Function Tests

A whole-body plethysmography system (Buxco Electronics, Sharon, CT) was used to assess both spontaneous breathing patterns and airway hyperresponsiveness (AHR) in unrestrained animals as described previously (Alessandrini et al., 2006). For the evaluation of spontaneous breathing patterns, tidal volumes, respiratory rates, and minute ventilation were measured for 40 min prior to UF-Ir exposures (asterisk, Figure 1, $n = 4$ /group). AHR following methacholine provocation was measured in NS and S mice 24 h after $1 \times$ or $2 \times$ OVA challenge and in untreated mice (plus sign, Figure 1, $n = 4$ –10/group). The doses of methacholine aerosol applied were the following: 10 mg/ml methacholine nebulized for 1, 2, or 4 min (M1–M3), and 40 mg/ml methacholine for 2 min (M4). The “enhanced pause” (PenH) determined before and after methacholine exposure was applied as an index of AHR (Alessandrini et al., 2006).

Aerosol Production and Characterization

Ultrafine ^{192}Ir aerosol was produced with a spark generator as previously described (Kreyling et al., 2002; Figure 2). Specific iridium activity of the aerosol was 10 GBq g^{-1} at the reference date. Mass concentration was calculated to be 0.2 mg m^{-3} from the radioactivity measurement of an integral filter sample during exposure and the sampled aerosol volume. Size distribution parameters were the following: particle number concentration $9.8 \pm 0.9 \times 10^6/\text{cm}^3$, count median diameter 35 nm; geometric standard deviation 1.7, the first being continuously monitored by a condensation particle counter (CPC 3022A, TSI, Aachen, Germany) and the second with a differential particle mobility analyzer (Classifier 3071 + CPC 3010, TSI, Aachen Germany). Particles were chain agglomerates consisting of 2- to 4-nm sized primary particles. Secondary ion mass spectroscopy revealed that the chain agglomerates were oxidized on their surface (Szymczak et al., 2006).

UF-Ir Inhalation and Sacrifice

The exposure chamber is depicted in Figure 2. Each mouse was placed in a restraint tube with a breathing hole (6 mm diameter) in the conical front and a plunger in the back inserted to gently push the mouse towards the breathing hole. Each restraint tube was inserted into a port of a slim tower-shaped aerosol chamber holding 20 ports at one of its sides. A uniform laminar vertical replacement flow assured uniform aerosol exposure of each mouse. Oxygen concentration was maintained at 21% at 22°C and relative humidity at about 50%. For radiation protection the entire aerosol system was maintained at $-(50\text{--}100) \text{ Pa}$ low pressure. Animals were selected for different UF-Ir exposures in a randomized fashion.

After exposure, the mice were housed in individually ventilated cages (VentiRack, cage type CU-31). Urine and feces

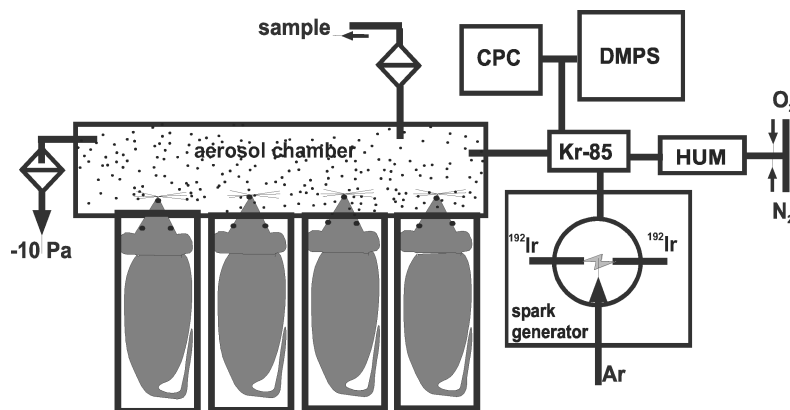


FIG. 2. Mouse exposure chamber for iridium ultrafine particles (UF-Ir) inhalation. The ultrafine ^{192}Ir aerosol was produced with a spark generator (Kreyling et al., 2002). The spark frequency was 3 Hz in an argon stream of 3 L min^{-1} ; the aerosol was neutralized by a radioactive ^{85}Kr source. The aerosol then was diluted with nitrogen and adjusted to 21–23% oxygen, 50–60% relative humidity, and $22\text{--}25^\circ\text{C}$. The aerosol was continuously monitored by a condensation particle counter (CPC) and a differential particle mobility analyzer (DMPS). Specific iridium aerosol activity was calculated from the radioactivity measurement of an integral filter sample and the sampled aerosol volume. For radiation protection the entire aerosol system was maintained at -10 Pa low pressure and the entire apparatus was built in a glove-box-like containment highly ventilated through an absolute particle filter.

