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Repeated inhalations of diesel exhaust particles and oxidatively damaged DNA in young oxoguanine DNA glycosylase (OGG1) deficient mice

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Abstract

DNA repair may prevent increased levels of oxidatively damaged DNA from prolonged oxidative stress induced by, e.g. exposure to diesel exhaust particles (DEP). We studied oxidative damage to DNA in broncho-alveolar lavage cells, lungs, and liver after 4 × 1.5 h inhalations of DEP (20 mg/m^3) in *Ogg1* $^{-/-}$ and wild type (WT) mice with similar extent of inflammation. DEP exposure increased lung levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in *Ogg1* $^{-/-}$ mice, whereas no effect on 8-oxodG or oxidized purines in terms of formamidopyrimidine DNA glycosylase (FPG) sites was observed in WT mice. In both unexposed and exposed *Ogg1* $^{-/-}$ mice the level of FPG sites in the lungs was 3-fold higher than in WT mice. The high basal level of FPG sites in *Ogg1* $^{-/-}$ mice probably saturated the assay and prevented detection of DEP-generated damage. In conclusion, *Ogg1* $^{-/-}$ mice have elevated pulmonary levels of FPG sites and accumulate genomic 8-oxodG after repeated inhalations of DEP.

Keywords: *Oxidative stress, DNA repair, 8-oxodG, OGG1, comet assay, diesel exhaust particles*

Abbreviations: *8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; BAL, broncho-alveolar lavage; BER, base excision repair; DEP, diesel exhaust particle; ERCC1, excision repair cross-complementing 1; ESCODD, European Standards Committee on Oxidative DNA Damage; FPG, formamidopyrimidine DNA glycosylase; GLM, general linear model; HO-1, heme oxygenase 1; HPLC-EC, high performance liquid chromatography-electrochemical; IL-6, interleukin 6; LSD, least significant difference; NER, nucleotide excision repair; OGG1, oxoguanine DNA glycosylase; ROS, reactive oxygen species; SB, strand breaks; SRM, standard reference material*

Introduction

The ability to repair DNA base damage is crucial in the protection against diseases such as cancer. Mammalian cells have evolved several types of repair systems that remove different types of damage generated by reactive oxygen species (ROS) [1]. Oxidized bases are generally repaired by base excision repair (BER) pathways [2,3]. The *OGG1* gene encodes oxoguanine DNA glycosylase, which removes 8-oxo-7,8-dihydroguanine (8-oxoGua) from the DNA as part of the BER pathway [4]. The deoxynucleoside, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), is one of the most studied forms of oxidatively damaged DNA due to the relative ease of measurement and mutagenic property [5]. The importance of OGG1 is underscored by the accumulation of 8-oxodG in DNA with age in the liver of repair deficient *Ogg1* $^{-/-}$ mice [6–8]. Moreover, *Ogg1* $^{-/-}$ mice excrete a lower level of the liberated damaged base (8-oxoGua) in the urine

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[9]. However, many tissues do not accumulate 8-oxodG with age in the *Ogg1* $^{-/-}$ mice [7]; this could be because backup repair systems have the capacity to keep the basal level of DNA damage low when the mice are not challenged with excessive oxidative stress. In situations of oxidative stress, 8-oxodG may accumulate excessively, e.g. shown in the kidney of potassium bromate-exposed *Ogg1* $^{-/-}$ mice, which also had increased mutation frequency [10].

Exposure to particulate air pollution generate ROS by surface-derived redox reactions and causes inflammation, oxidative stress and oxidatively damaged DNA, which may all play a part in the related carcinogenesis [11,12]. Elevated levels of 8-oxodG in the lung have been observed in various experimental rodent models following intratracheal instillation or inhalation of diesel exhaust particles (DEP) [13,14]. In previous experiments, we observed that a single exposure of DEP by inhalation increased the level of 8-oxodG in the lungs of mice. In contrast, if the same dose was fractionated to smaller exposures inhaled on four consecutive days there was no increase in the levels of oxidatively damaged DNA, whereas mRNA expression of *OGG1* increased, suggesting compensatory transcriptional up-regulation of repair [15].

The aim of this study was to investigate the level of 8-oxodG following repeated inhalations of DEP in wild type (WT) and *Ogg1* $^{-/-}$ mice. It is well recognized that determination of 8-oxodG by chromatographic assays may suffer from spurious oxidation of guanine resulting in high levels, unless sufficient precautions are taken during DNA extraction and sample preparation [16,17]. Oxidation of purines, including 8-oxodG, in cellular DNA can also be measured as formamidopyrimidine DNA glycosylase (FPG) sensitive sites by, e.g. the comet assay with levels that are at least 2.5 lower than 8-oxodG levels by the most reliable high performance liquid chromatography (HPLC) measurements [17]. We used this sensitive FPG-based method for detection of oxidatively damaged DNA in WT mice, in which we expected no or minimal effect on 8-oxodG in addition to determination by HPLC with electrochemical (EC) detection. Nucleotide excision repair (NER) may act as backup for deficient BER [18,19] and we measured the mRNA level of excision repair cross-complementing 1 (*ERCC1*), which may be a rate limiting protein in NER. Oxidative stress effects and inflammatory responses were studied in broncho-alveolar lavage (BAL) cells and lung tissue as heme oxygenase 1 (*HO-1*) and interleukin 6 (*IL-6*) mRNA expression, and as the recruitment of macrophages and neutrophils in the BAL fluid.

