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

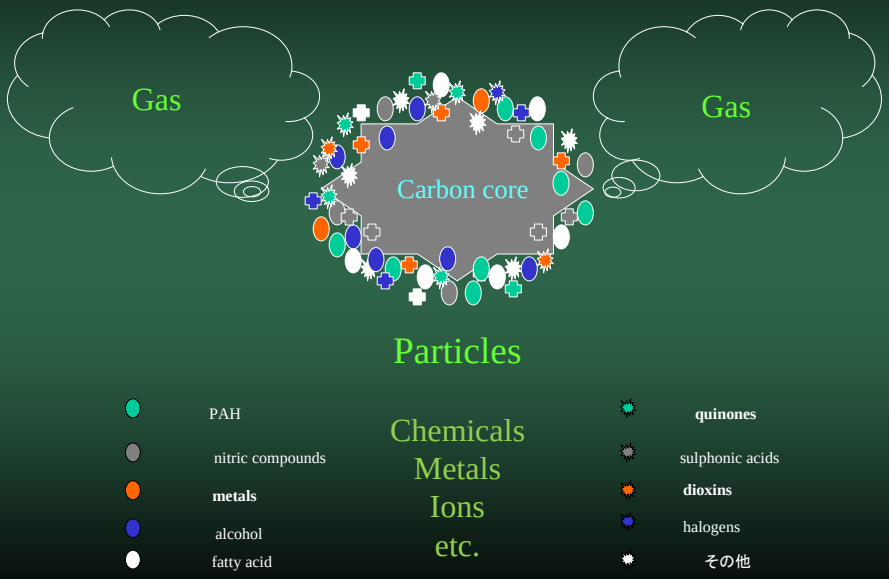
PM_{10}: with diameter less than 10 μm
derived chiefly from natural sources
with less health effects (?)

$PM_{2.5}$: with diameter less than 2.5 μm
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 μm（getting smaller and smaller!!)

Significant proportion of PM2.5 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

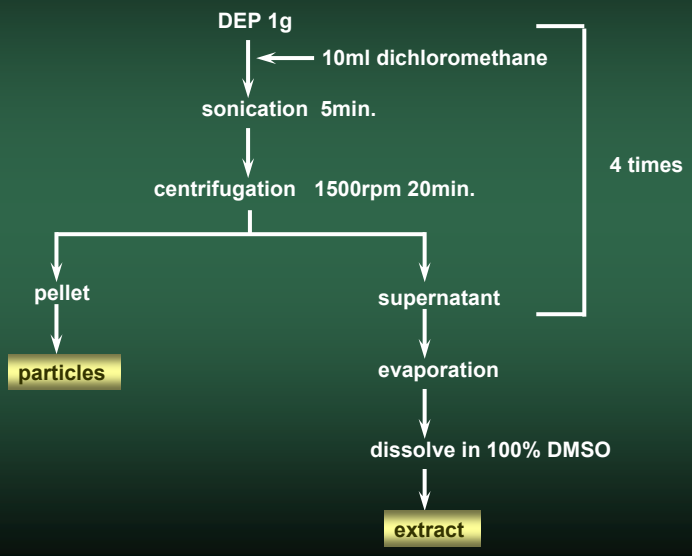
6) visit by upper respiratory infection, bronchitis and asthma

correlate with concentration of PM-DEP .

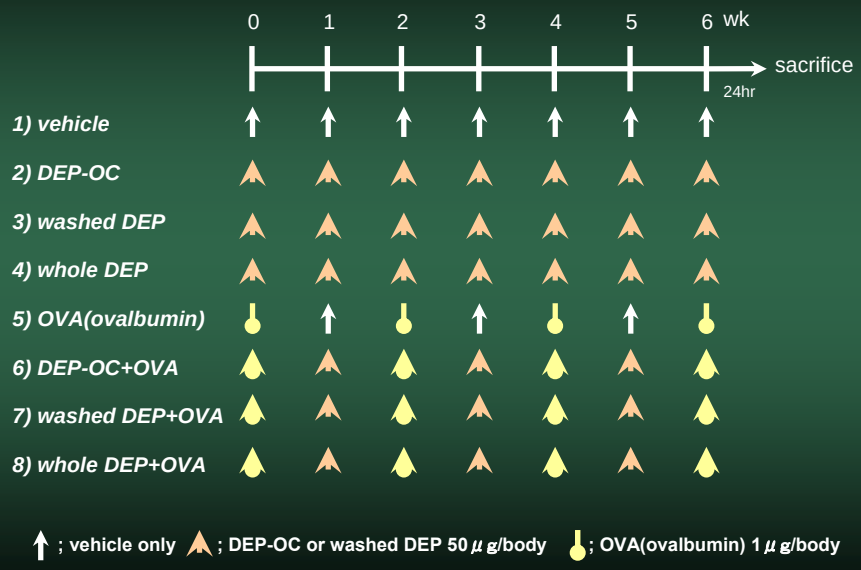
DEP and their components enhance a murine model of allergic asthma.

Protocol

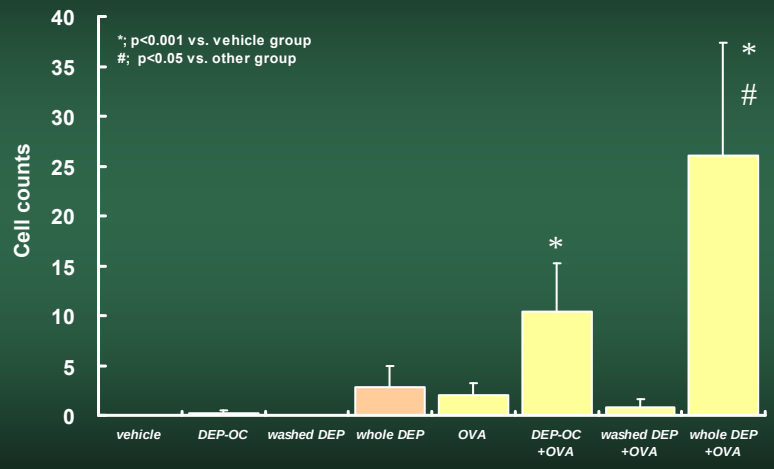
(Extraction of DEP)



