

Experimental approach to biological effects of particulate matters

粒子状物質の生体影響に関する実験的アプローチ

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Suspended particulate matters : SPM

粒子状物質

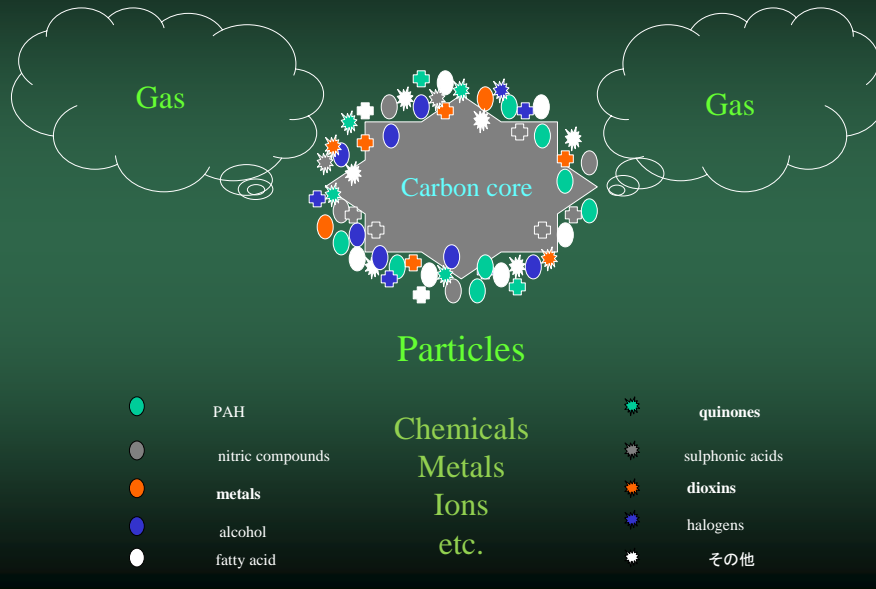
PM₁₀: with diameter less than 10 μ m
derived chiefly from natural sources
with less health effects (?)

PM_{2.5}: with diameter less than 2.5 μ m
derived chiefly from combustion sources
contain vast number of chemicals, metals, and ions
with more severe health effects

ディーゼル排気粒子(diesel exhaust particles : DEP):
with diameter less than 0.4 μ m (getting smaller and smaller!!)

Significant proportion of PM_{2.5} in metropolitan area
is occupied by DEP.

Composition of DEP

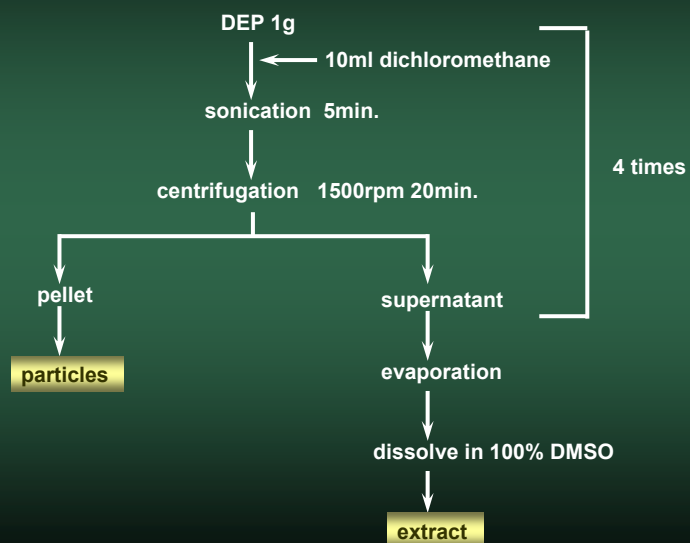


Recent epidemiological reports on PM-DEP

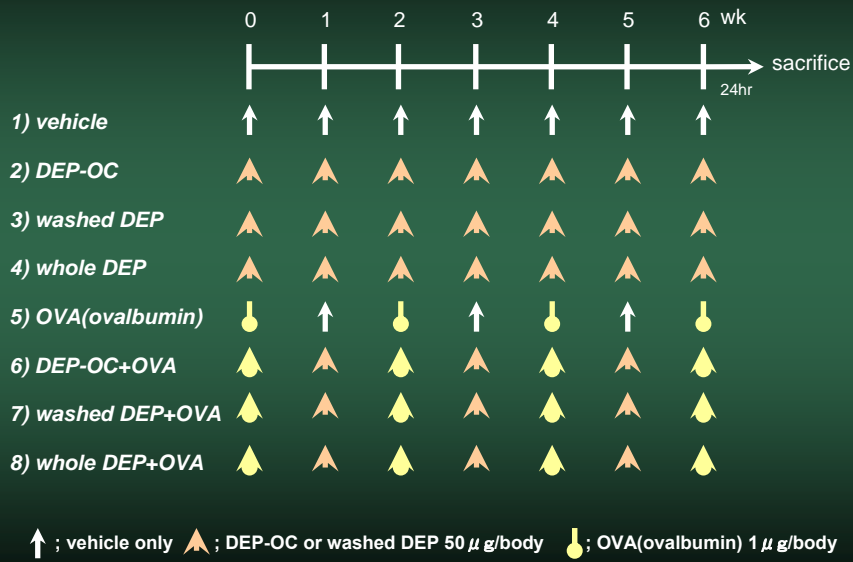
- 1) total mortality
mortality from COPD, DM, and cardiovascular diseases
 - 2) emergency on respiratory diseases
admission on cerebrovascular diseases
 - 3) total mortality in children and aged individuals
 - 4) respiratory symptom in children with atopic prone
 - 5) symptom and severity of asthma
 - 6) visit by upper respiratory infection, bronchitis and asthma
- correlate with concentration of PM-DEP .

DEP and their components enhance a murine model of allergic asthma.