Materials and methods

Animals

Breeder pairs of *Ogg1* $^{-/-}$ mice were from Institute of Medical Microbiology, The National Hospital, Oslo, Norway. Female 8–10 weeks-old (~20 g body weight) *Ogg1* $^{-/-}$ mice were allocated to the experiment. WT C57BL/6 mice of the same age and weight were purchased from Taconic Europe, Ry, Denmark. Prior to the experiment, the animals were acclimatized for minimally 7 days in polypropylene cages with sawdust bedding (Lignolcel S8, Brogaarden, Gentofte, Denmark). All mice had access to standard diet (Altromin Standard Diet no. 1324, Brogaarden, Gentofte, Denmark) and tap water *ad libitum*. The animals were housed in groups of 10 in an environmentally controlled animal facility operating at 18–22°C, 40–60% humidity and a 12 h light/dark cycle. Institutional guidelines for animal welfare were followed and the Danish government's Danish Ethical Committee for Animal Studies approved the animal experiments.

Inhalation of DEP

Mice were exposed to DEP (Standard Reference Material (SRM) 2975, National Institute of Standards and Technology Gaithersburgh, MD, USA) by nose only inhalation in a 18 l glass/stainless steel chamber as described previously [15] in groups of 7–8 animals. The mice were exposed by repeated 1.5 h bouts of inhalation on 4 consecutive days of 20 mg/m^3 DEP with 9.5×10^5 particles/cm^3 (measured by a condensation particle counter (TSI model 3022A)) and a geometric mean of 215 nm as previously described in Saber et al. (2005) [20]. Control mice were exposed to filtered air. The mean number of particles for the control group was below 500 particles/cm^3.

Preparation of cells and tissue

The mice were sacrificed 1 h after the last exposure. They were anaesthetized by i.m. injection of a mixture of 1.5 mg/kg Hypnorm (Janssen, Titusville, NJ, USA) and 1.5 mg/kg Dormico (Roche, Basel, Switzerland). Isolation of BAL cells was performed as described previously [21]. The lungs and liver were isolated and snap frozen in liquid nitrogen and stored at −80°C.

Identification of BAL fluid cells

Aliquots of the BAL cell suspension were used to determine the total cell number by haemocytometer counting. For cell identification, cells were collected on microscope slides by centrifugation at 1000 rpm for 4 min in a Cytofuge 2 (StatSpin, Bie and Berntsen, Rødovre, Denmark). The slides were fixed with 96% ethanol and stained with May-Grünwald-Giemsa stain. 200 cells were evaluated for each preparation.

Oxidatively damaged DNA

The level of oxidatively damaged DNA was measured as 8-oxodG relative to dG in tissues of the right lung and liver by HPLC-EC detection after isolation by the

sodium iodine precipitation method and digestion of the DNA as recommended by the European Standards Committee On Oxidative DNA Damage (ESCODD) [22]. The level of DNA strand breaks (SB) and oxidized purines was determined by the comet assay with enzymic digestion of nuclei by FPG from *E. coli* (the FPG enzyme preparation was a gift from Professor Andrew Collins, University of Oslo, Norway). Detailed description of the comet assay has been reported previously [23]. The level of DNA damage was scored in 100 randomly selected nuclei as classes from zero to four, thus giving a range of 0–400 for each sample. The score of FPG sites was translated into lesions per 10^6 dG by using our own individual X-ray calibration curve with the slope of 0.0238 Gy per score and assuming that 1 Gy generates 1160 lesions per genome [24]. We have used a conversion factor of 0.0105 lesions/10^6 dG per score (assuming that a diploid cell contains 4×10^{12} Da DNA, corresponding to 6×10^9 bp and 22% of the nucleotides are guanines). The level of SB is reported in arbitrary units. All samples were analysed in duplicate. We have participated in the ESCODD, and in inter-laboratory tests our assays have demonstrated low background levels and clear dose-responsiveness of 8-oxodG as well as of FPG sites at low levels.

The level of FPG sensitive sites was relatively high in the lungs from *Ogg1* $^{-/-}$ mice and we worried that a ceiling level of the assay had been reached. To test this we treated gel-embedded nucleoids from WT and *Ogg1* $^{-/-}$ lungs with Ro 19-8022 (gift from Hoffmann-LaRoche, Switzerland), a well-known photosensitizer and illuminated the samples with white light from a 1000 W halogen lamp before enzyme treatment. This exposure generates oxidized guanines with very little concomitant generation of SB [16]. The experiments were planned as a two-factor study with Ro 19-8022 and mouse strain as determinants. Cryopreserved samples of lung tissue from one WT mice and one *Ogg1* $^{-/-}$ were investigated in three independent experiments.

Measurements of mRNA expression

Tissue from the left lung was homogenized in 2 ml RLT buffer using an Ultra-Turrax T25 (IKA-Werke GmbH, Staufen, Germany). Aliquots of 350 or 700 µl were spun through a QIAshredder column, and total RNA was purified using RNeasy Mini Kit (QIAGEN Nordic, West Sussex, UK). The integrity of the RNA was checked by gel electrophoresis. The cDNA was synthesized using the TaqMan® Gold RT-PCR kit as recommended by the manufacturer (Applied Biosystems, Foster city, CA, USA).