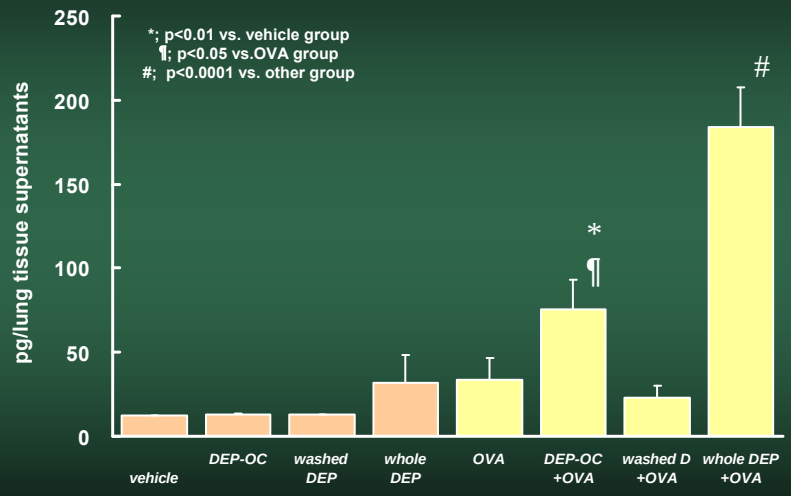
Experimental Protocol



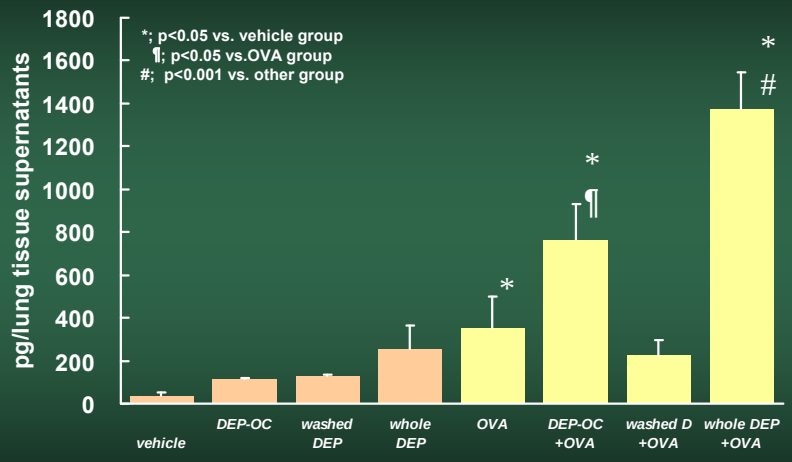
Eosionophils in BALF



Expression of IL-5 in lung

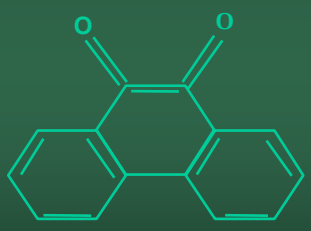


Expression of Eotaxin in lung

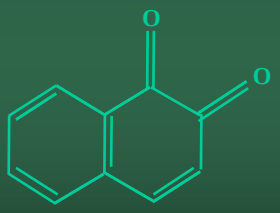


Quinone compounds in DEP partly enhance a murine model of allergic asthma.

Phenanthraquinone（PQ）



naphthoquinone（NQ）

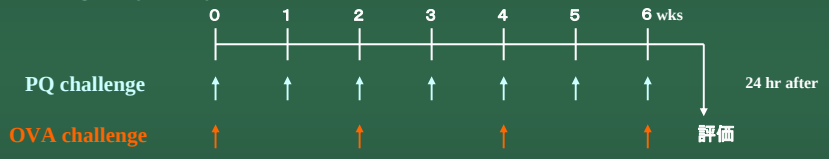


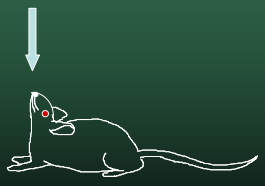
Protocol

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 μg/body（1/2week） NQ：1.58 ng/body（every week）