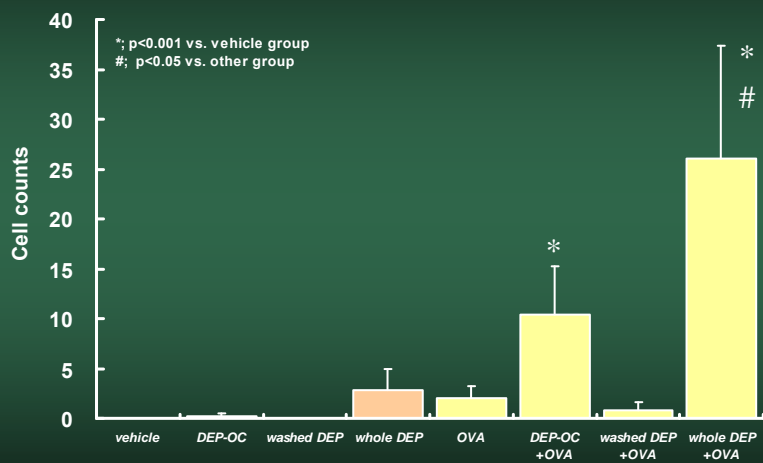
Protocol (Extraction of DEP)



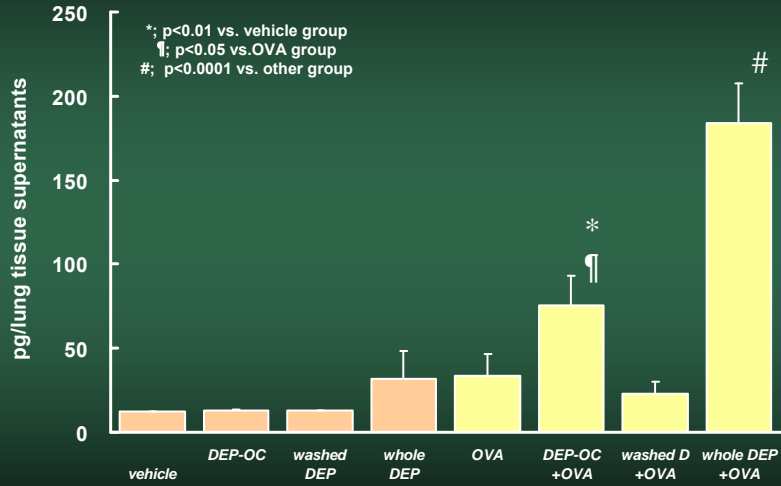
Experimental Protocol



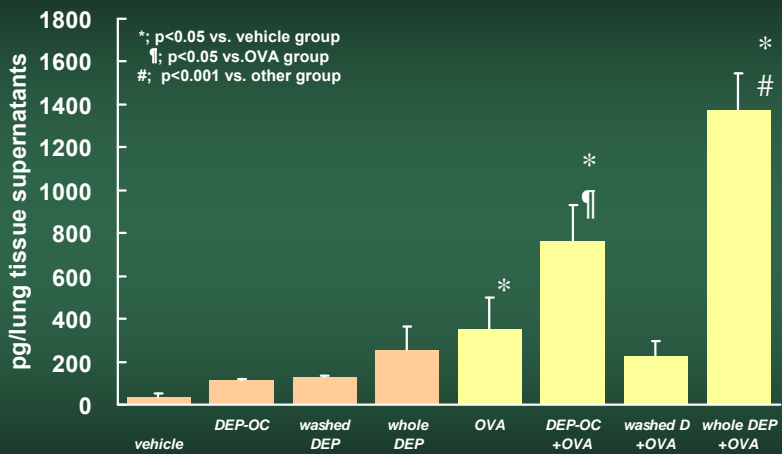
Eosinophils in BALF



Expression of IL-5 in lung



Expression of Eotaxin in lung

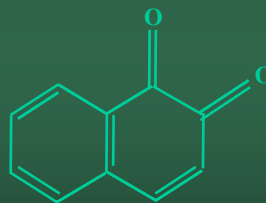


**Quinone compounds in DEP
partly enhance a murine model
of allergic asthma.**

Phenanthraquinone (PQ)



naphthoquinone (NQ)



Protocol

Animals : ICR male mice

exposure : intratracheal instillation (PBS(pH 7.4)-0.025% Tween 80-0.5%DMSO) 0.1 ml

PQ : 2.5 ng/body (every week) OVA 1 μ g/body (1/2week) NQ : 1.58 ng/body (every week)