Real-time PCR quantification of *OGG1*, *ERCC1*, and *HO-1* mRNA levels relative to *18S* rRNA were performed by the procedure described previously in Risom et al. (2003) [23]. For quantification of *IL-6* mRNA levels, pre-developed TaqMan® primer/probe assay reagents were used as previously described in Dybdahl et al. (2004) [21].

Statistics

All data were tested for normal distribution using the Shapiro–Wilks test. The groups were also tested for homogeneity of variance with Levene's Test ($P > 0.05$). To fulfil the criteria for normality and homogeneity of variance data on lymphocytes in BAL fluid, lung 8-oxodG, liver FPG, lung *ERCC1* and *OGG1* mRNA, and SB in the liver were logarithmically transformed using the base of ten. The effects of exposure and strain were tested as 2×2 factor design in the General Linear Model (GLM) with $P < 0.05$ as level of significance, and Fisher least significant difference (LSD) test as *post hoc* analysis. The analysis of *OGG1* mRNA level did not include interactions between strain and DEP exposure because of unbalanced statistical design. Because of inhomogeneity of variance between the groups despite transformations, data on *IL-6* mRNA level and SB in BAL cells were analysed with a 2×2 non-parametric Kruskal-Wallis test and correction for the disproportional replication of values in the group of *Ogg1* $^{-/-}$ mice inhaling DEP. The statistical analysis of parametric tests was performed in Statistica 6.0 for Windows, StatSoft, Inc. (1997), Tulsa, OK, USA.

Results

Body weight

Mice at 8–10 weeks of age were treated with DEP as described previously [15]. The *Ogg1* $^{-/-}$ and WT mice had similar bodyweight and the bodyweight did not change in the group of DEP-exposed mice. The average body weight of the *Ogg1* $^{-/-}$ and WT mice exposed to filtered air were 20.1 and 20.8 g, respectively, and the corresponding average values for the DEP-exposed mice were 20.3 g for the *Ogg1* $^{-/-}$ group and 19.0 g for the WT group.

Oxidatively damaged DNA

The levels of 8-oxodG in the lung and liver of WT and *Ogg1* $^{-/-}$ mice are outlined in Figure 1. There was an interaction between the strain and DEP exposure in the lung ($P < 0.05$, GLM); *Ogg1* $^{-/-}$ mice had significantly higher level of 8-oxodG after DEP-inhalation compared to the other groups, whereas there were no significant differences between the 8-oxodG levels in *Ogg1* $^{-/-}$ and WT mice exposed to filtered air or DEP. In the liver, there was no significant differences in the 8-oxodG level between the exposed and unexposed *Ogg1* $^{-/-}$ and WT mice.

The levels of FPG sensitive sites in BAL cells, lung, and liver tissue are shown in Figure 2. In WT mice there was no difference in FPG sensitive sites between filtered

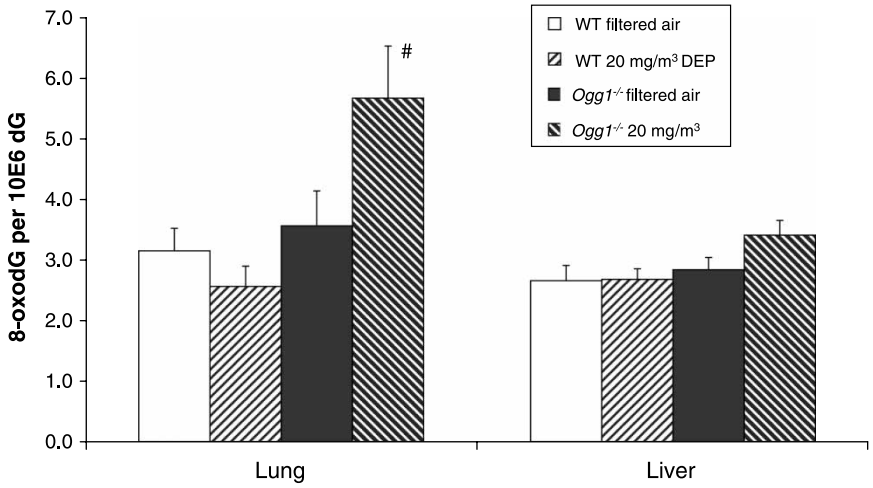

Figure 1. Oxidatively damaged DNA expressed as 8-oxodG by HPLC-EC detection in lung and liver tissue in WT and *Ogg1* $^{-/-}$ mice. Bars are means ± standard error of mean (SEM) ($n = 7–8$). #Interaction between strain and DEP exposure ($P < 0.05$, GLM); *Ogg1* $^{-/-}$ mice exposed to DEP had higher 8-oxodG compared to filtered air exposed, whereas no effect of DEP exposure was observed in WT mice.

