

## **IA1 (SL6)      *Effects of diesel exhaust and its particles on respiratory and reproductive systems in mice***

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### **1. Introduction**

In recent year, there has been a progressive increase in urban air pollution that is characterized by high concentrations of atmospheric particulate matter (PM10), resulting primarily from increase of automobiles, especially diesel engine powered cars. Although the mechanisms of underlying respiratory morbidity due to PM10 are not nuclear, it is thought that the fine particles (PM2.5) are of greatest concern to health since they can be breathed most deeply into the lung, where they are likely to be more toxic than the larger particles. Major parts of PM10 or PM2.5 in big cities in Japan are consist of diesel exhaust particles (DEP).

It is well known that DEP cause lung cancers and allergic rhinitis in experimental animal, but it is not known whether they can cause asthma. On the other hand, many epidemiologic studies have reported that there is a clear association between episodes of air pollution and impaired lung function, cough, infections of the lower respiratory tract and respiratory symptoms in asthmatics. Recent studies have reported very high associations between fine particles (PM2.5) and daily mortality rates.

More recently, we found that (DEP) or diesel exhaust (DE) caused asthma-like features in experimental animals. It has been reported from Swedish scientists that human sperm mortality was decreased by diesel particle extracts. Therefore, we examined whether DE inhalation caused similar effects on reproductive system in mouse.

In this paper, I would like to present a possible mechanisms asthma-like symptoms by DEP or DE, and decrease of sperm production ability and occurrence of teratogenic changes by DE or DEP.

### **2. Onset of asthma-like features by repeated instillation of DEP**

#### **2.1. Non allergic mechanism via involvement of active oxygen species**

We previously reported that DEP produced  $O_2^-$  and  $\cdot OH$  via xenobiotic metabolizing reactions of quinone like compounds and polyaromatic hydrocarbons in DEP, and phagocytic reaction of DEP by alveolar macrophages.<sup>1,5)</sup> These active oxygens easily damage endothelial and epithelial cells in lungs.<sup>2)</sup> The cell damage causes the infiltration of neutrophils in the submucosal layer of bronchioli in an early phase, and then eosinophils were infiltrated by chronically repeated instillation of DEP.<sup>3)</sup> The chronical instillation of DEP has induced increases of  $O_2^-$  and NO producing enzymes and decreases of two types of SOD in bronchial cells.<sup>4)</sup> These results suggest that ONOO $\cdot$  are produced from of  $O_2^-$  and NO, and endothelial and epithelial cells are damaged by  $O_2^-$ , NO,  $\cdot OH$  and ONOO $\cdot$  (Fig.1). Recently, it is reported that ONOO $\cdot$  scavenging reagent, ebselen, is more effective to prevent asthma-like features than steroid hormones (in press). This evidence supports strongly our mechanism. These active oxygens may induce the infiltration of eosinophils. The eosinophils and active oxygens may release toxic granule proteins such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and neurotoxin (NT). It is well known that these toxic granule proteins cause desquamation of epithelial cells from the basement of the airway. Numerous nerve lying under the epithelium

may therefore be sensitive, and the smooth muscle would constrict markedly. This may be a possible mechanism inducing the asthma-like features mediated by active oxygen derived from DEP (Fig.2).

## 2.2. Allergic mechanism via involvement of IgG1 antibody, IL-5 and eosinophils<sup>6-10)</sup>

We investigated the effects of DEP instilled intratracheally on antigen-induced airway infiltration, local expression of cytokines, and antigen-specific immunoglobulin (Ig) production in mice. DEP enhanced ovalbumin (OA)-induced airway inflammation characterized by infiltration of eosinophils and lymphocytes and an increase of goblet cells in bronchial layer. DEP with OA markedly increased interleukin-5 (IL-5) in lung tissue compared with either antigen or DEP alone. The combination of DEP and OA induced significant increases in local expression of IL-4, and GM-CSF. DEP exhibited adjuvant activity for the antigen-specific production of IgG1, but not IgE.<sup>6)</sup> It is reported that IgG1 can cause degranulation from eosinophils via the combination with Fc $\gamma$ R2 receptor on eosinophils. Furthermore, we found that there are strain differences in allergic airway inflammation by a combination of DEP + OA, and there is a high relationship between the inflammation and the ability of IgG1 production.<sup>7, 8)</sup> From these evidences, we would like to propose that DEP can enhance the manifestation of allergic asthma-like features by a mechanism via IgG1, IL-5 productions and increased infiltration of eosinophils (Fig.3).