samples were collected quantitatively together with the cage bedding until the end of the experiments. After sacrifice, the skin was completely removed and sampled and the lungs and gastrointestinal tract (GIT), head (without the larynx), and the remaining carcass were sampled separately. A complete balance of ^{192}Ir activity retained in the body and cleared by excretion out of the body (Kreyling et al., 1993) was quantified by gamma spectroscopy (Kreyling et al., 1986, 2002). Hence, total activity A_{tot} represents the deposited activity in the respiratory tract and on the pelt of each mouse.

Calculation of Deposition Parameters

Total deposited fraction (TDF). TDF is the fraction of inhaled UFP deposited in the respiratory tract and is defined as total particle deposition in the mouse (TD) divided by inhaled particles (D_{in}). TD of UFP is calculated from the quantitatively determined ^{192}Ir radioactivity (A_{tot}) found in each mouse after subtracting mean external pelt contamination (A_{pe}) measured in skin samples of mice killed within 5 min after UF-Ir exposure and put on ice immediately after sacrifice (time 0). D_{in} is estimated from the inhaled volume (V_{in}) using lung function data of subsets of spontaneously breathing mice, described earlier (asterisk, Figure 1), mean particle number concentration (C_{ae}), and specific aerosol activity (A_{sp}). A_{sp} was determined from parallel filter sampling (Figure 2).

$$\text{TDF} = \frac{\text{TD}}{D_{\text{in}}} = \frac{A_{\text{tot}} - A_{\text{pe}}}{V_{\text{in}} C_{\text{ae}} A_{\text{sp}}} \quad [1]$$

For the calculation of TDF in S and NS mice, TDF data of S and NS mice not challenged (NS and S/UF-Ir) were pooled with

the data of S and NS mice challenged after UF-Ir exposure (NS and S/UF-Ir/OVA and NS and S/UF-Ir/OVA+24) because here allergen challenge was performed at a time point where particle deposition had already occurred.

Regional deposited fraction (RDF). RDF was calculated for extrathoracic airways in the head versus intrathoracic airways (trachea and bronchial tree) and versus alveoli given as fraction of the UFP deposition in the entire respiratory tract. Extrathoracic particle deposition fraction (ETDF) was determined from the radioactivity measurement of the head, A_{head} , after removal of its pelt in those animals killed at time 0.

$$\text{ETDF} = \frac{\text{Avg}[A_{\text{head}}(t = 0)]}{(A_{\text{tot}} - A_{\text{pe}})} \quad [2]$$

Mean ETDF were used for mice killed at later time points. In order to compare ETDF of control NS mice (NS/UF-Ir) versus all animals, ETDF of each mouse either only sensitized (S/UF-Ir) or only challenged (NS/2×OVA-24h/UF-Ir and NS/2×OVA-96h/UF-Ir) or both sensitized and challenged (S/2×OVA-24h/UF-Ir and S/2×OVA-96h/UF-Ir) was normalized to the mean ETDF relative to control (NS/UF-Ir). RDF data were similarly corrected for pelt contamination as for TDF. Deposited fractions in airways were distinguished functionally from alveolar fraction ADF by fast particle clearance into the GIT and fecal excretion versus retained particles in the lungs 72 h after exposure. Tracheobronchial tree deposition ITDF was then calculated from the radioactivity found in the GIT A_{GIT} and total excretion A_{Excr} in mice killed up to 72 h after exposure after subtraction of ETDF.

$$\text{ITDF} = \frac{A_{\text{GIT}} + A_{\text{Excr}}}{(A_{\text{tot}} - A_{\text{pe}})} - \text{ETDF} \quad [3]$$

It should be noted that clearance from extra- and intrathoracic airways will have started and some will have taken place during the exposure itself, which took 1 h. This would lead to an underestimation of ETDF and ITDF. Alveolar deposition ADF was determined from radioactivity found in the lungs of mice killed 72 h after exposure.