air and DEP exposed groups. On the other hand, markedly higher levels of FPG sensitive sites were observed in BAL cells and lung tissue of untreated *Ogg1* $^{-/-}$ mice as compared with WT mice ($P < 0.001$, GLM). No further significant increase was found in DEP exposed *Ogg1* $^{-/-}$ mice. To test if the detection of FPG sites had reached saturation in the comet assay as we performed it, nucleoids from WT and *Ogg1* $^{-/-}$ lung tissue were embedded in the gels and exposed to 1 μM Ro 19-8022 and irradiated 5 min with white light. We could not detect more FPG sensitive sites in samples of *Ogg1* $^{-/-}$ lung nucleoids treated with Ro 19-8022 compared to non-irradiated samples (mean ± standard deviation (SD): 3.3 ± 0.2 lesions/10^6 dG for control and 3.2 ± 0.2 lesions/10^6 dG for Ro 19-8022 treated nucleoids), whereas the corresponding treatment in lung nucleoids of WT mice increased (mean ± SD: 1.0 ± 0.3 lesions/10^6 dG for control and 1.9 ± 0.2 lesions/10^6 dG for Ro treated nucleoids, $P < 0.01$ for interaction between treatment and strain). The level of FPG sites in the *Ogg1* $^{-/-}$ samples was somewhat higher than in the mice used for the DEP-inhalation study, but it is worthwhile to point out that we used samples from a much older mouse (18 months) that had not yet been killed after we stopped the breeding of the *Ogg1* $^{-/-}$ mice. The level of FPG sites in the WT lung samples was also higher than observed in the main study. The difference between the samples in the control experiment is about 2 FPG sites per 10^6 dG; this is similar to the range between the lowest and highest measurements in the WT and *Ogg1* $^{-/-}$ lung samples in the main study, respectively (Figure 2). In the main study, the level of FPG sites in the lung of WT mice was similar to the level detected in a previous study where oxidized DNA lesions

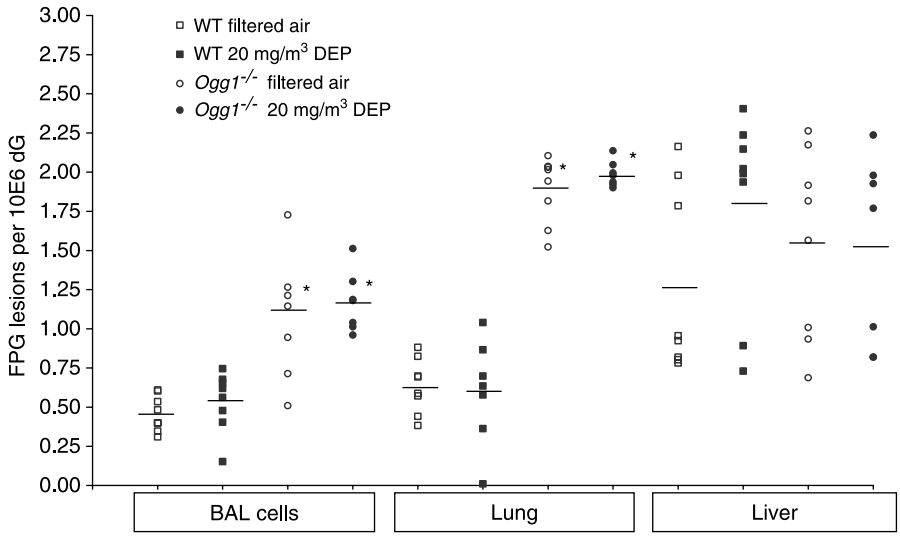

Figure 2. Scatterplot of oxidatively damaged DNA measured as FPG lesions per dG in BAL, lung and liver tissue in WT and *Ogg1* $^{-/-}$ mice. Horisontal lines are means. *$P < 0.01$ for single factor effect of strain (GLM).

Table I. Strand breaks in WT and *Ogg1* $^{-/-}$ mice after exposure to filtered air or DEP.

	BAL cells	Lung	Liver
WT filtered air	113 ± 10	45 ± 6	57 ± 19
WT 20 mg/m^3	83 ± 12	41 ± 5	38 ± 5
Ogg1 $^{-/-}$ filtered air	109 ± 27	55 ± 5	32 ± 5
Ogg1 $^{-/-}$ 20 mg/m^3	83 ± 6	48 ± 4	47 ± 6

Data represent mean ± SEM ($n = 7–8$) in arbitrary units of 100 nuclei per sample, corresponding to a range of 0–400 arbitrary units.

were analysed in fresh samples (0.5 FPG sites/10^6 dG as compared to 0.6 FPG sites/10^6 dG in this study) [23]. Since the samples in this control experiment and the main study were cryopreserved in the same manner, we do not consider that the storage influenced the results.

Because of the intriguing finding of elevated FPG sites in the pulmonary tissue of *Ogg1* $^{-/-}$ mice, we also measured oxidatively damaged DNA in the liver. The level of hepatic FPG sites was similar between WT and *Ogg1* $^{-/-}$ mice, although these sites were markedly more abundant compared to the lung and BAL cells. The levels of FPG sites were not elevated in DEP exposed *Ogg1* $^{-/-}$ or WT mice as compared with counterparts exposed to filtered air. The lack of effect in *Ogg1* $^{-/-}$ mice cannot be related to saturation of the assay related to SB because the levels were similar in *Ogg1* $^{-/-}$ and WT mice (Table I).

Gene expression of ERCC1, HO-1, *and* OGG1

The level of mRNA was measured only in lung tissue (Figure 3). *OGG1* mRNA expression could not be detected in the *Ogg1* $^{-/-}$ mice, thus, verifying the specificity of the probe and the knockout of the OGG1 gene in the strain. For the WT mice there was no change in the mRNA expression level of *OGG1* after repeated DEP-inhalations. The *ERCC1* mRNA level showed no effect of DEP-inhalation exposure or strain type. There was an interaction between the strain and DEP exposure on the mRNA levels of *HO-1* ($P < 0.05$, GLM); *Ogg1* $^{-/-}$ mice had significantly higher mRNA expression levels of *HO-1* after DEP-inhalation ($P < 0.001$, *post hoc* LSD) whereas in the WT mice *HO-1* mRNA levels were unchanged. There was no significant difference in *HO-1* mRNA expression between the two groups of un-exposed mice.

