Diesel Exhaust Exposure Enhances the Persistence of Endometriosis Model in Rats

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Diesel exhaust (DE) is known to be one of the main causes of air pollution. Several studies have suggested that DE causes lung cancer, cardiovascular disease, abnormal reproductive function, and central nervous system damage as well as type I allergy in the airway. Type I allergy also plays a role in pathogenesis of endometriosis. In the present study, we examined the effect of exposure to DE on a rat model of endometriosis. Endometriosis was induced by autotransplantation of endometrium to the peritoneum in female Sprague-Dawley rats exposed to DE during prenatal and postnatal periods. Endometriotic lesions, normal peritoneum, and blood samples were obtained on days 4, 7, and 14 after autotransplantation. The extent of stromal proliferative lesions in the endometriosis model was greater in the rats of the DE exposure group than in those of the control group on day 14. Serum monocyte chemoattractant protein (MCP)-1 level was significantly higher in rats with endometriosis in the DE exposure group than in those in the control group on day 14. Results of this study suggest that DE exposure enhances the histologic and molecular pathology of endometriosis in rats.

Key words —— endometriosis, diesel exhaust, monocyte chemoattractant protein-1, stromal proliferation

INTRODUCTION

Air pollution is a serious problem in urban areas all over the world. One of the major air pollutants is diesel exhaust (DE), which contains carbon monoxide, sulfur oxides, nitrogen oxides, and DE particles (DEPs). Numerous studies have indicated that DE and DEPs have various detrimental effects on health; it has been reported that DE in humans causes lung cancer,[1] chronic obstructive pulmonary disease,[2] cardiovascular disease,[3–6] and in mice, reduced male reproductive function,[7–10] abnormal fetal development of female reproductive function,[11] abnormal expression of immune-related genes in the placenta,[12] and central nervous system damage.[13] It has also been reported that DE exposure promotes allergic reactions in the airway and exaggerates the pathology of respiratory allergic diseases. Diaz-Sanchez *et al.*[14, 15] reported that *in vivo* nasal challenge with DEPs enhances local expression and production of cytokines and CC chemokines in humans. Matsumoto *et al.*[16] showed that repeated exposure to low-dose DE (100 μg DEP/m^3) increased airway hyper-responsiveness and exaggerated allergic responses in ovalbumin-induced asthmatic mice.

Endometriosis is a common gynecological disorder, the prevalence of which is 6–10% in women of reproductive age[17] and which is now on the rise. It is well known that retrograde seeding of endometrial cells during menstruation partially contributes to the pathogenesis of endometriosis;[18] however, the details involved in such pathogenesis have not been clarified. To identify the pathogenic factors of this disorder, we created a rat endometriosis model by autotransplantation of endometrium to peritoneal tissue. Uchiide *et al.*[19] found interstitial stromal hyperplasia and degranulation of mast cells in a endometriosis model induced by autotransplantation of endometrial tissue to peritoneum. We previously reported increase of local expression of cytokines and chemokines in the rat endometriosis model, consistent with the characteristics of endometriosis in humans.[20] These observations suggest that

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immunological reactions play a role in the pathogenesis of endometriosis. In the present study, we focused on the effects of exposure to DE on allergic reactions associated with endometriosis and analyzed them using a rat endometriosis model.

MATERIALS AND METHODS

Animals and Treatments —— Pregnant Sprague-Dawley rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). Four of these rats were exposed to DE through the airway starting on gestational day (GD) 2 and four others were used for control (no exposure to DE). Neonatal rats from the DE-exposed pregnants were exposed to DE until 7 weeks of age (DE exposure group), whereas neonatal rats from the control group were maintained in clean air. Endometriosis was induced in female pups at 8 weeks of age. A 5-mm × 5-mm piece of uterine tissue was attached to each side of the peritoneum by surgical autotransplantation. Induced endometriotic lesions ($n = 2$ per rat) and blood were obtained from anesthetized rats of the DE exposure group on days 4 ($n = 4$), 7 ($n = 4$), and 14 ($n = 4$) after autotransplantation and from those of the control group on days 4 ($n = 4$), 7 ($n = 4$), and 14 ($n = 3$). Normal peritoneal tissues were also obtained from nontreated rats on days 4 ($n = 4$), 7 ($n = 4$), and 14 ($n = 3$). All animals were handled in accordance with institutional and national guidelines for the care and use of laboratory animals.

DE Exposure —— Rats were exposed to DE 7 hr per day, 5 days per week, in an inhalation chamber at the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association (Tokyo, Japan). A 2369-cc diesel engine (Isuzu Automobile, Inc., Tokyo, Japan) was operated at 1050 rpm and 80% load with commercial diesel oil. The exhaust was introduced into a stainless steel dilution tunnel and mixed with clean air that was passed through a high-efficiency particulate air filter and a charcoal filter. The concentration of DEPs was adjusted to approximately 1.0 mg DEP/m^3.