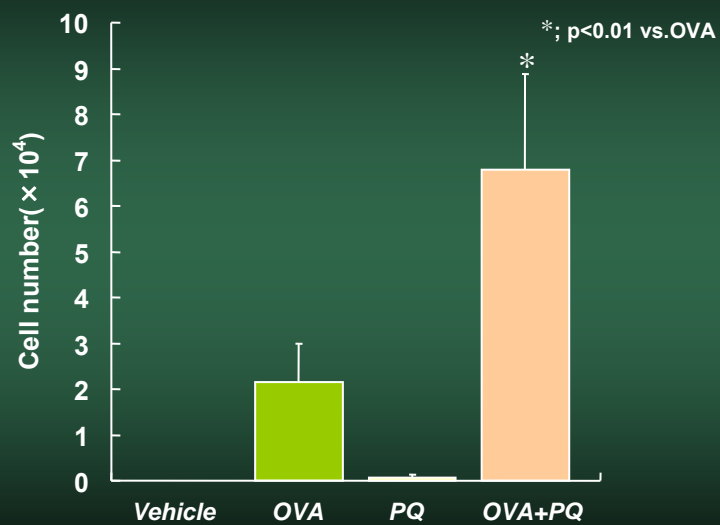
evaluation : Cellular profile in BALF

Cytokines and chemokines in the lung

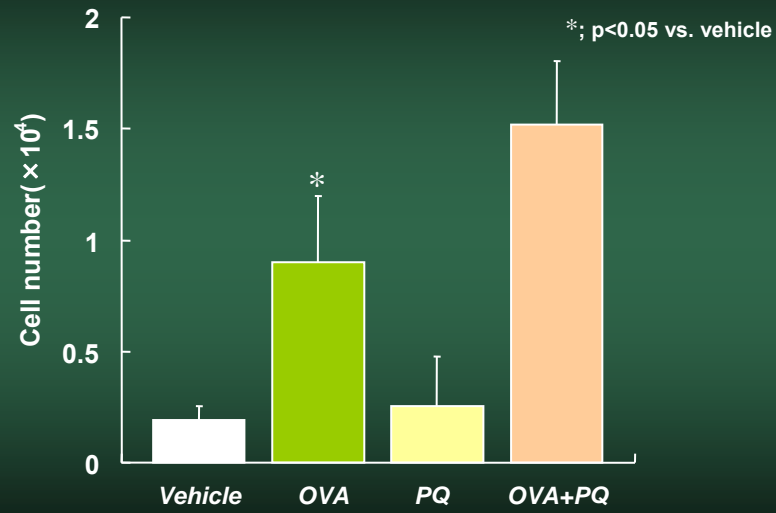
Antigen-specific IgG1, IgE

Histology

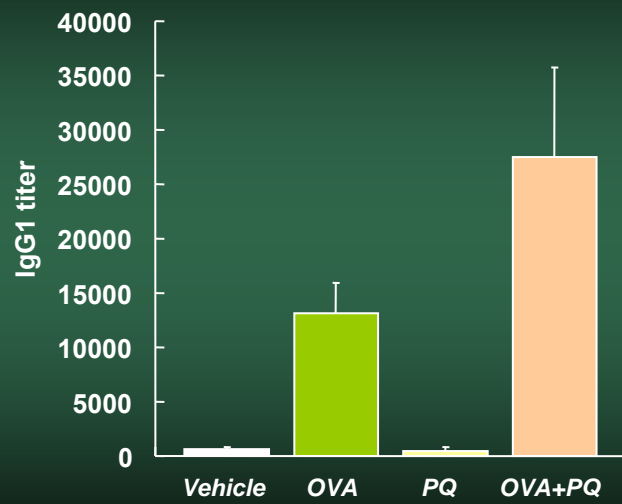
Number of Eosinophils in BALF



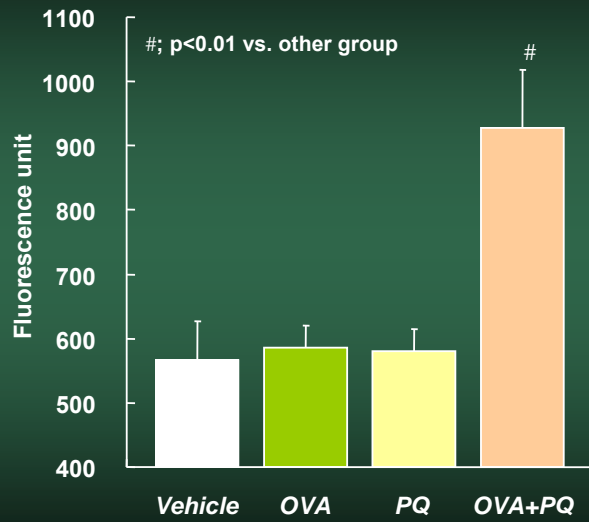
Number of lymphocytes in BALF



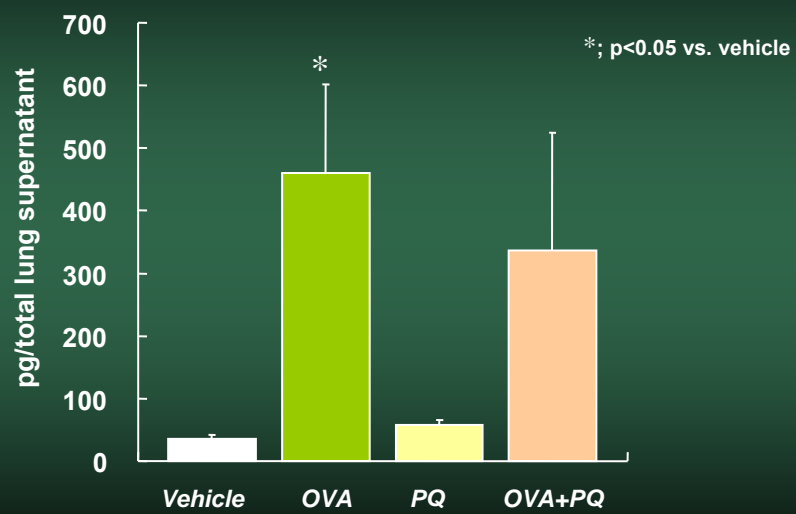
OVA specific IgG1 titer



Production of OVA-specific IgE



Eotaxin in lung tissue supernatants



IL-5 in lung tissue supernatants



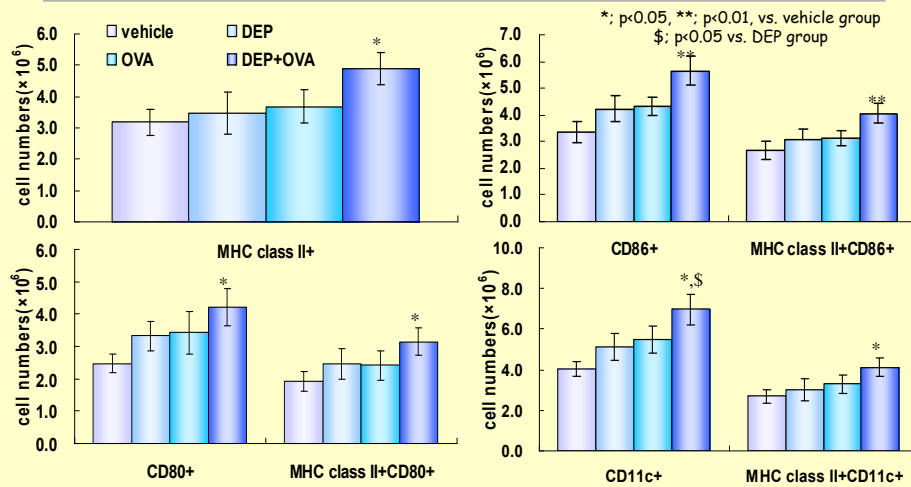
Conclusions

- Organic chemicals in DEP, rather than particles themselves, enhance a murine model of allergic asthma.
- The enhancing effects are accelerated by the combination of organic chemicals and particles.
- Quinone compounds in DEP are partially responsible for the enhancing effects of DEP. However, other compounds or complicated effects should be further determined in future.