Inflammation

The number of cells and population in the BAL fluid are summarized in Table II. Total cell number revealed interaction of strain and DEP exposure ($P < 0.05$, GLM), and the number of cells in BAL fluid was increased more in WT than in *Ogg1* $^{-/-}$ mice (*post hoc* LSD: $P < 0.001$ and $P < 0.05$ for WT and *Ogg1* $^{-/-}$ mice, respectively). This may be because of higher background total cell level in *Ogg1* $^{-/-}$ mice ($P < 0.01$, *post hoc* LSD). The BAL fluid from mice exposed to filtered air was dominated by macrophages. In the DEP exposed mice the composition of cells in the BAL fluid showed a marked influx of neutrophils at the expense of macrophages, whereas the concentration of lymphocytes remained relatively constant. The number of neutrophils in BAL fluid was increased in both types of mice exposed to DEP ($P < 0.05$, GLM). The level of *IL-6* mRNA is depicted in Figure 4. There was a single-factor effect related to the mouse strain (*Ogg1* $^{-/-}$ mice had lower *IL-6* mRNA levels than the WT mice; $P < 0.05$, Kruskal-Wallis) and the inhalation of DEP strongly increased the *IL-6* mRNA ($P < 0.001$,

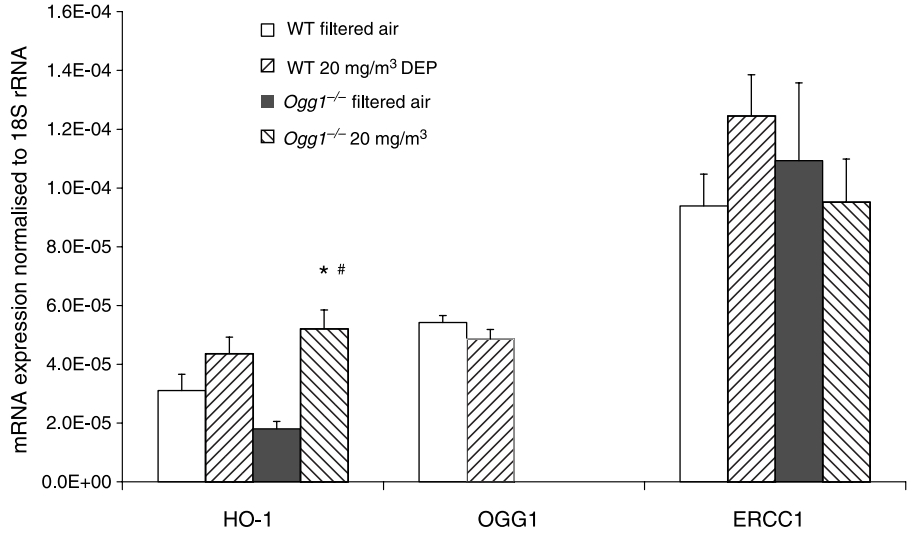

Figure 3. Relative mRNA expression in lung tissue quantified by real-time PCR in WT and *Ogg1* $^{-/-}$ mice. Bars are means ± SEM ($n = 7–8$). #Interaction of strain and DEP exposure on the mRNA levels of *HO-1* ($P < 0.05$, GLM). *$P < 0.001$ (*post hoc* LSD) DEP versus filtered air exposed *Ogg1* $^{-/-}$ mice.

Table II. Cellular profile of BAL cells in WT and *Ogg1* $^{-/-}$ mice after exposure to filtered air or DEP.

	Total cells ($\times 10^4$)⋆	Macrophages/100 cells†	Neutrophils/100 cells†	Lymphocytes/100 cells‡
WT filtered air	2.9 ± 0.9 (4)	89.4 ± 8.5 (8)	2.5 ± 2.5 (8)	8.1 ± 6.8 (8)
WT 20 mg/m^3	8.5 ± 1.4¶ (8)	65.6 ± 9.8¶ (8)	26.4 ± 4.4¶ (8)	7.9 ± 8.6 (8)
Ogg1 $^{-/-}$ filtered air	5.6 ± 1.8§ (7)	92.8 ± 11.4 (8)	4.7 ± 11.9 (8)	2.5 ± 2.0 (8)
Ogg1 $^{-/-}$ 20 mg/m^3	7.8 ± 1.7¶ (7)	66.1 ± 13.4¶ (7)	30.1 ± 14.2¶ (7)	3.9 ± 1.5§ (7)

Mean ± SD ($n = 7–8$) of total cell number in lavage fluid (3 ml); ⋆ $P < 0.05$, GLM: interaction of strain and DEP exposure; † $P < 0.05$, GLM: single-factor effect of DEP; ‡ $P < 0.05$, GLM: single-factor effect of strain; ¶ $P < 0.05$, *post hoc* LSD: versus filtered air exposed control (WT or *Ogg1* $^{-/-}$ mice); § $P < 0.05$, *post hoc* LSD: versus WT (filtered air or DEP exposed).

Kruskal-Wallis). There was no interaction between the strain of mice and DEP exposure indicating that the *Ogg1* $^{-/-}$ and WT had similar response to the DEP exposure.