## 2.3. Diesel exhaust (DE) can enhance allergic asthma-like features<sup>11-15)</sup>

The intratracheal instillation of DEP enhances allergic asthma-like features as mentioned above. However, it is not known whether the effects of such instillation are similar to those of the daily inhalation of DE. We, therefore, examined whether the inhalation of DE would also enhance allergic reactions.

ICR mice were exposed to clean air or DE at a soot concentration of 0.3mg/m<sup>3</sup>, 1.0mg/m<sup>3</sup> and 3 mg/m<sup>3</sup> for 12 hr daily up to 8 months. Four months after exposure to DE, mice were sensitized intraperitoneally with 10  $\mu$ g of OA, and challenged by an aerosol of 1% OA six times at 3-week intervals during the last 4 months of the exposure.<sup>14)</sup>

DE exposure caused a dose-dependent increase of nonciliated cell proliferation and epithelial cell hypertrophy in the airway, but showed no effects of goblet cell proliferation in the bronchial epithelium and eosinophil recruitment in the submucosa of the airway. OA-treatment alone induced very slight changes in goblet cell proliferation and eosinophil recruitment. The combination of OA and DE exposure produced dose-dependent increases of goblet cells and eosinophils, in addition to further increases of the typical changes induced by DE. OA treatment induced OA-specific IgG1 and IgE production whereas the adjuvant effects of exposure on Ig production were not observed. Inhalation of DE led to increased levels of IL-5 in the lung at a soot concentration of 1.0 and 3.0 mg/m<sup>3</sup> with OA, although these increases did not reach statistical significance. We conclude that the combination of antigen challenge and chronic exposure to DE produces increased eosinophilic inflammation, and cell damage on the airway epithelium may depend on the degree of eosinophilic inflammation in the airway.<sup>11-15)</sup>

From these results, we think that DE exposure combined with antigen produces asthma-like features via a similar mechanism obtained with the instillation of DEP + OA.

## **3. Effects of diesel exhaust (DE) on the reproductive system in mice**

It is reported that human sperm counts have declined worldwide approximately 50% since the beginning of

Word War II. In 1993, Fredericsson et al reported that organic solvent extract of DEP decreased human sperm motility in vitro experiment.<sup>16)</sup> That evidence suggests that inhalation of DE also may affect on sperm motility.

Therefore, we examined whether DE exposure affects on animal sperm motility and production. By exposure to diesel exhaust for 6 months, daily sperm production per gram(g) testis was observed at the degree of 29%, 36% and 53% of the control (100%) in 3 groups of 0.3 mg/m<sup>3</sup>, 1.0 mg/m<sup>3</sup> and 3 mg/m<sup>3</sup> as DEP concentration, respectively.<sup>17)</sup> Ultrastructural change and reduction of LH receptor mRNA expression in Leydig cells were observed in mice exposed to DE. These evidences suggest that DE damages to Leydig cells and decreases testosterone synthesis.<sup>17)</sup>

Male mice exposed to DE for 5 months were mated with normal 10 week-old female mice. We observed a half deficiency of a tail and abnormal ovally and vagina in the F1 babies.<sup>18)</sup> In other rat experiment, histopathological changes of Sertoli cells are also observed in testis of mice exposed to DE for long time. From these results, DE exposure affects serious abnormal effects to male reproductive system. I would like to emphasize that usage of diesel engine powered car should be limited.

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