Statistical Analysis

For statistical evaluation, one-way analysis of variance (ANOVA) with post hoc Scheffé comparisons was used for cellular and biochemical analysis of bronchoalveolar lavage fluid (BALF) and lung function tests. *t*-Tests were used to compare data of total and regional deposition of S versus NS mice in all experimental groups. In addition, a multiway between-groups ANOVA with multiple dependent variables (MANOVA) with post hoc Scheffé comparisons was used to compare data of TDF in nonsensitized versus sensitized mice with different OVA challenge conditions. All data were expressed as mean $\pm$ SD. A *p* value $< .05$ was considered to be significant.

RESULTS

Allergic Airway Inflammation Induced by Allergen Aerosol Challenge

Differential cell counts and protein concentration in BALF from S and NS mice at different time points after $1 \times$ and $2 \times$ OVA challenge are shown in Figure 3, a and b, respectively. In both cases, S mice showed a significantly increased number of neutrophils, lymphocytes, eosinophils, and BALF total protein compared to NS mice. No significant variation was detected in the number of alveolar macrophages (data not shown). Histological evaluation of lung samples from S mice showed inflammatory infiltrate and mucus hypersecretion starting 48 h after $1 \times$ OVA challenge and 24 h after $2 \times$ OVA challenge and increasing at later time points, as shown in a representative sample (Figure 3d). NS mice showed no lung histological alterations following OVA challenge (Figure 3c). Lung function tests performed 24 h after the last OVA challenge showed increased PenH following increasing methacholine concentrations in S compared to NS and untreated mice (Figure 3e).

Increased Total Particle Deposition in Sensitized Versus Nonsensitized Mice

NS and S mice showed no significant difference in spontaneous breathing patterns measured before UF-Ir exposure (Figure 4a). Mean TDF in NS mice either not challenged or challenged after UF-Ir exposure was 34% and in the corresponding S mice was 42% of total inhaled particles (Figure 4b). The multivariate analysis of variance showed that the difference between NS and S mice was statistically significant ($p < .01$). In addition, the difference was statistically significant independently from the challenge condition ("OVA challenge" effect: $p = .8$). ETDF represented a very low fraction of TDF (Figure 4b, insert). No significant differences in ETDF were detected between