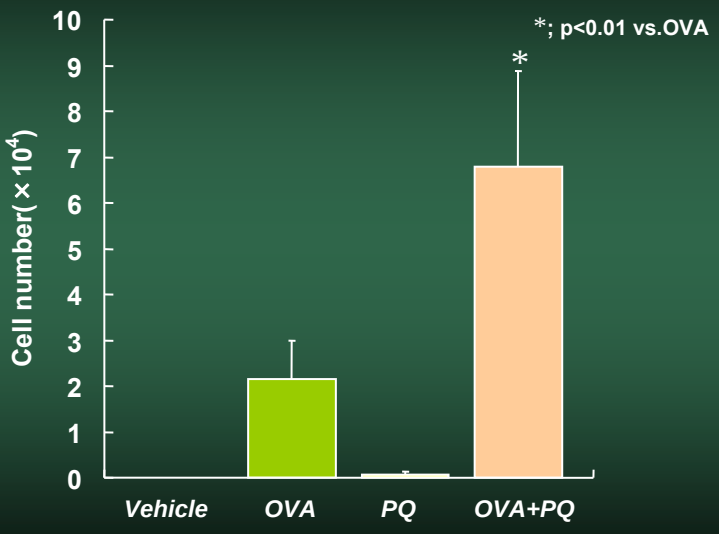
evaluation：Cellular profile in BALF

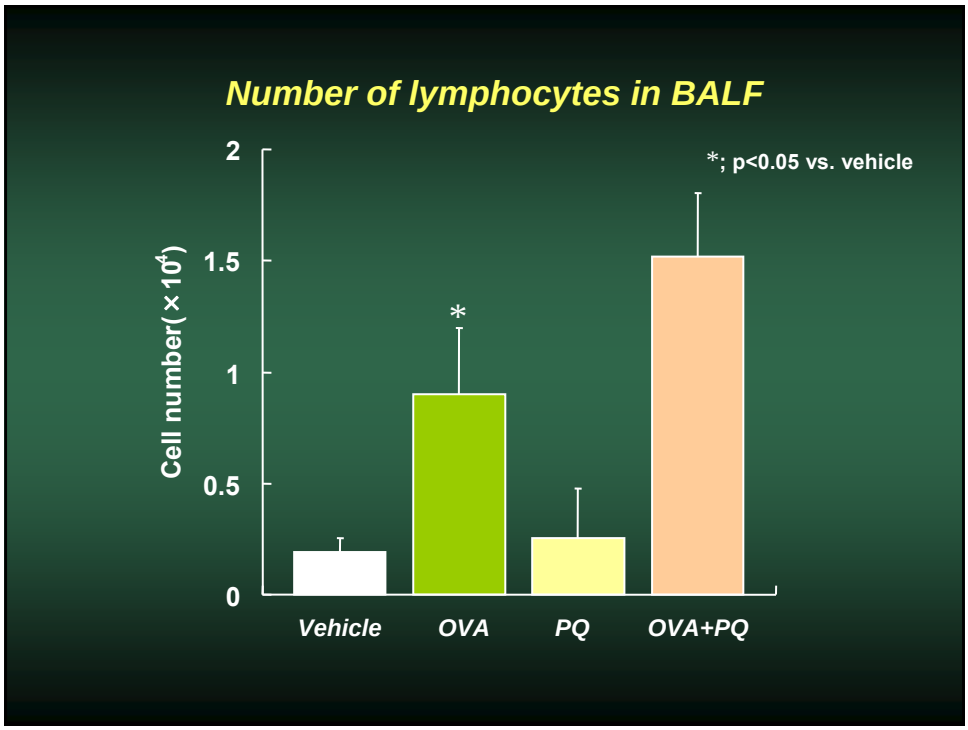
Cytokines and chemokines in the lung

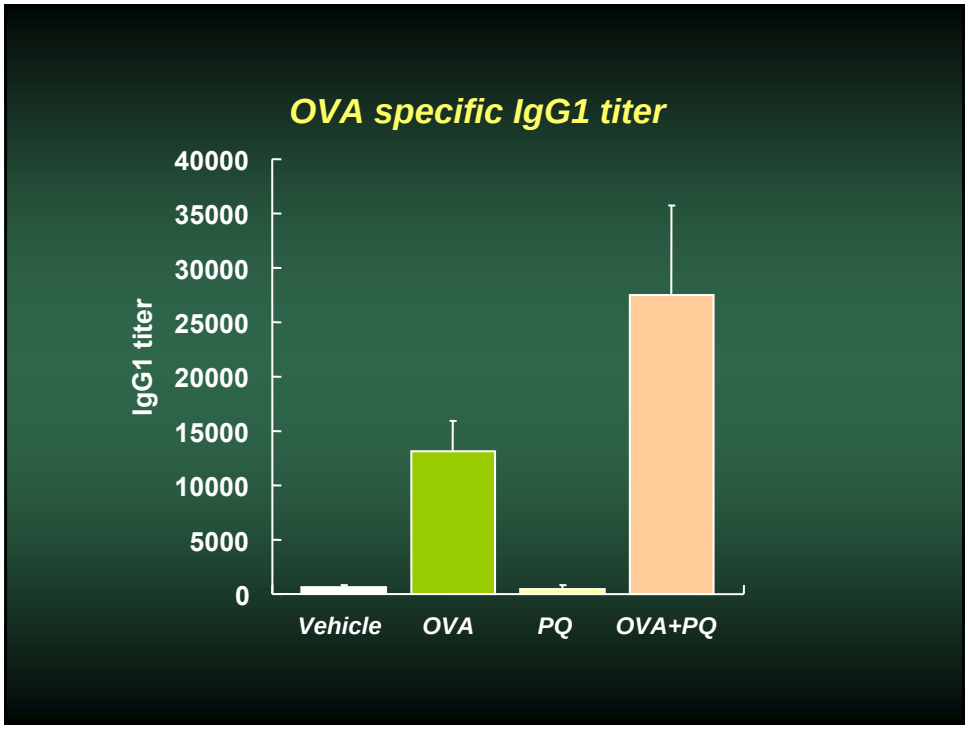
Antigen-specific IgG1、IgE

Histology

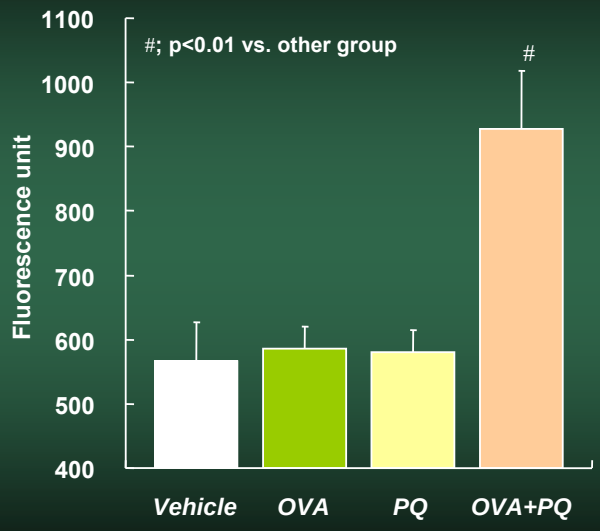
Number of Eosinophils in BALF



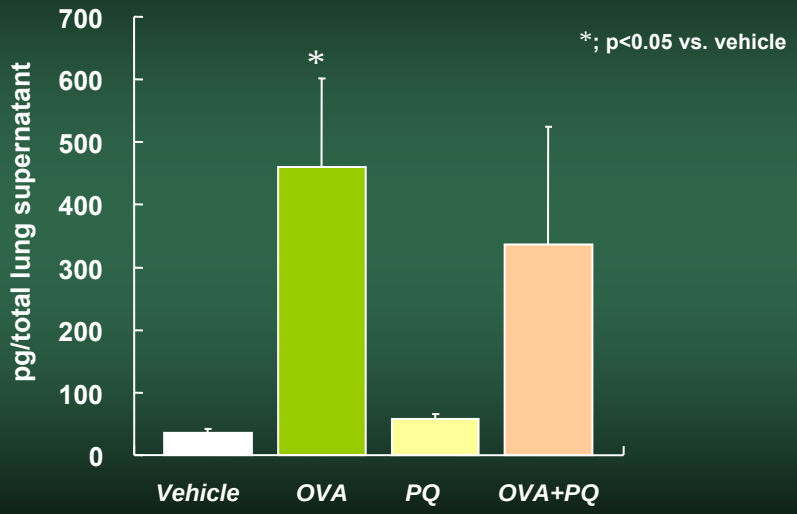

Number of lymphocytes in BALF
*; p<0.05 vs. vehicle
Cell number(× 10⁴)
2
1.5
1
0.5
0
*
Vehicle
OVA
PQ
OVA+PQ