Light Microscopic Analysis —— Tissue samples were fixed in formaldehyde-glutaraldehyde fixative (4% formaldehyde and 1% glutaraldehyde in 0.1 M phosphate-buffered saline; pH 7.4). Fixed tissues were washed with phosphate buffer (pH 7.4), dehydrated in a graded series of ethanol, and embedded in paraffin. Paraffin-embedded tissue sections were stained with hematoxylin and eosin (HE) for histologic analysis.

Serum Monocyte Chemoattractant Protein (MCP)-1 Level —— Serum MCP-1 level was measured with the use of a commercially available ELISA kit (Biosource Inc., Camarillo, CA, U.S.A.) according to the manufacturer's instructions.

Statistical Analysis —— Data are shown as mean ± standard error of the mean (SEM). Unpaired *t*-test was used to analyze differences in values between two groups. A p value of <0.05 was considered statistically significant.

RESULTS

Effect of DE Exposure on Litter Size, Sex Ratio and Body Weight of Pups

Eight pregnant rats in this study delivered their pups on GD 22. There was no significant difference in litter size and sex ratio of pups between the DE exposure group and the control group. Body weight of female pups in the DE exposure group was significantly lower than that in the control group when the pups were 4 days old and 8 weeks old (Table 1).

Effect of DE Exposure on Changes in Histopathologic Features

In rats, the abdominal wall is made up of three muscles, the internal abdominal muscle, which is nearest the abdominal cavity, the medial abdominal muscle, and the external abdominal muscle (Fig. 1 A). Analysis of which abdominal muscles have been affected by interstitial stromal cell hyperplasia, a proliferative lesion, is useful for investigating the pathogenesis of endometriosis.[19] Proliferative stromal lesions in the peritoneum adjacent to the autotransplanted endometrial tissue reached the internal abdominal muscle within 4 days after autotransplantation (Fig. 1B). The lesions reached their maximum extent within 7 days (Fig. 1C), and they were decreased on day 14 (Fig. 1D, E). Endometriotic lesions induced in the

Table 1. Body Weight of Female Pups in the DE Exposure and Control Groups

	Control group	DE exposure group
4 days old	7.7 ± 0.2 g ($n = 23$)	6.4 ± 0.4 g ($n = 22$)**
3 weeks old	35.8 ± 0.8 g ($n = 23$)	36.3 ± 0.6 g ($n = 21$)
8 weeks old	201.8 ± 3.3 g ($n = 12$)	187.5 ± 1.9 g ($n = 12$)**

Data are mean ± SEM. **Statistically significant difference between two groups ($p < 0.01$).

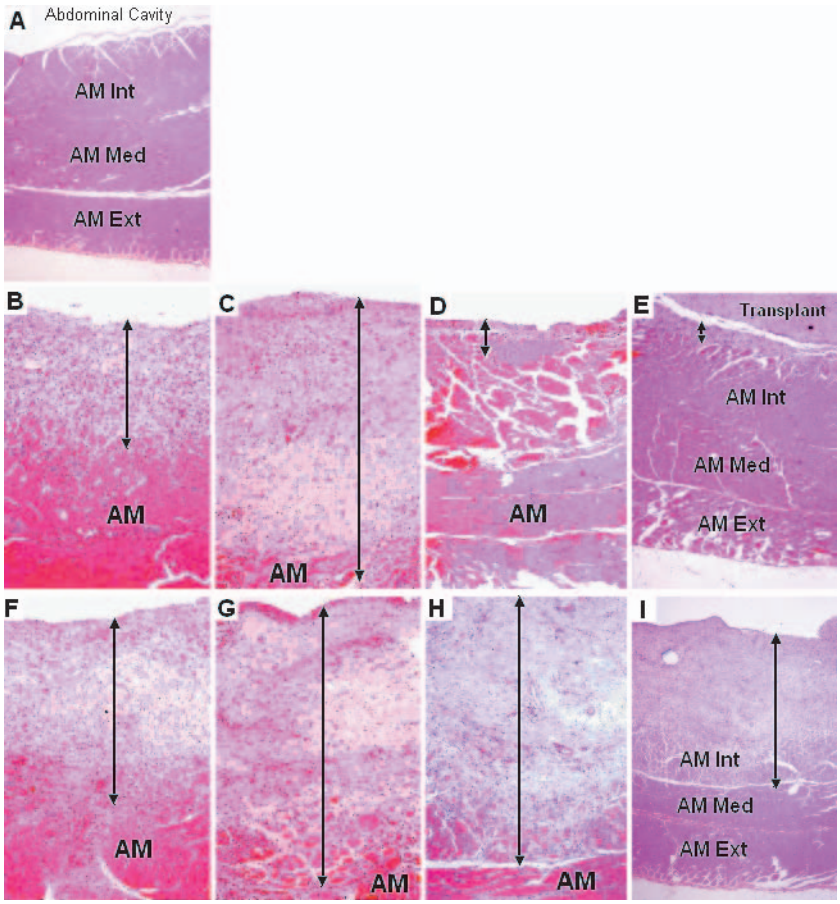

Fig. 1. Histopathological Features of the Rat Endometriosis Model in the DE Exposure Group and Control Group