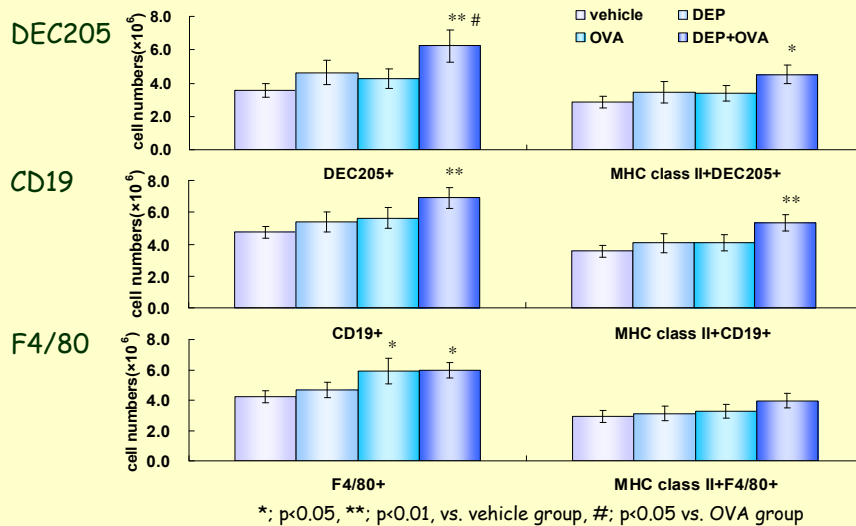
Hiyoshi, Takano, et al: *Clin Exp Allergy*, 2005
Yanagisawa, Takano, et al: *Clin Exp Allergy*, 2006

Effects of Diesel Exhaust Particles on Antigen-Presenting Cells and Antigen-Specific Th2 Immunity in Mice

DEP activate the expression of MHC class II and costimulatory molecules in lung cells under antigen stimulation in mice



DEP increase the number of APC in murine lung under antigen stimulation



Conclusion

- Pulmonary exposure to DEP can enhance allergic responses via the enhancing effects on APC including DC, which culminates in the promotion of local and systemic Th2 immunity.

Recent epidemiological reports on PM-DEP

- 1) total mortality
mortality from COPD, DM, and cardiovascular diseases
 - 2) emergency on respiratory diseases
admission on cerebrovascular diseases
 - 3) total mortality in children and aged individuals
 - 4) respiratory symptom in children with atopic prone
 - 5) symptom and severity of asthma
 - 6) visit by upper respiratory infection, bronchitis and asthma
- correlate with concentration of PM-DEP .

**DEP enhance infection-related
lung inflammation in mice.**

Methods

Intratracheal administration

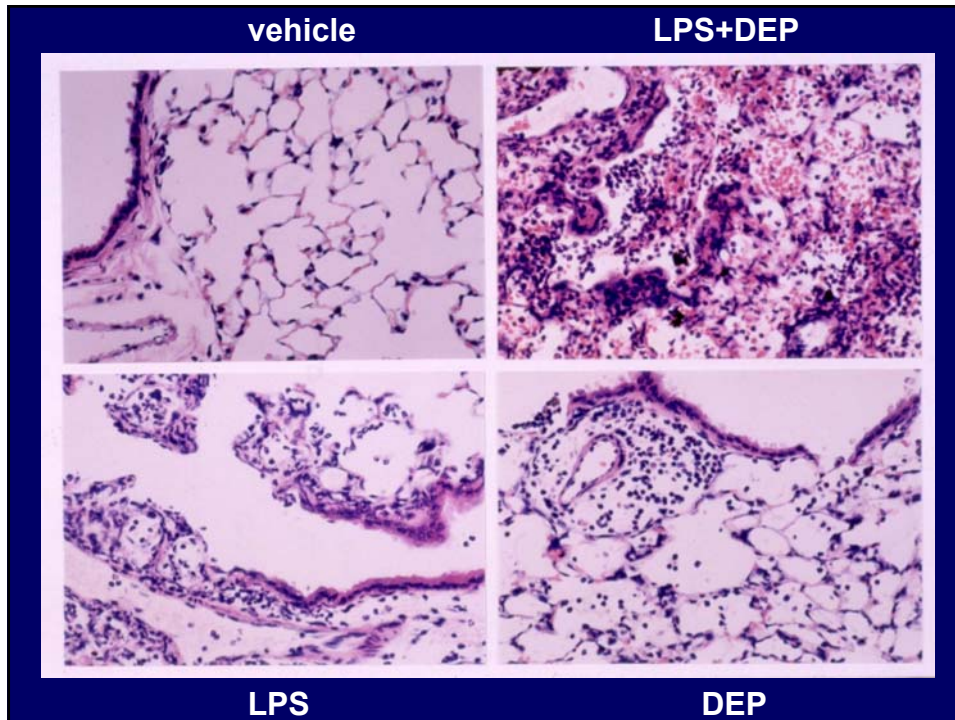
- 1) vehicle
 - 2) lipopolysaccharide (LPS: 4 or 100 $\mu\text{g}/\text{body}$)
 - 3) diesel exhaust particles (DEP: 10 or 250 $\mu\text{g}/\text{body}$)
 - 4) LPS + DEP
- ICR mouse



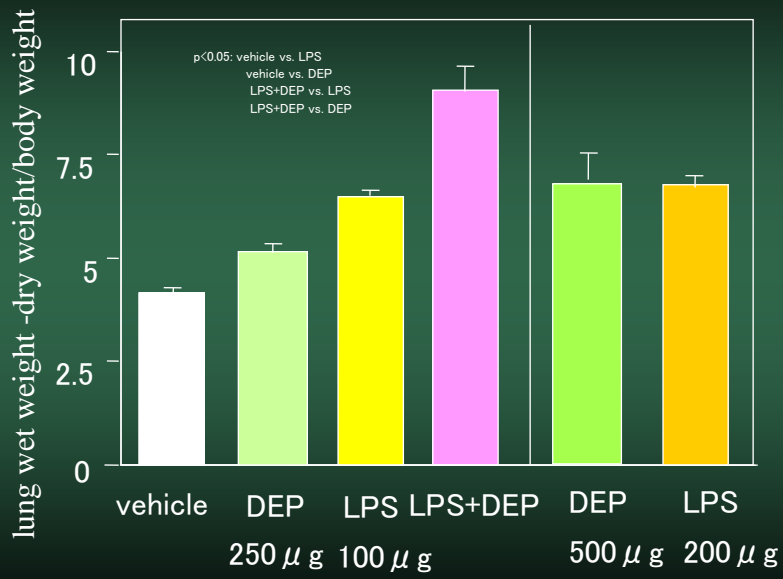
Cytoplasmic and nuclear protein
NF- κ B (p65, p50) etc.

mRNA
IL-1 β , MIP-1 α ,
Toll-like receptors,
ICAM-1, etc.