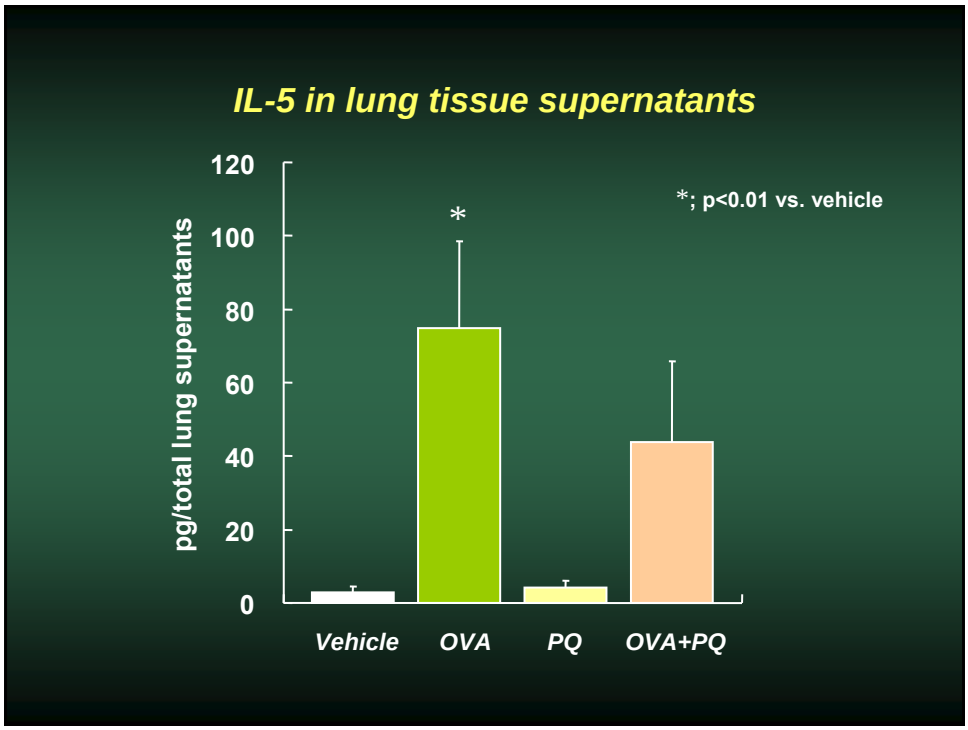

OVA specific IgG1 titer
IgG1 titer
40000
35000
30000
25000
20000
15000
10000
5000
0
Vehicle
OVA
PQ
OVA+PQ

Production of OVA-specific IgE



Eotaxin in lung tissue supernatants





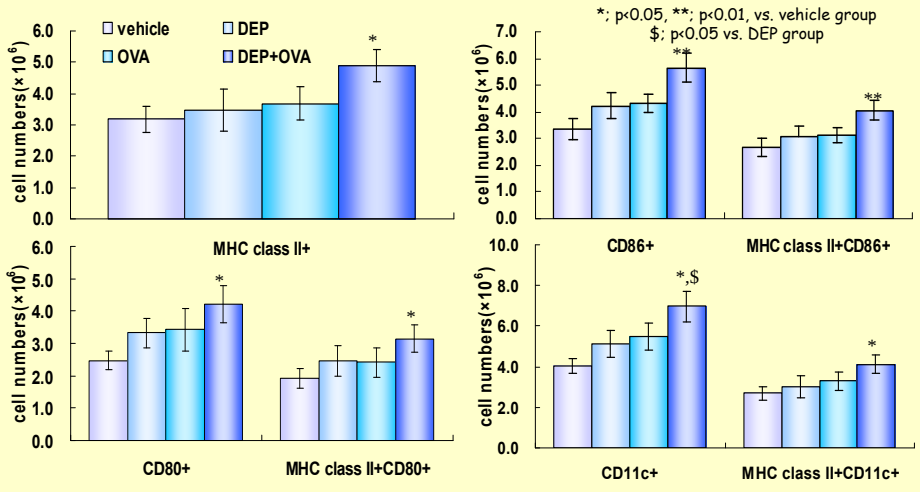
Conclusions

- 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.

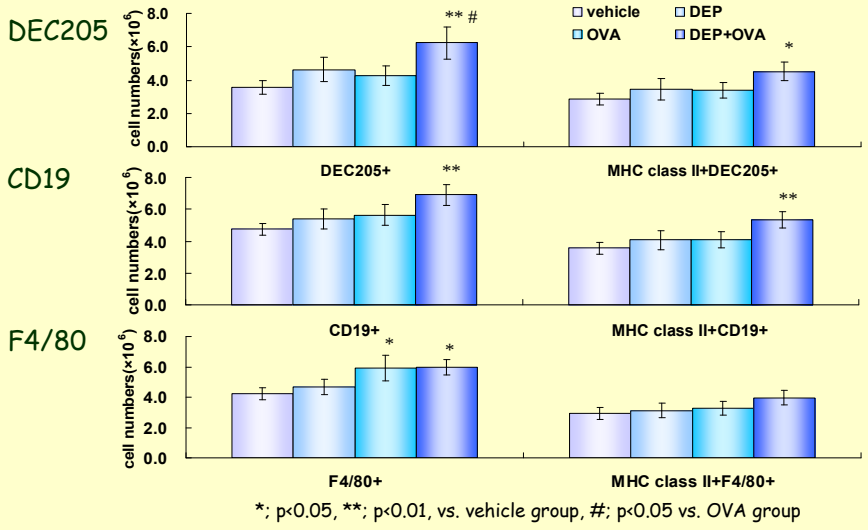
Hiyoshi, Takano, et al: Clin Exp Allergy, 2005
Yanagisawa, Takano, et al: Clin Exp Allergy, 2006

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



*; p<0.05, **; p<0.01, vs. vehicle group, #; p<0.05 vs. OVA group

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.