Discussion

The *Ogg1* $^{-/-}$ mice have provided a valuable tool for analysing genotoxic effects of ROS generating exposures as a model for animals compromised in DNA repair. *Ogg1* $^{-/-}$ mice have previously been shown to accumulate 8-oxodG in hepatocytes with age [6,7]. Curiously, the basal level of oxidatively damaged DNA has not been investigated in the lung of young *Ogg1* $^{-/-}$ mice even though this tissue is exposed to the highest level of oxygen among the internal organs. In this study, we found that young *Ogg1* $^{-/-}$ mice had much higher basal levels of FPG sites in nuclear DNA of BAL cells and lung tissue. Although slightly elevated FPG sites were detected in the liver of *Ogg1* $^{-/-}$ mice this did not reach statistical significance. We used young (8–10 weeks) female mice and our results are not in conflict with the previous report showing indisputable evidence of elevated levels of FPG sites in male mice at 10 months of age [6]. The finding that *Ogg1* $^{-/-}$ mice had elevated levels of oxidatively damaged DNA in the lung even at young age is interesting considering that the lung is exposed to high oxygen tension and a number of environmental exposures, including particulate matter in ambient air, are associated with oxidative stress as well as pulmonary cancers [11,12,25]. A similar level of 8-oxodG was observed in the liver and lung of the unexposed *Ogg1* $^{-/-}$ mice, which most likely reflects the baseline level of 8-oxodG measured by the HPLC method. On the other hand, the liver of WT mice contained higher levels of FPG sites than the lung and BAL cells. This difference could not be detected by the HPLC measurement and may have been below the threshold of the HPLC method. The higher level of FPG sites in the liver compared to the lung of untreated mice is intriguing in the sense that it may be related to a higher level of oxidative stress, slower repair or differences in cell turnover. However, it should be stressed that we have not observed this difference in the level of FPG sites in other of the most recent studies (unpublished observations).

In a previous study, we found increased 8-oxodG levels in lung DNA after a single inhalation with a concentration of 80 mg/m^3 DEP [15]. If the same dose was delivered as 4 × 20 mg/m^3 on four consecutive days there was no induction of 8-oxodG but increased expression of *OGG1* mRNA. In this study, we found

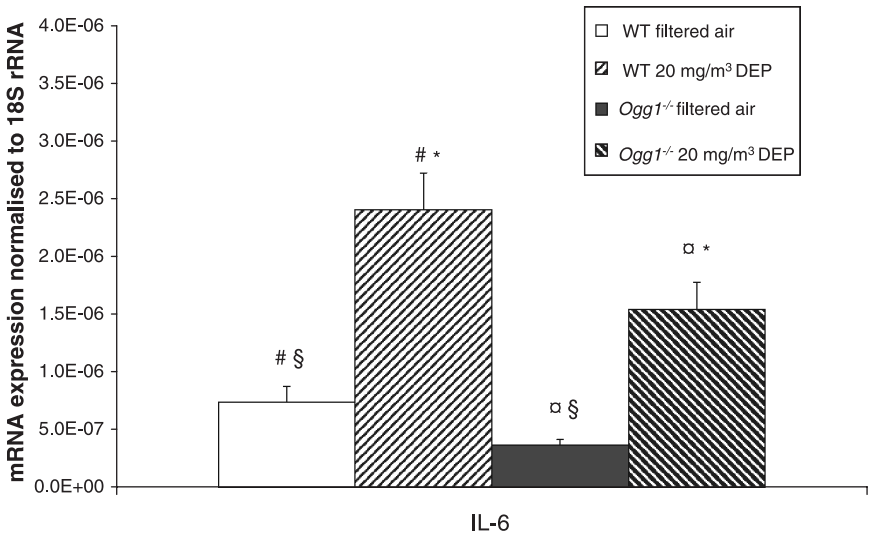

Figure 4. Relative *IL-6* mRNA expression in lung tissue quantified by real-time PCR in WT and *Ogg1* $^{-/-}$ mice. Bars are means ± SEM ($n = 7–8$). Symbols indicate single-factor effects of strain and DEP exposure. WT mice (#) had higher expression than *Ogg1* $^{-/-}$ mice (¤), ($P < 0.05$ for Kruskal-Wallis). Mice exposed to DEP (⋆) had higher expression than filtered air exposed mice (§) ($P < 0.001$, Kruskal-Wallis).