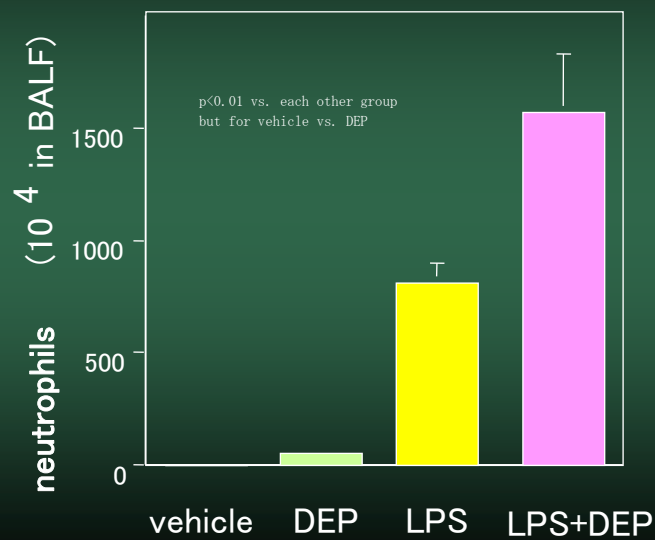
Histology
Water content (edema)
BAL
Protein expression
IL-1 β , IFN- γ , TNF- α ,
MIP1- α , MCP-1, KC,
 σ -ICAM-1, etc.