Inoue, Takano, et al: Exp Biol Med, 2009

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

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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 μg/body)
3) diesel exhaust particles (DEP: 10 or 250 μg/body)
4) LPS +DEP

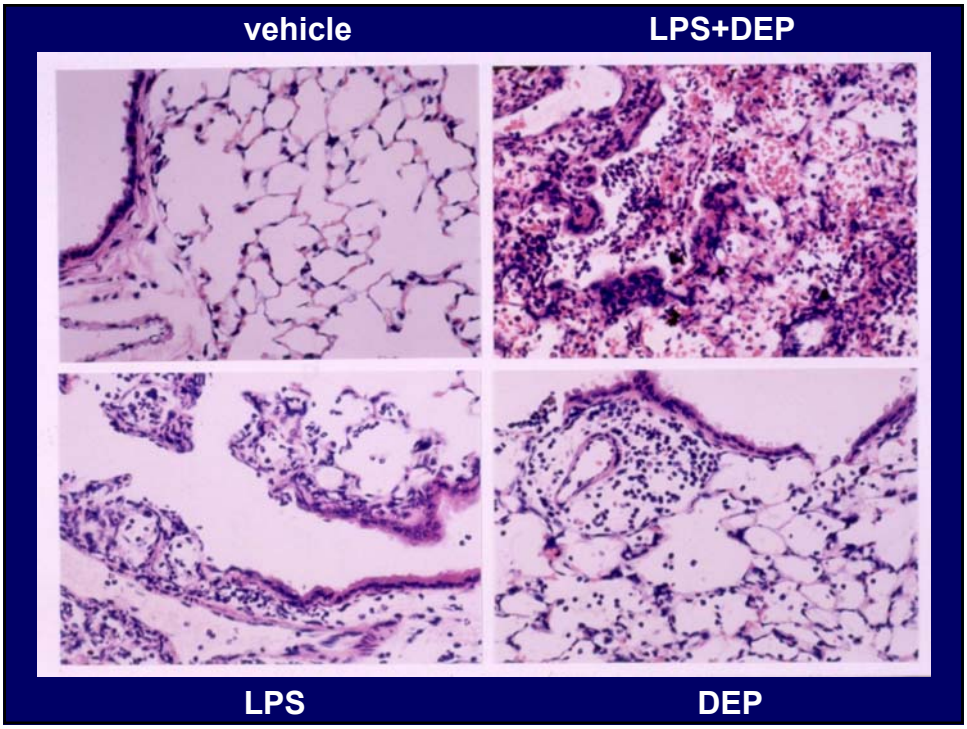
ICR mouse

2h 4h 24h

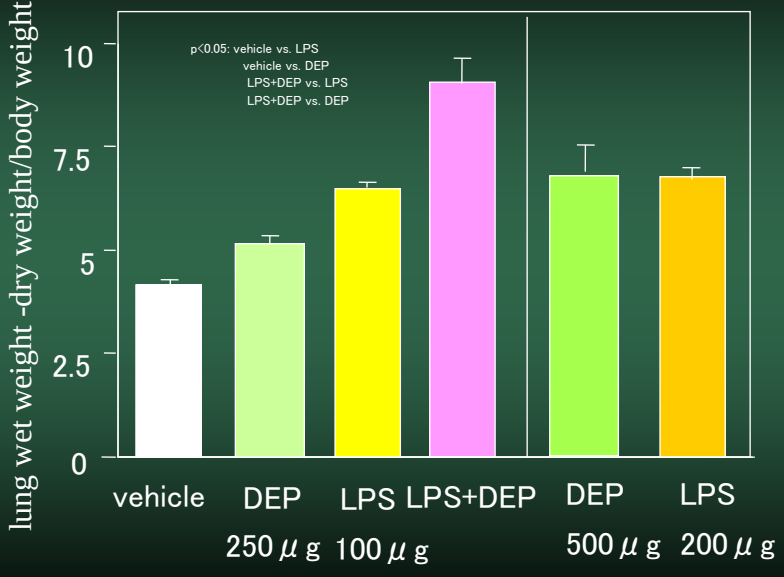
Cytoplasmic and nuclear protein
NF-κB (p65, p50) etc.

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

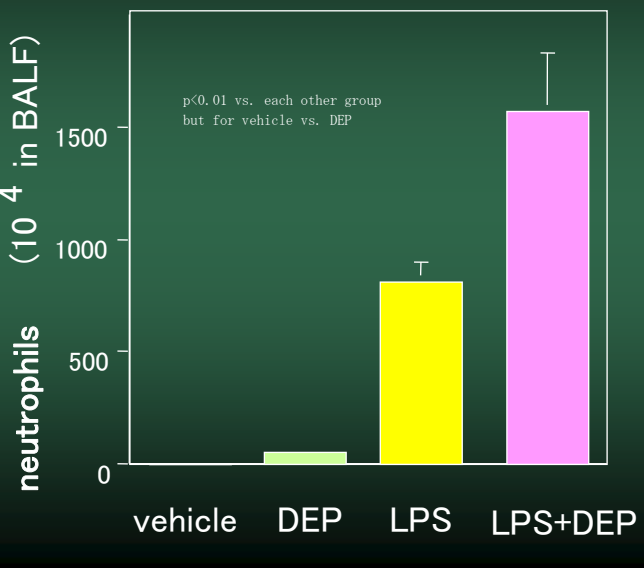
Histology
Water content (edema)
BAL
Protein expression
IL-1β, IFN-γ, TNF-α,
MIP1-α, MCP-1, KC,
s-ICAM-1, etc.