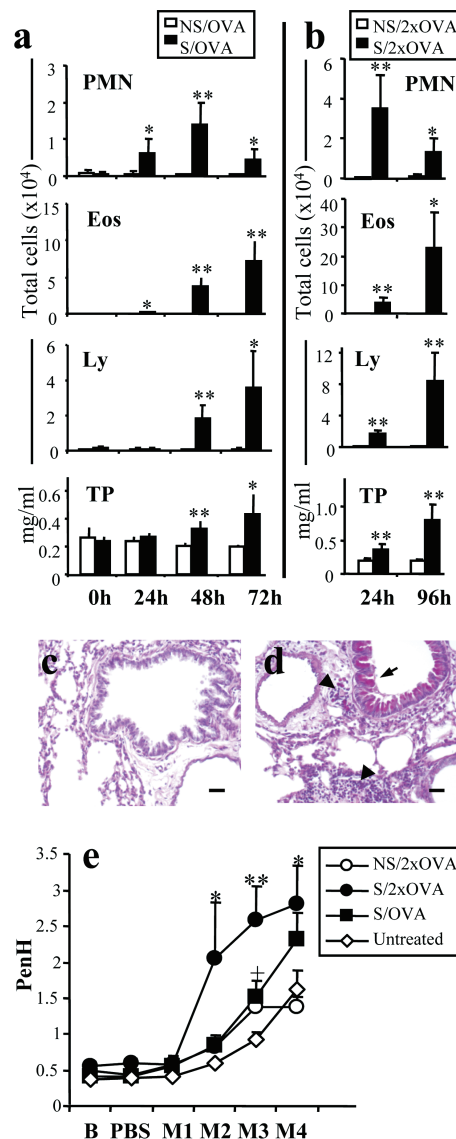


FIG. 3. BAL cellular and biochemical constituents, lung histology, and lung function. (a) Nonsensitized and OVA challenged mice (white bars) and OVA sensitized and challenged mice (black bars) were evaluated 0, 24, 48, and 72 h after $1 \times$ OVA challenge and (b) 24 and 96 h after $2 \times$ OVA challenge. Results are expressed as mean $\pm$ SD ($n = 4$ /group/time point; $*p < .05$, $**p < .01$ vs. NS/OVA (a) or vs. NS/ $2 \times$ OVA (b)). PMN, polymorphonuclear granulocytes; Eos, eosinophils; Ly, lymphocytes; TP, total protein. (c, d) Representative lung sections from nonsensitized mice (NS/ $2 \times$ OVA, c) and from sensitized mice (S/ $2 \times$ OVA, d) retrieved 96 h after $2 \times$ OVA challenge and stained with PAS. Inflammatory infiltrate and mucus hypersecretion are indicated by arrowheads and arrows, respectively. Scale bar, 30 μ m. (e) Lung function measurements were performed by noninvasive whole-body plethysmography. Airway hyperresponsiveness (represented by PenH) was measured 24 h after the last OVA challenge. Data are expressed as mean $\pm$ SEM ($n = 4-10$). Significance indicated by $*p < .05$, $**p < .01$ vs. untreated; $^+p < .05$ vs. S/ $2 \times$ OVA.

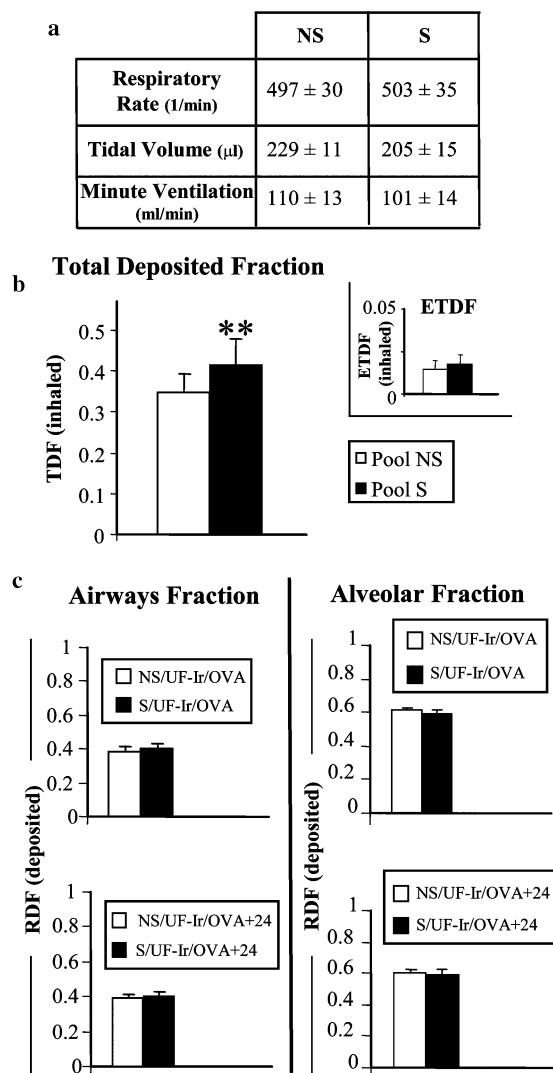


FIG. 4. (a) Spontaneous breathing patterns in untreated mice and OVA-sensitized mice. Data are represented as mean $\pm$ SD measured over 40 min ($n = 4$). (b) Total deposited fraction TDF of inhaled ultrafine ^{192}Ir radiolabeled iridium particles (UF-Ir) in nonsensitized mice (NS, white bars) and OVA-sensitized mice (S, black bars). The data represent the mean of the data obtained from the 0-, 24-, and 72-h time points of sacrifice. Results are expressed as mean $\pm$ SD ($n = 28$; significance indicated by $**p < .01$ vs. NS). Insert: extrathoracic particle deposition fraction (ETDF) in nonsensitized (NS, white bar) and OVA-sensitized (S, black bar) mice depicted in (b) sacrificed at time 0 (mean $\pm$ SD, $n = 8$). (c) Regional particle deposition (as a fraction of total deposited UF-Ir) in nonsensitized (NS, white bars) and OVA-sensitized mice (S, black bars), exposed to UF-Ir at 0 h (NS/UF-Ir/OVA; S/UF-Ir/OVA) and 24 h (NS/UF-Ir/OVA+24; S/UF-Ir/OVA+24) prior to OVA challenge. The data represent the 72-h time points of sacrifice after UF-Ir exposure. The airways fraction is the sum of extra- and intrathoracic deposition.