that only *Ogg1* $^{-/-}$ mice had increased levels of 8-oxodG in DNA of lungs when exposed to DEP, despite generally similar level of inflammatory response as in WT mice. The higher induction of HO-1 in the *Ogg1* $^{-/-}$ mice suggests that an apt response to the combat of oxidative stress in these mice requires a more pronounced activity of the antioxidant defence system. It clearly illustrates, that OGG1-mediated repair pathways are pivotal for the defence against oxidatively damaged DNA in the lung. Our data are in accordance with a previous study of *Ogg1* $^{-/-}$ mice exposed to potassium bromate in the drinking water during a 12 week period where the level of 8-oxodG in liver and kidney increased by 25 and 70-fold, respectively [10]. The repair of 8-oxodG in WT mice must be remarkably efficient as judged by the unaltered levels of FPG sites in WT mice exposed to DEP. The induction of repair activity appears to proceed over a few days. Rodents exposed to DEP or crocidolite asbestos fibres had increased 8-oxodG repair activity 5–7 days following intratracheal instillation, whereas shorter time periods indicated a decreased repair activity in DEP-exposed animals [26,27]. In line with this effect we have observed increased levels of 8-oxodG in guinea pig lung tissues 4 days after intratracheal instillation of DEP [14]. Clearly it is difficult to compare inhalation and intratracheal instillation of particles or fibres. Intratracheal instillation may be associated with excessive deposition in the alveolar region, whereas inhalation results in relatively homogeneous distribution of particles in the lung and the particles deposit according to size in the respiratory tract [28]. The deposited dose of DEP after each inhalation exposure with 20 mg/m^3 DEP was 0.06 mg DEP/g lung, assuming a deposition rate of 15%, a tidal volume of 0.15 ml, an average lung weight of 130 mg, 250 inhalations/min, and 90 min exposure period. This yields a deposited dose of 0.24 mg DEP/g lung after four repeated bouts of inhalation assuming that the particles are not cleared from the airways. It has been estimated that the pulmonary clearance system is increasingly impaired when the lung burden of particles exceeds a threshold of 0.5–2 mg DEP/g lung tissue [29–31]. It is likely that the inhalation of DEP in this study provided less deposition in the lower respiratory pathways and better regulation of the repair system as compared to intratracheal instillation. This is supported by the increased *OGG1* mRNA levels that we observed previously in a study of consecutive DEP inhalations [15].

We used two different assays for detection of oxidized guanines with somewhat different results, which may be due to differences in the dynamics of the assays. Low levels of FPG sensitive sites could be detected sensitively and it was possible to detect the differences between lung cells from the two types of mice, but inhalation of DEP did not generate more FPG sites. A possible explanation could be saturation of the current assay protocol as indicated by the inability of detecting additional FPG sites in nucleoids from *Ogg1* $^{-/-}$ mice treated with Ro 19-8022 and white light, which is relatively specific for these lesions [24,32]. Refinements of the comet assay procedure may increase the dynamic range of FPG sites, although this may also hamper the sensitivity in the low end of the range. Altered comet assay procedures could be variations in the pH or length of the alkaline treatment and electrophoresis time [33]. The HPLC-EC could detect the increase in 8-oxodG due to DEP exposure in *Ogg1* $^{-/-}$ mice although the WT only showed minor, and far from significant differences. The lack of elevated hepatic 8-oxodG in the *Ogg1* $^{-/-}$ mice is at odds with previous reports that indicated levels of about 3–12 8-oxodG/10^6 dG in liver DNA [6,8,34]. We cannot explain this discrepancy, but do not doubt the finding for reasons as follows: (1) the level of 8-oxodG in WT mice, which we have measured, is similar to the levels reported in previous publications; (2) the level of 8-oxodG does not exceed the threshold of five 8-oxodG/10^6 dG that is accepted by ESCODD as being close to the true level in normal cells; (3) We have participated in the ESCODD project and could detect a dose-response relationship in coded samples; (4) elevated levels of 8-oxodG in lung cells could be detected following DEP exposure. It is likely that lack of difference in 8-oxodG related to the absence of OGG1 is due to possible spurious oxidation during sample preparation despite the use of the optimum recommended methodology and relatively low background levels of around three 8-oxodG per 10^6 dG as compared with around 0.7 FPG sites per 10^6 dG in WT mouse lung [24]. Recent experimental evidence indicates that FPG detects DNA damage induced by alkylating agents [35,36]. It is also well recognized that the FPG protein has similar catalytic activity toward 8-oxodG and 2,6-diamino-4-hydroxy-5*N*-methyl-formamidopyrimidine, which is a ring-opened derivative of *N*7-methylguanine [37]. ROS generate a number of formamidopyrimidine lesions that are structurally similar to 2,6-diamino-4-hydroxy-5*N*-methyl-formamidopyrimidine. These are commonly regarded as oxidatively generated bases, whereas *N*7-methylguanine is rather a traditional alkylated lesion. The FPG protein originally earned its name for its glycosylase activity toward various formamidopyrimidine lesions, including those generated by alkylating agents. As pointed out by Speit et al. (2004) detection of elevated FPG sites of unknown compounds do not unambiguously depict the mode of action to generation of oxidatively damaged DNA. Although this may outline evidence that FPG is unspecific toward oxidatively damaged DNA, it is worthwhile to consider a few weaknesses if these results are being interpreted beyond the application in genetic toxicology. First, the FPG enzyme is isolated as a cell extract from an over-producing bacterial strain. This extract most likely contains low concentrations of, e.g. the AlkA glycosylase that excises alkylated bases [38]. Cell culture experiments with high concentrations

of alkylating agents may induce high numbers of alkylated bases that are repaired by glycosylases with specificity toward methylated bases. Second, it has been shown that methylating agents cause lipid peroxidation in cell cultures, as a consequence of glutathione depletion [39,40]. Basically, this means that it cannot be ruled out that the elevated levels of FPG sites observed following exposure to alkylating agents is a consequence of oxidative stress in the cells. Third, there is not a uniform consensus about the existence of these alkylated ring-opened derivates of primary alkylated bases. For example, the generation of 2,6-diamino-4-hydroxy-5*N*-methyl-formamidopyrimidine from the primary 7-methylguanine lesions occurs slowly at physiologically pH, whereas there is a fast conversion in strong alkaline solution [41]. In this experiment, the elevated levels of FPG sites in the *Ogg1* $^{-/-}$ mice are not likely to be ring-opened lesions generated by alkylating agents, and the SRM2975 DEP preparation, to the best of out knowledge, does not contain alkylating agents. Accordingly, the two types of assays for measurement of guanine oxidation, based on chromatographic and enzymatic principles may be best suited to relatively high and low levels of damage, respectively.