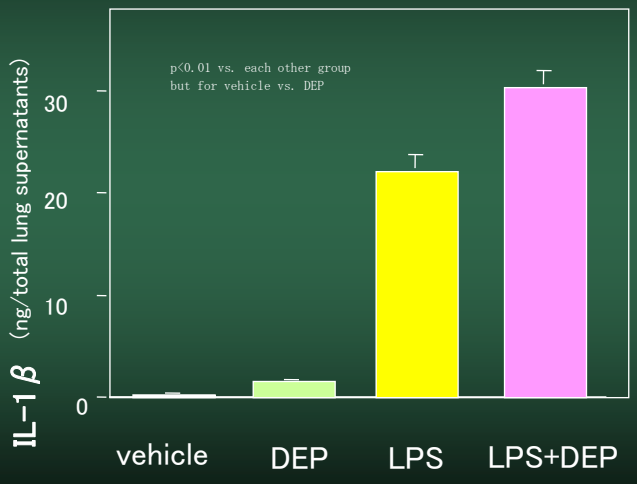
Evaluation of pulmonary edema



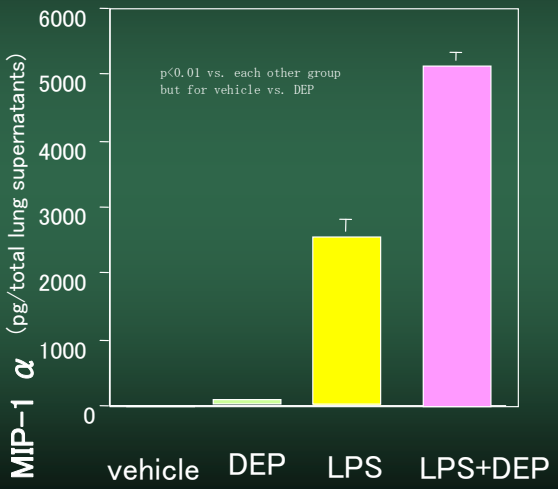
Number of neutrophils in BAL fluid



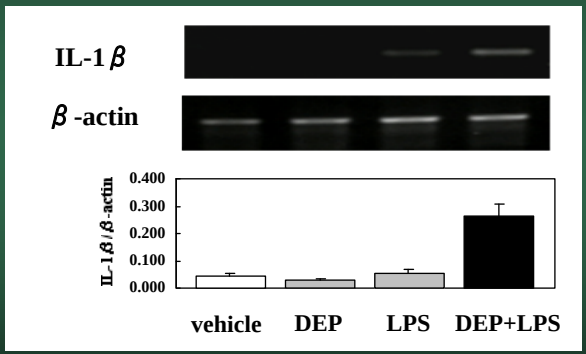
IL-1 in lung tissue supernatants



MIP-1 α in lung tissue supernatants

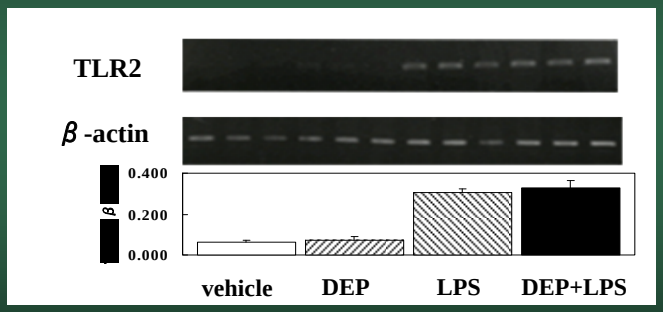


PCR for IL-1 β

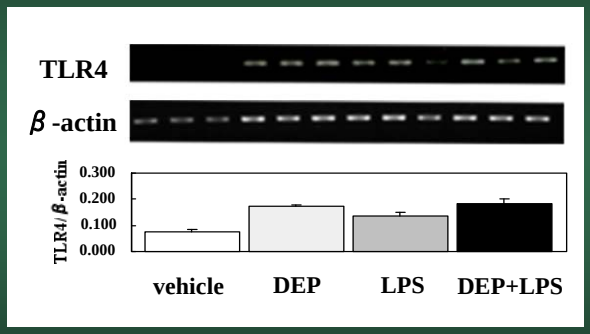


Western blot for p65

PCR for Toll-like receptor 2



PCR for Toll-like receptor 4

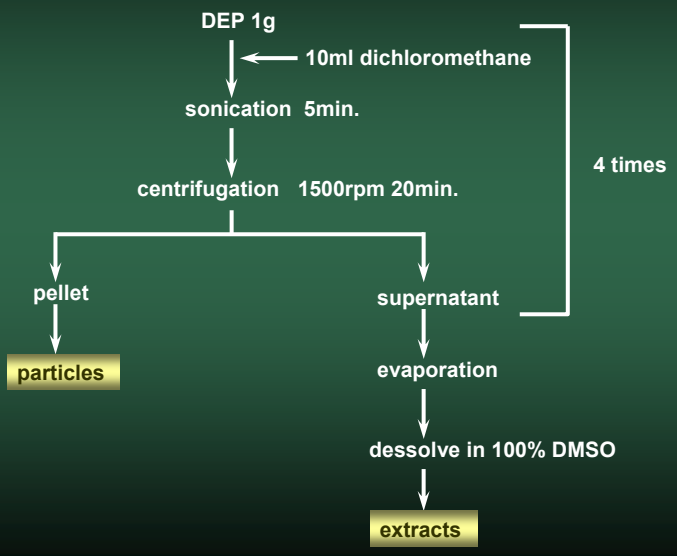


Conclusions

- 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

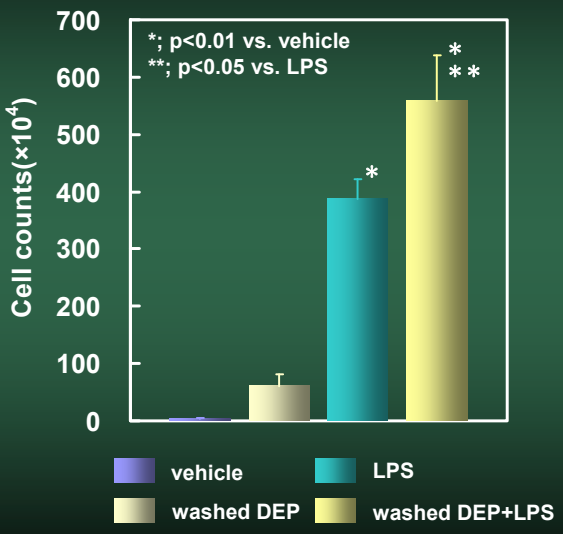
Group
1) vehicle(PBS(pH7.4)-0.025%Tween80-0.25%DMSO)
2) washed DEP (125μg/body)
3) LPS (75μg/body)
4) washed DEP(125μg)+LPS (75μg)