S and NS mice exposed to UF-Ir followed or not by OVA challenge. The ETDF data were calculated also relatively to control NS/UF-Ir (1 ± 0.3 in NS vs. 1.2 ± 0.4 in S). Similarly, no significant differences in RDF (Figure 4c), calculated as a fraction either of the inhaled or of the deposited particles, were detected between S and NS mice, exposed to UF-Ir followed or not by OVA challenge at any of the time points evaluated.

Allergen-Induced Airway Inflammation Has No Effect on Total and Regional Particle Deposition

NS and S mice evaluated 24 and 96 h after $2 \times$ OVA challenge showed no significant difference in spontaneous breathing patterns (Figure 5a). Upon UF-Ir exposure, no changes in mean TDF were observed between NS and S mice with $2 \times$ OVA challenge (Figure 5b). Furthermore, no difference was detected between S mice with and without OVA challenge (Figures 5b and 4b, respectively). However, $2 \times$ OVA challenge significantly increased deposited fractions in NS and in S/ $2 \times$ OVA-24/UF-Ir (Figure 5b) as compared to NS mice without OVA challenge ($p < .01$ vs. NS, Figure 4b). No significant differences in extrathoracic deposition as calculated relative to control NS/UF-Ir (1 ± 0.3 in NS vs. 1.3 ± 0.6 in S mice and 1 ± 0.2 in NS vs. 1.1 ± 0.5 in S mice, exposed to UF-Ir 24 and 96 h after challenge, respectively) or in RDF were detected between S and NS mice exposed to UF-Ir after OVA challenge, in any of the time points evaluated (Figure 5c).

DISCUSSION

Increased levels of air particle concentrations are associated with increased respiratory and cardiovascular mortality and morbidity, as well as worsening of asthma. Ultrafine particles (UFP) have been shown to contribute to these health effects (von Klot et al., 2002; Peters et al., 1997; Alessandrini et al., 2006). In order to investigate whether UFP deposition in the lung was altered by allergen sensitization and whether regional lung deposition of UFP was modified during an allergic inflammation of the lung, we used an allergic mouse model and performed inhalation of ultrafine insoluble iridium particles radiolabeled with ^{192}Ir (UF-Ir). UF-Ir exposures were performed prior to or at two different time points during the elicitation phase of the allergic response. Mice were sacrificed 0, 24, and 72 h after UF-Ir exposure because these times were shown to allow for identification of regional deposition fractions in the respiratory tract (Kreyling et al., 2002).

First, we investigated the effect of allergen sensitization on the deposition of UF-Ir in the lung and evaluated the effect of allergen challenge on the total and regional fractions of UF-Ir deposited in sensitized and nonsensitized mice. Our results showed that OVA sensitized mice had significantly increased UFP deposition compared to nonsensitized mice. Few studies on UFP deposition in humans under normal and pathologic conditions are described in the literature. UFP deposition in healthy human