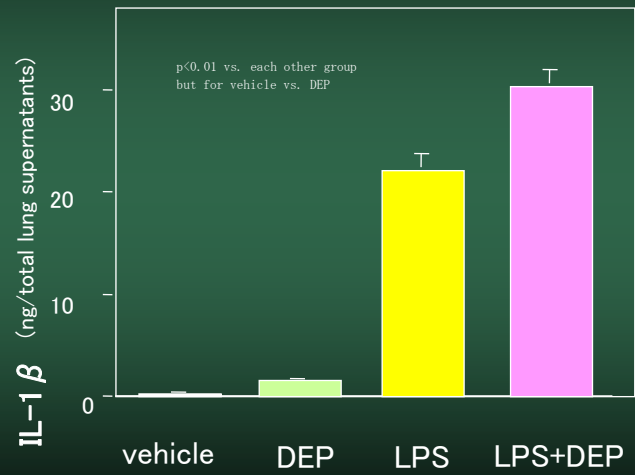
Evaluation of pulmonary edema



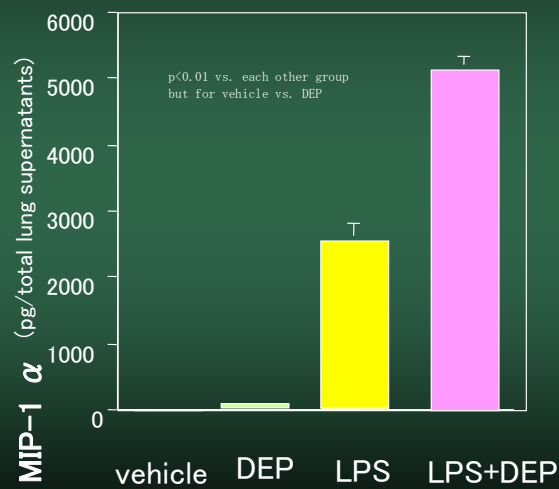
Number of neutrophils in BAL fluid



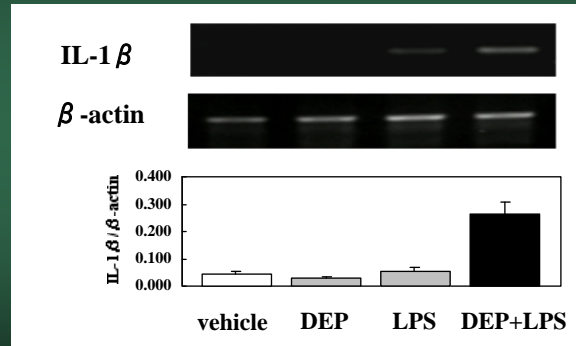
IL-1 β in lung tissue supernatants



MIP-1 α in lung tissue supernatants



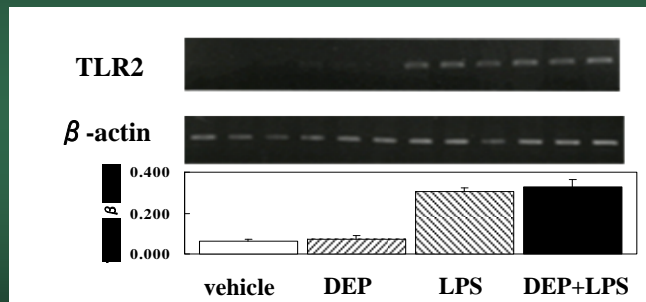
PCR for IL-1 β



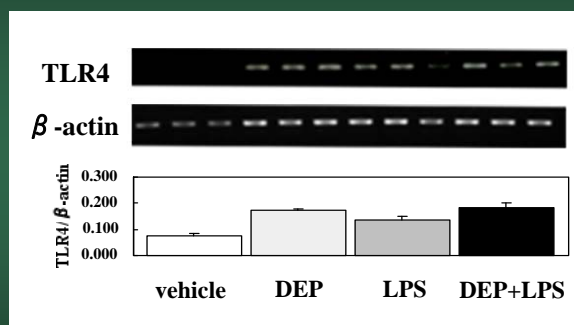
Western blot for p65



PCR for Toll-like receptor 2



PCR for Toll-like receptor 4



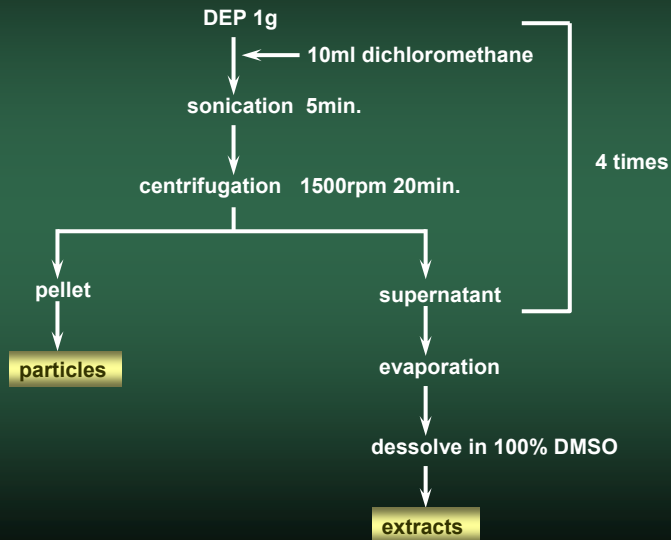
Conclusions

- DEP enhance acute lung inflammation related to bacterial endotoxin.
- The enhancing effects are concomitant with the enhanced expression of proinflammatory cytokines, chemokines, and adhesion molecules.
- The enhanced expression of these proinflammatory molecules are possibly mediated, at least partly, through the activation of nuclear transcriptional factors and the increased expression of Toll-like receptors.

Takano et al: Am J Respir Crit Care Med, 2002

**Components of DEP
diversely enhance
infection-related lung
inflammation in mice.**

Protocol (Extraction of DEP)



Protocol (protocol for washed DEP)



ICR ♂ 7-8w 32-38g

Group

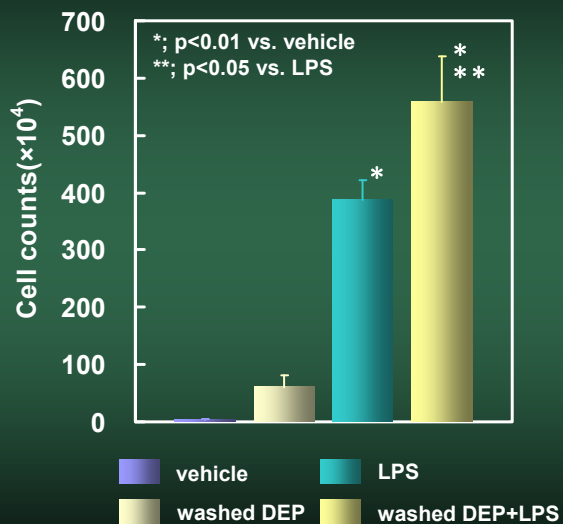
- 1) vehicle(PBS(pH7.4)-0.025%Tween80-0.25%DMSO)
- 2) washed DEP (125 μ g/body)
- 3) LPS (75 μ g/body)
- 4) washed DEP(125 μ g)+LPS (75 μ g)

i.t. 100 μ l

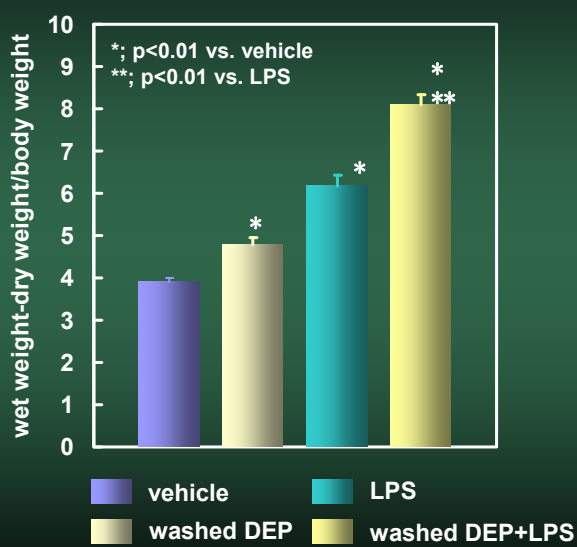
24hr

cell counts in BALF
(total cells, neutrophils, macrophages)
←Turk,Diff-Quik stain
lung water content
cytokine or chemokine in lung tissue
(IL-1 β , MIP-1 α , MCP-1, KC)←ELISA

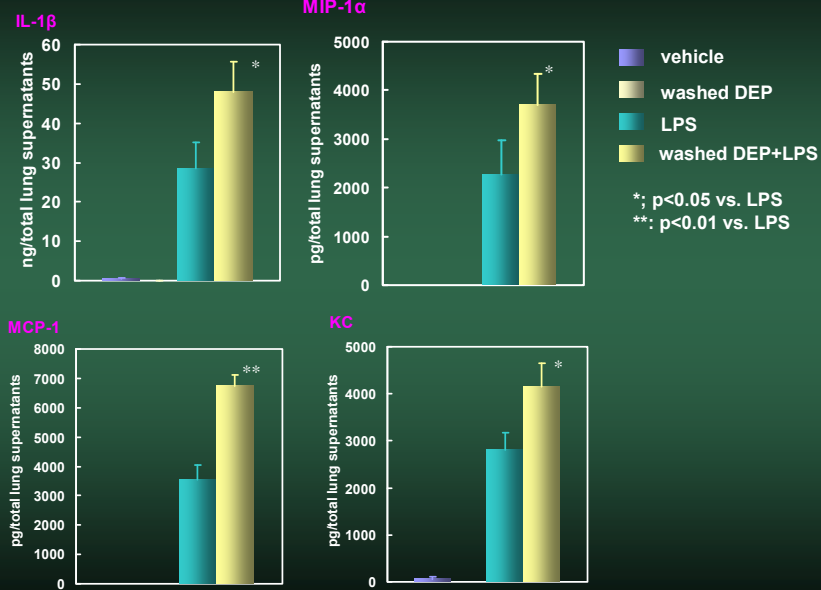
Neutrophils in BALF



Lung water content



Cytokine or chemokine in lung tissue



Protocol (protocol for DEP-OC)