i.t. 100μl

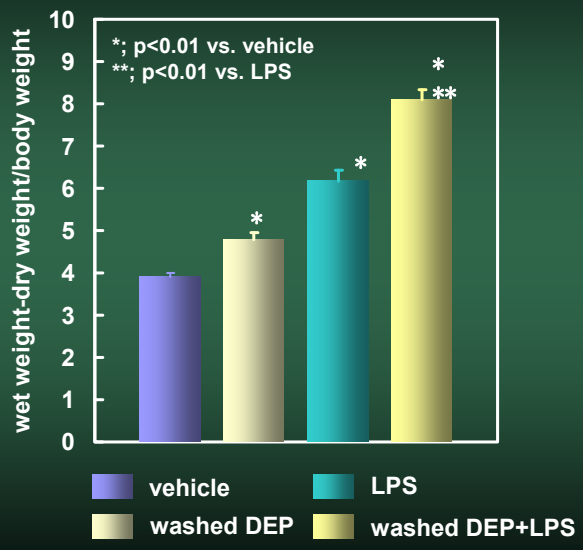
24hr

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

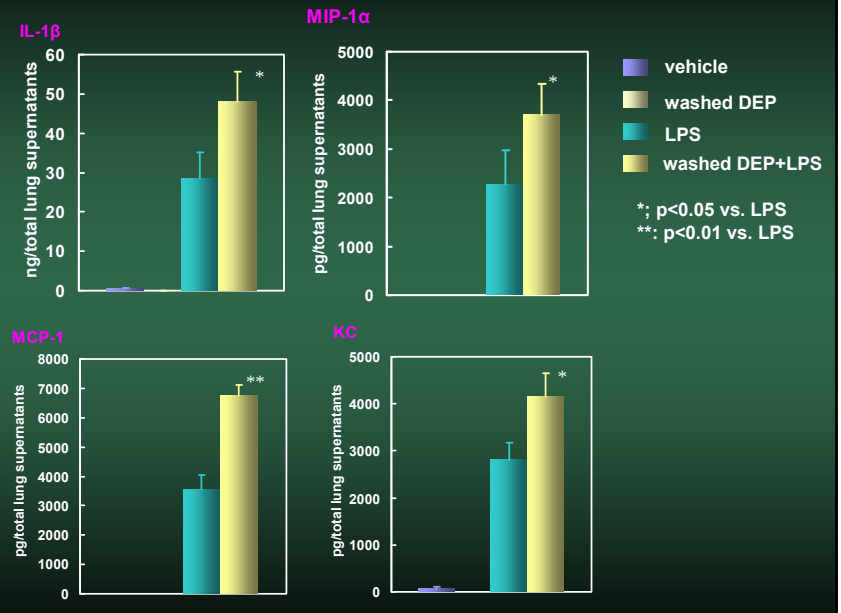
Neutrophils in BALF



Lung water content



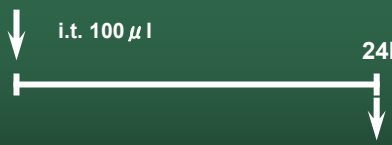
Cytokine or chemokine in lung tissue



Protocol
(protocol for DEP-OC)

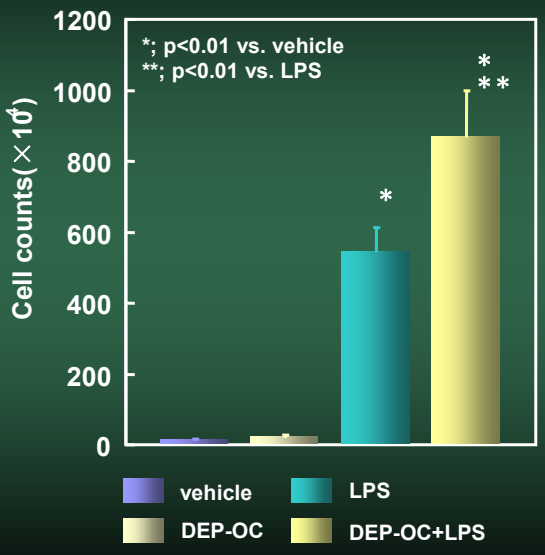
ICR ♂ 7-8w 32-38g

Group
1) vehicle(PBS(pH7.4)-0.025%Tween80-0.25%DMSO)
2) DEP-OC(125 μg/body)
3) LPS (75 μg/body)
4) DEP-OC(125 μg)+LPS (75 μg)