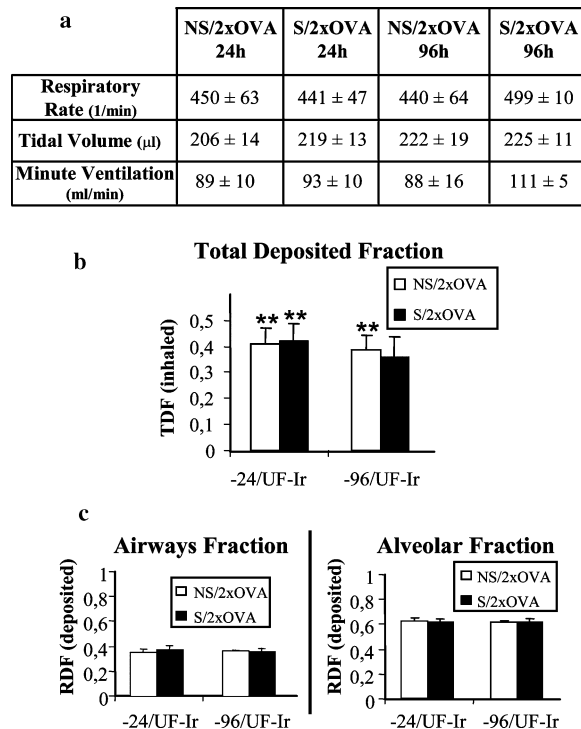


FIG. 5. (a) Spontaneous breathing patterns in nonsensitized and OVA-sensitized mice exposed to OVA challenge on two consecutive days (NS/2×OVA and S/2×OVA, respectively) and evaluated 24 and 96 h after OVA challenge. Data are expressed as mean ± SD measured over 40 min ($n = 4$). (b) Total deposited fraction of inhaled UF-Ir in nonsensitized (NS/OVA-24/UF-Ir and NS/OVA-96/UF-Ir, white bars) and OVA-sensitized mice (S/OVA-24/UF-Ir and S/OVA-96/UF-Ir, black bars), exposed to UF-Ir 24 and 96 h after OVA challenge. Results are expressed as mean ± SD of the data obtained from the 0-, 24-, and 72-h time points of sacrifice [significance indicated by $**p < .01$ vs NS (Figure 4b); $n = 12$ /time point]. (c) Regional particle deposition (as a fraction of total deposited UF-Ir) in nonsensitized (NS, white bars) and OVA-sensitized mice (S, black bars), exposed to UF-Ir 24 or 96 h after 2×OVA challenge (2×OVA-24/UF-Ir and 2×OVA-96/UF-Ir, respectively). The data represent the 72-h time point of sacrifice after UF-Ir exposure (mean ± SD, $n = 4$ /time point). The airways fraction is the sum of extra- and intrathoracic deposition.

adults following inhalation via mouth breathing is not age dependent but sex dependent and increases during exercise (Daigle et al., 2003; Kim & Jaques, 2005; Jaques & Kim, 2000). Mouth-breathing subjects with mild to moderate asthma showed increased carbon UFP deposition compared to normal healthy subjects (Chalupa et al., 2004; Frampton et al., 2004). Although it is difficult to compare human results with ours, because of different breathing patterns [in mice characterized by high-frequency, low-tidal-volume ventilation, which results in extremely different flow patterns in the airways (Slutsky et al., 1985)] and ex-

posure conditions employed (mouthpiece versus nose-only exposures), we can draw some points of discussion. Interestingly, we showed that allergen sensitization alone is enough to cause a 21% relative increase in UFP deposition. We have previously shown that allergen-sensitized mice are more susceptible in their allergic response to UFP inhalations (Alessandrini et al., 2006). Whether the increase in particle deposition that we observed in sensitized mice compared to nonsensitized mice could affect the susceptibility to UFP inhalation in sensitized mice has still to be elucidated. To date, we have no clear-cut explanation for the alterations in deposition in sensitized animals. Increased particle deposition can be due to increased airway hyperresponsiveness, resulting in narrowing of airways, as it has been described in humans (Kohlhauf et al., 1999). We measured lung physiological parameters of sensitized versus nonsensitized animals in order to evaluate whether an increase in tidal volume or a decrease in breathing frequency of sensitized animals could be the cause of increased particle deposition. We registered minimal alterations of spontaneous breathing patterns between nonsensitized and sensitized mice prior to particle exposure, which were not sufficient to explain the difference in total UF-Ir particle deposition (Figure 4a). A weak point in our study is that we lack lung function measurements during particle exposures. In fact, we cannot exclude the possibility that the breathing pattern changed during the exposures and this can influence particle deposition. Similarly, no morphometric analysis of airspaces and alveoli has been performed yet in order to evaluate if there is a difference in the lungs of sensitized and nonsensitized animals. Recent studies have shown that the cytokine TNF- α is increased in BAL fluid from sensitized rats and that alveolar macrophages are mainly responsible for this increase (Careau et al., 2006). This cytokine is known to cause bronchial hyperresponsiveness (Kips et al., 1992). We can speculate that allergen-sensitized animals, because of increased TNF- α release in BAL fluid, could be more prone to develop particle-induced bronchial hyperresponsiveness upon UF-Ir exposure. In addition, alveolar space narrowing and alterations in the composition of the bronchiolar fluid can be due to morphological and functional alterations of Clara cells. Clara cells have been shown to undergo functional alterations not only in mouse allergic asthma models (Reader et al., 2003), but also following particle inhalation (Van Miert et al., 2005). Whether alterations in Clara-cell morphology are present following particle exposure in allergen sensitized animals is a possibility that remains to be elucidated.