Curiously, in this study the expression of *OGG1* mRNA in the lung tissue was not increased in the DEP-exposed WT mice in contrast to our previous study on Balb/c mice [15]. In theory, an unaltered expression level of *OGG1* could be explained by the up-regulation of other repair pathways such as NER, or alternatively, by a high capacity of OGG1 enzyme at normal expression level, which may be high in the presently used C57BL/6 mice. ERCC1 is a key nuclease of the NER pathway suggested to be involved in the repair of FPG sites in *Ogg1* $^{-/-}$ mice [7]. We were not able to detect changes in the level of *ERCC1* mRNA expression in the *Ogg1* $^{-/-}$ mice whether exposed to filtered air or DEP. The ERCC1 protein plays a central role in the NER pathway as subunit in the 5′-incision complex [3]. The hallmark of the NER pathway is the excision of damaged nucleotides; using ERCC1 as a marker for the NER process rests on the assumption that it needs to be upregulated if the activity of the whole NER process is increased at the level of transcription. However, it should be noted that measurement of mRNA level does not detect alterations in the activity due to post-translational regulation. Nevertheless, a recent study found excellent agreement between mRNA levels and incision activity of OGG1 when comparing a number of different cell lines [42]. In earlier experiments we have observed increased gene expression of both *ERCC1*, *OGG1*, and *HO-1* mRNA in the lung following thoracic X-ray irradiation of Balb/c mice [23]. There are two possibilities for this discrepancy in *ERCC1*, *OGG1* and *HO-1* mRNA expression in this study compared with our earlier observations: (1) the genetic background of the mice is different, i.e. the earlier experiments were carried out in Balb/c mice and this study was in C57BL/6 mice; (2) the DEP preparation differed, i.e. we used the SRM1650 preparation in the former experiment and the SRM2975 in the present study. We switched to the SRM2975 preparation because the SRM1650 stock is not commercially available anymore. There is evidence that the SRM1650 preparation was associated with increased levels of SB in BAL cells of Balb/c mice [21], whereas the level of SB in BAL cells of C57BL/6 mice was unaltered following the dose of SRM2975 [20]. As such we did not observe alterations in SB of BAL cells. The null effect in terms of mRNA expressions and SB in this experiment could in principle be due to either the DEP preparation or the strain, or it could even be due to an unfortunate combination of both less potent preparation (SRM2975) and a less sensitive strain (C57BL/6). We used C57BL/6 mice in this experiment because the *OGG1* knockout model was generated in that strain. Various strains of mice may respond differently to toxicological exposures, e.g. C57BL/6 are resistant and Balb/c are sensitive to ionizing radiation [43]. Also C57BL/J mice have higher hepatic non-heme iron content than Balb/c mice [44]; this may generate differences in the response of oxidative stress. The aggregated data from this and previous studies [15,23] indicate that Balb/c mice induce HO-1 to a greater extent than C57BL/6 mice after exposure to oxidative stress generating agents including DEP and ionizing radiation. The HO-1 mRNA expression level is commonly used as a marker of oxidative stress; the expression is highly responsive to oxidative stress and has potent antioxidant and cytoprotective properties that probably are important in the response that protects cells against oxidative stress [45,46]. A firm conclusion about the varying strain-specific sensitivity to DEP induced effects in terms of mRNA regulations of genes involved in DNA repair and the antioxidant response system warrants a thorough investigation of several strains of mice. However, the lack of oxidatively damaged DNA and unaltered mRNA levels of *HO-1*, *OGG1*, and *ERCC1* indicate that C57BL/6 mice are protected from some effects of DEP exposure, although not the pulmonary inflammation in terms of elevated neutrophilic granulocyte infiltration and elevated *IL-6* mRNA levels. Pulmonary inflammation was reported in a recent study after intraperitoneal injection of an acute lethal dose of endotoxin, where the response in neutrophil infiltration in lungs and circulating cytokine levels were lower in *Ogg1* $^{-/-}$ mice, whereas the survival was prolonged [47]. The collected data from our analysis show only minor differences between *Ogg1* $^{-/-}$ and WT mice with respect to inflammation following inhalation of DEP. This could be due to several reasons, including that the systemic inflammation evoked by lipopolysaccharide is more severe than DEP, the exposure routes were different, and the anticipated toxic response was different (survival versus sub-clinical effects on biomarkers). However, keeping in mind that inflammation is associated with oxidative stress and

elevated levels of oxidized DNA, further analysis in the role of OGG1 in regulation of inflammatory responses to exogenous agents certainly warrants further research.

In conclusion, this study demonstrated that *Ogg1* $^{-/-}$ mice have higher levels of purine oxidation products as determined by FPG sites in lung cells compared to WT mice. Moreover, *Ogg1* $^{-/-}$ mice were specifically sensitive to DEP, which caused increased levels of 8-oxodG in lung DNA as determined by HPLC-EC after repeated pulmonary exposures, whereas WT mice appeared resistant to this effect, despite similar levels of inflammation. The two types of assays for measurement of guanine oxidation, based on chromatographic and enzymatic detection may be best suited at relatively high and low level of damage, respectively.

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