HE-stained images show normal peritoneal tissue (A), and endometriosis model in rats of the control group on days 4 (B), 7 (C), and 14 (D and E) after autotransplantation and of the DE exposure group on days 4 (F), 7 (G), and 14 (H and I). AM, abdominal muscle; Int, internal; Med, medial; Ext, external; Magnification: B–D, F–H, and J, ×100; A, E, and I, ×40. Double-headed arrows indicate proliferative lesions.

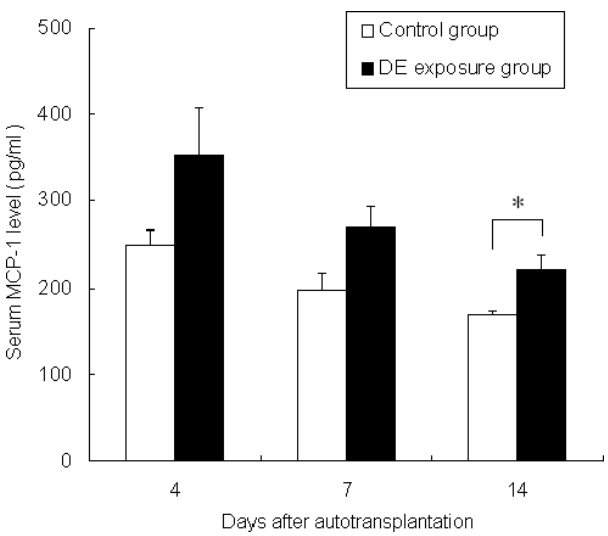

Fig. 2. Effects of DE Exposure on Serum MCP-1 Level in Rat Endometriosis Model

Serum MCP-1 levels were analyzed with ELISA. Data are mean ± SEM. *Statistically significant difference in the serum MCP-1 level between two groups ($p < 0.05$).

DE exposure group did not differ from those induced in the control group on days 4 and 7 after autotransplantation (Fig. 1F, G), but proliferative lesions in the DE exposure group had reached to the medial abdominal muscle by day 14 (Fig. 1H–J). To reveal the effect of DE more clearly, we measured the thickness of the proliferative lesions on day 14. The thickness of the lesions increased by a factor of 7.7 times in the DE exposure group compared to that in the control group on day 14.

Effects of DE Exposure on Serum MCP-1 Level in Rat with Endometriosis Model

The serum MCP-1 level of rats with endometriosis was significantly higher in the DE exposure group than in the control group on day 14 ($p < 0.05$) (Fig. 2).

DISCUSSION

A number of factors contribute to the pathogenesis of endometriosis. Although critical factors for the pathogenesis were not determined, we focused on the contribution of immunological factors because increase of activated mast cells has been observed in the rat endometriosis model[19] and in human endometriosis,[21] and the rat model was reduced by the effect of leukotriene antagonist.[22] In the present study, we analyzed the effects of DE exposure on histologic features and serum MCP-1 level in a rat endometriosis model.

Body weight of female pups in the DE exposure group was significantly lower than that in the control group. This weight loss corresponded with those of previous reports.[7,23] Recent studies reported that some components of DE, for example, carbon monoxide and sulfur oxides, induce weight loss in rats[24] and in humans.[25] The decreased body weight observed in the present study could be induced by these components.

Endometriosis was induced in female pups at 8 weeks of age. Proliferative stromal lesions reached the internal abdominal muscle by day 4 after uterine autotransplantation, and were decreased by day 14 in the control rats. In contrast, proliferative lesions reached the medial abdominal muscle on day 14 in the DE exposure group. This observation suggests that DE exposure enhance the persistence of this rat endometriosis model.

Serum MCP-1 level is increased in rats with endometriosis in comparison to those without endometriosis.[20] Several clinical researchers reported that serum MCP-1 is higher in women with endometriosis than in those without[26–28] and suggested its usefulness as a biomarker for nonsurgical prediction of endometriosis in humans.[29] Gmyrek *et al.*[27] reported that serum MCP-1 level in women with endometriosis was correlated with severity of the disease. In the present study, we showed that the serum MCP-1 level of rats with endometriosis was significantly increased in the DE exposure group relative to that in the control group on day 14 after autotransplantation ($p < 0.05$). This finding supports the histologic observation of persistence of the endometriosis model in the DE exposure group.

In summary, DE exposure enhances the persistence of proliferative stromal lesions of rat endometriosis model and increases serum MCP-1 level in rats with endometriosis. Present results suggest that DE exposure exaggerates the pathology of endometriosis and that it may contribute to the increasing prevalence of this disease.

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