In our study we evaluated also whether UF-Ir total and regional deposition was modified during the elicitation phase of the allergic response of the lung. We chose an early phase (24 h after 2× OVA challenge, characterized by a peak in PMNs), and a late phase of the allergic inflammation (96 h after 2× challenge, characterized by high eosinophils and lymphocytes, Figure 3b). Surprisingly, our results showed no difference in UF-Ir deposition between sensitized and nonsensitized mice exposed to UF-Ir during the elicitation phase of the allergic response (Figure 5). In S mice OVA challenge did not increase further

UF-Ir deposition, as we would have expected by the known allergen challenge-induced structural and functional alterations, as reported by Blyth et al. (1996), (shown also in Figure 3), and by the results shown in human studies (Chalupa et al., 2004; Frampton et al., 2004).

However, 2× OVA challenge significantly increased TDF in NS mice which reached the level observed in S mice (Figures 5b and 4b). Obviously, OVA challenge affected the respiratory frequency, which in NS/2×OVA mice was slightly lower compared to NS, at similar tidal volumes (Figures 4a and 5a). This results in increased residence times of UF-Ir in the airways, which could in part explain the increased diffusion-dependent deposition. This concept could be applied also in the interpretation of the deposition data of S/2×OVA-96/UF-Ir (Figure 5b). In this group we observed no significant increase in TDF compared to NS mice, but rather a slight yet nonsignificant decrease in particle deposition compared to S mice in Figure 4b. S/2×OVA-96/UF-Ir was in fact characterized by a relative high respiratory rate compared to the other challenged groups shown in Figure 5a and a relatively high minute ventilation compared to S mice shown in Figure 4a.

We evaluated extrathoracic deposition in animals sacrificed at time 0 after particle inhalation and the deposited fractions in extra- and intrathoracic airways versus alveoli during 72 h following UF-Ir inhalation. No significant differences were detected either in extrathoracic deposition between NS, S, and S-and-challenged mice or in regional particle deposition between S and NS mice, challenged either before or after UFP inhalation. The increased TDF observed in S mice and in S and NS mice following 2× OVA challenge occurred equally in both airway and alveolar region, since the fractions shown in Figures 4c and 5c do not differ between different groups. Therefore, not only the fraction deposited in the airways but also the fraction deposited in alveoli were obviously also increased in S mice and in S and NS mice following OVA challenge. This may be an important new finding that may be caused by epithelial thickening, resulting in small decreases of the alveolar spaces with consequent increased diffusion-determined deposition of UF-Ir particles.

Long-term clearance analysis of UF-Ir inhalation in the rat showed that a prominent fraction of UFP was retained in the lungs for up to 6 mo after inhalation (Semmler et al., 2004). Whether or not there is a difference in long-term alveolar retention and clearance pathways of UFP in allergen-challenged sensitized and nonsensitized mice has yet to be elucidated.

To the best of our knowledge, this is the first study evaluating UFP total and regional deposition in a mouse model of allergic inflammation of the lung. Taken together, our data are consistent with the assumption that allergen sensitized individuals are more susceptible to the health effects of UFP exposure. Whether higher UFP deposition in S individuals compared to NS individuals contributes to the higher susceptibility has still to be elucidated. There certainly may be other factors that increase the susceptibility of allergic individuals, e.g., changes in

long-term clearance kinetics or biologic response to particles, which should be a matter for future research efforts.

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