ICR ♂ 7-8w 32-38g

Group

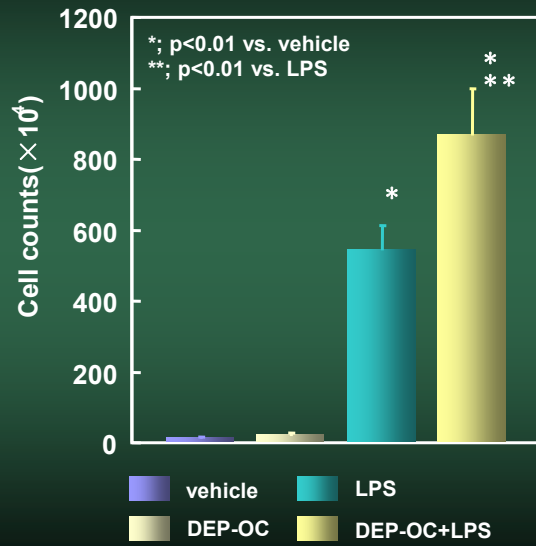
- 1) vehicle (PBS (pH 7.4) - 0.025% Tween 80 - 0.25% DMSO)
- 2) DEP-OC (125 μ g/body)
- 3) LPS (75 μ g/body)
- 4) DEP-OC (125 μ g) + LPS (75 μ g)

i.t. 100 μ l

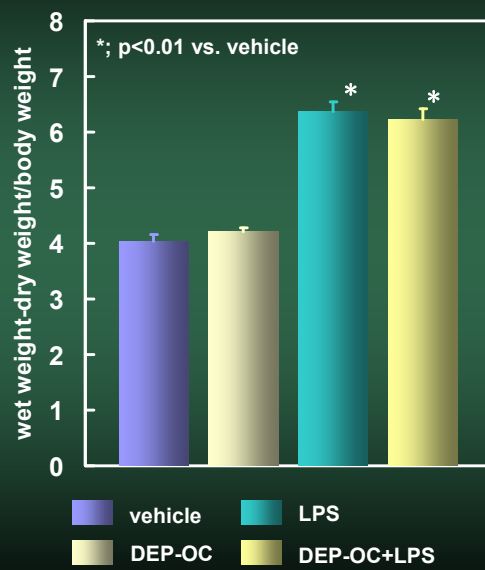
24hr

cell counts in BALF
lung water content
cytokine or chemokine in lung tissue

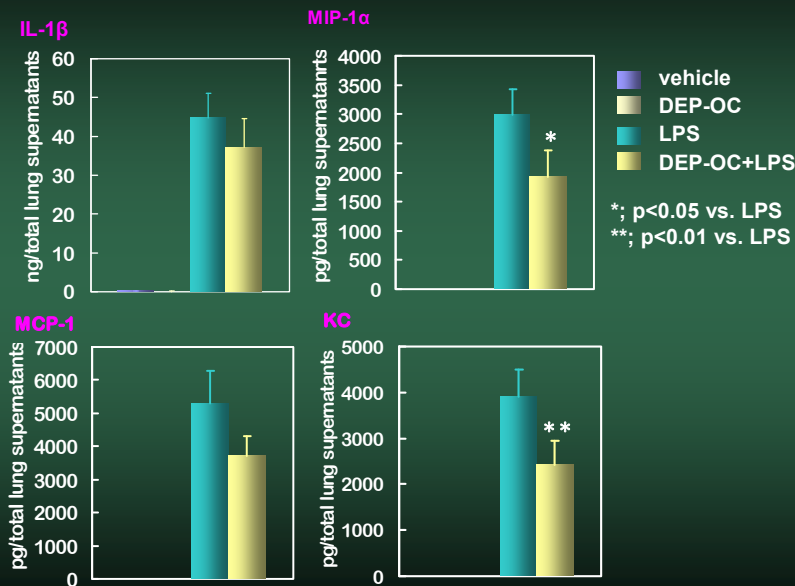
Neutrophils in BALF



Lung water content



Cytokine or chemokine in lung tissue



Conclusions

- Particles themselves, rather than organic chemicals in DEP, enhance neutrophilic lung inflammation related to bacterial toxin.
- The enhancing effects are accelerated by the combination of particles and organic chemicals.

Yanagisawa, Takano, et al: Thorax, 2003

- **The different components of DEP are responsible for the aggravation of different respiratory diseases. However, the combination of particles and organic chemicals provides the most prominent deterioration of respiratory health.**

Recent epidemiological reports

on PM-DEP

- 1) total mortality
mortality from COPD, DM, and cardiovascular diseases
 - 2) emergency on respiratory diseases
admission on cerebrovascular diseases
 - 3) total mortality in children and aged individuals
 - 4) respiratory symptom in children with atopic prone
 - 5) symptom and severity of asthma
 - 6) visit by upper respiratory infection, bronchitis and asthma
- correlate with concentration of PM-DEP .

**DEP enhance
fatty liver changes
in obese mice.**

Tomaru, Takano et al: Int J Mol Med, 2007

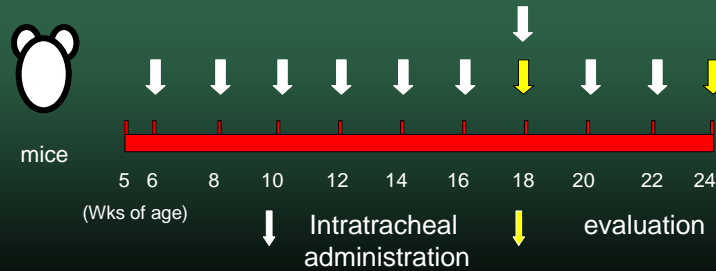
Protocol

Animals

- db/db (diabetic obese) mice, ♀, 5 weeks old (23.6-29.1g)
- db/+m (control) mice, ♀, 5 weeks old (15.5-20.4g)

Groups

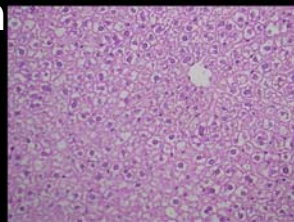
- (1) db/+m-vehicle (PBS (pH7.4)-0.05% Tween 80: 0.1ml/body)
- (2) db/+m-DEP (100 μ g/body in 0.1ml vehicle)
- (3) db/db-vehicle (PBS (pH7.4)-0.05% Tween 80: 0.1ml/body)
- (4) db/db-DEP (100 μ g/body in 0.1ml vehicle)



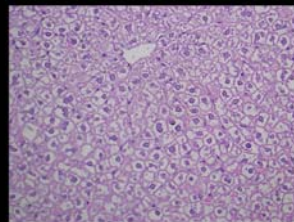
Histological effects of pulmonary exposure to DEP on fatty change in the liver

H&E Stain

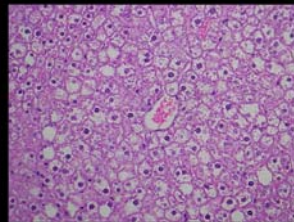
db/+m
Vehicle



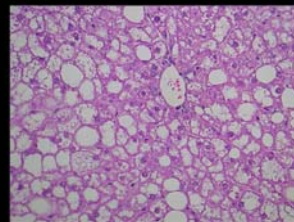
db/+m
DEP



db/db
Vehicle

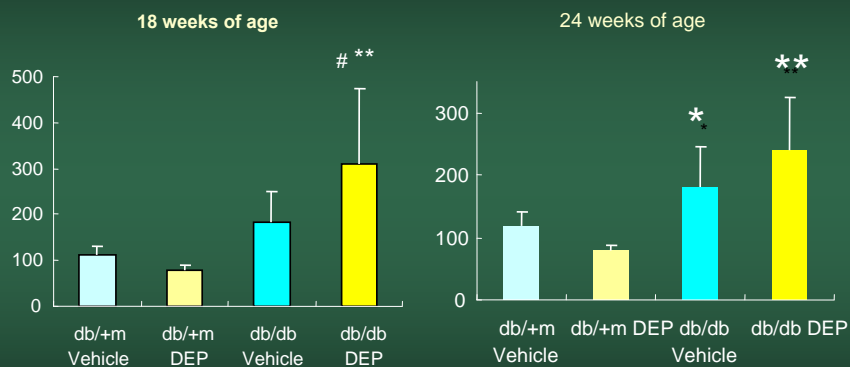


db/db
DEP



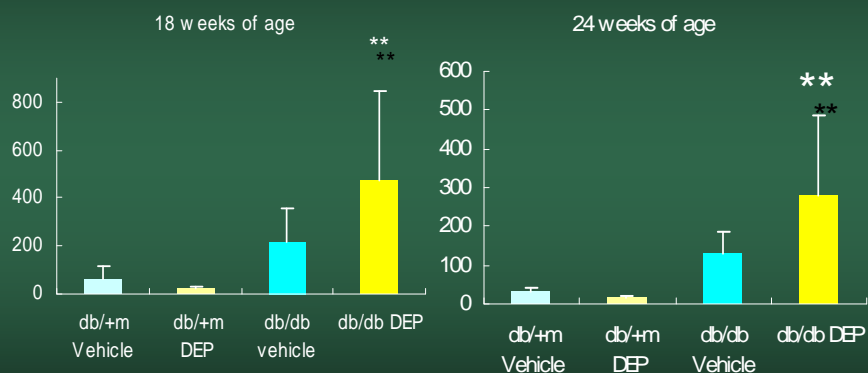
× 400

Aspartate aminotransferase (AST)



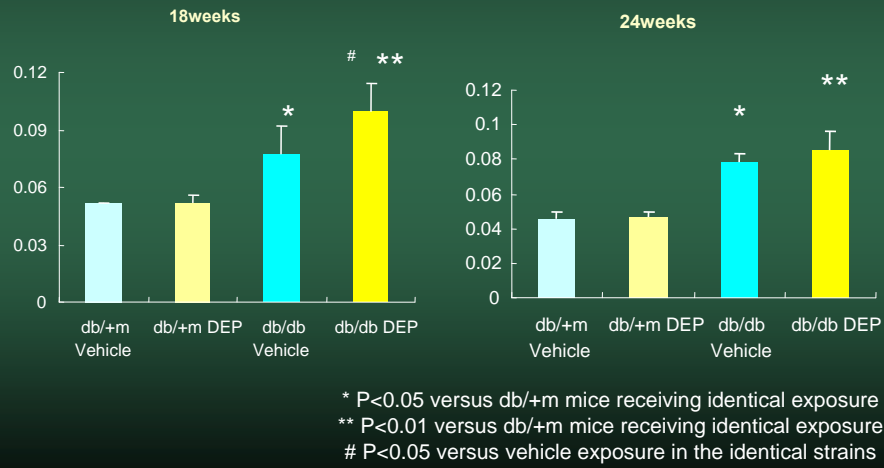
* P<0.05 versus db/+m mice receiving identical exposure
 ** P<0.01 versus db/+m mice receiving identical exposure
 # P<0.05 versus vehicle exposure in the identical strains

Alanine aminotransferase (ALT)

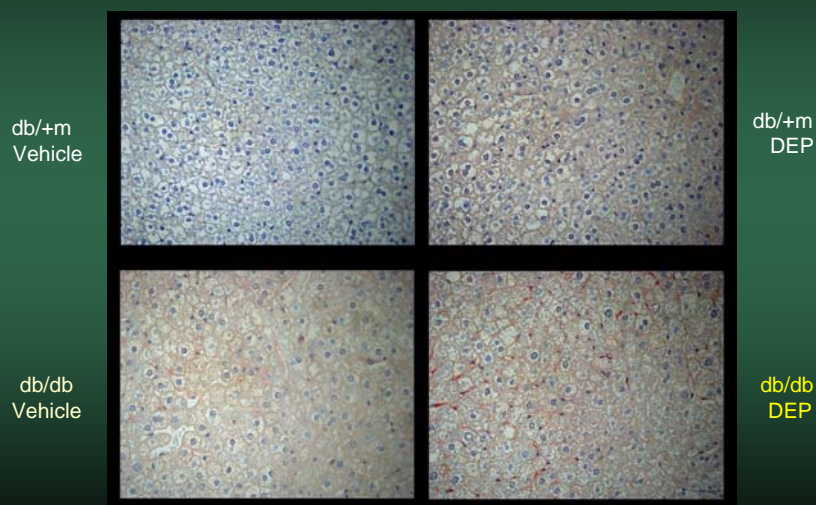


** P<0.01 versus db/+m mice receiving identical exposure

Effects of pulmonary exposure to DEP on liver weight



Effects of pulmonary exposure to DEP on the oxidative stress (HEL) in the liver



×400

Final conclusions

- **PM such as DEP and their components can affect susceptible populations including subject with respiratory diseases and diabetes mellitus with obesity.**

with special thanks to Ken-ichiro Inoue, Rie Yanagisawa, Eiko Koike, Miho Sakurai, Naoko Ueki (NIES), Takamichi Ichionose, Kaori Sadakane, Kyoko Hiyoshi (Oita), Akinori Shimada (Tottori)