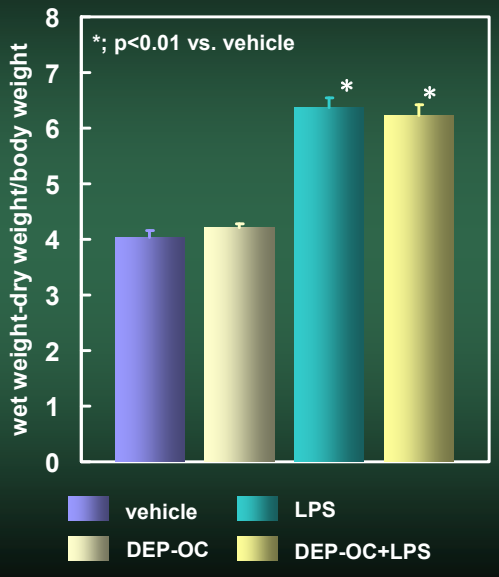


cell counts in BALF
lung water content
cytokine or chemokine in lung tissue

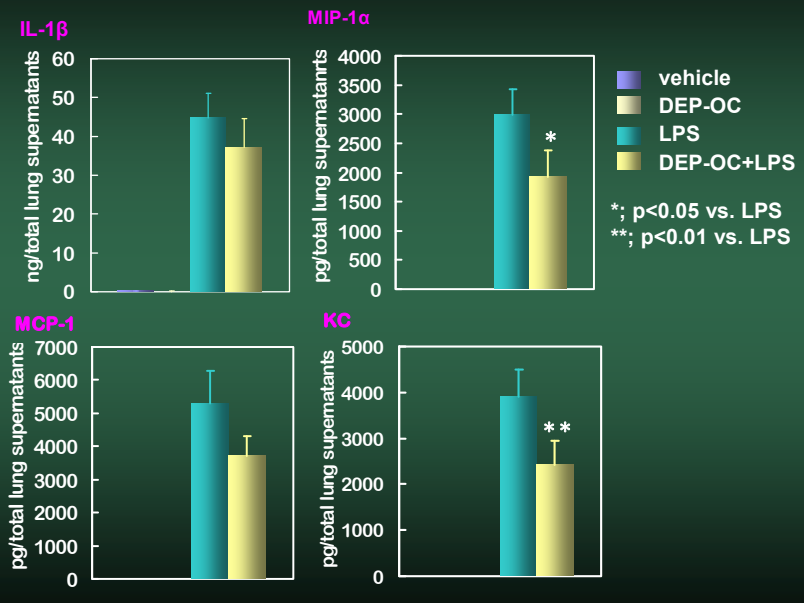
Neutrophils in BALF



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
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3) total mortality in children and aged individuals

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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)

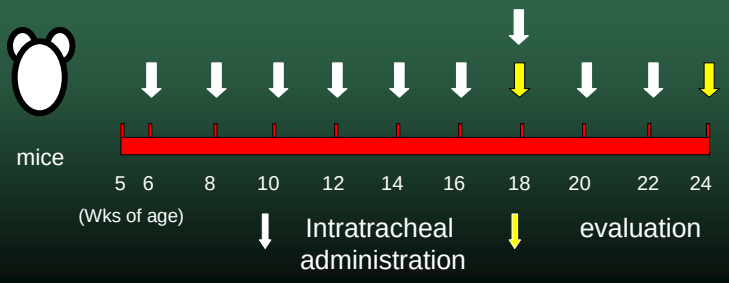
Groups

(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)

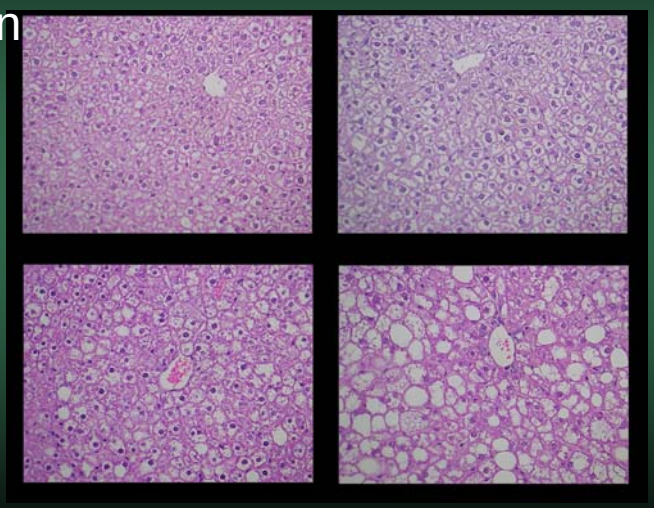
(3) db/db-vehicle (PBS (pH7.4)-0.05% Tween 80: 0.1ml/body)

(4) db/db-DEP (100 μ g/body in 0.1ml vehicle)



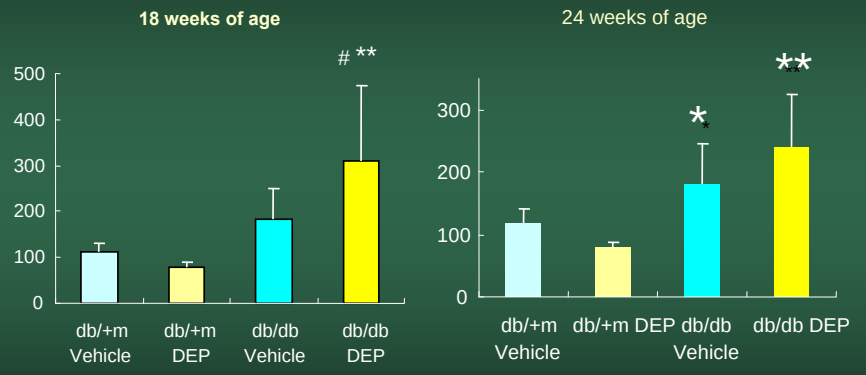
Histological effects of pulmonary exposure to DEP on fatty change in the liver

HE Stain



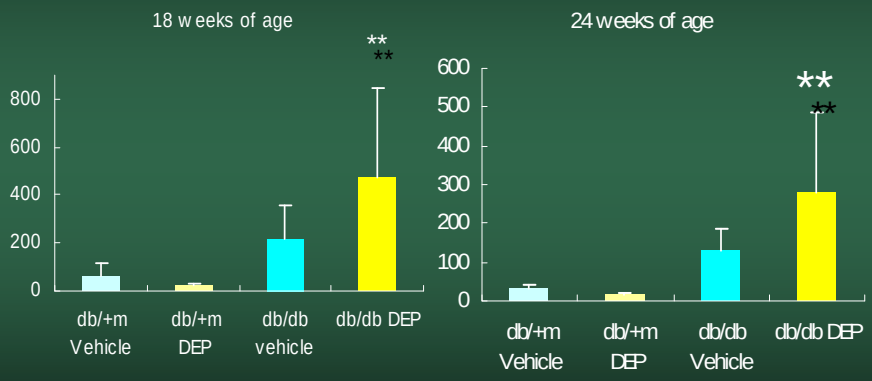
×400

Aspartate aminotransferase (AST)



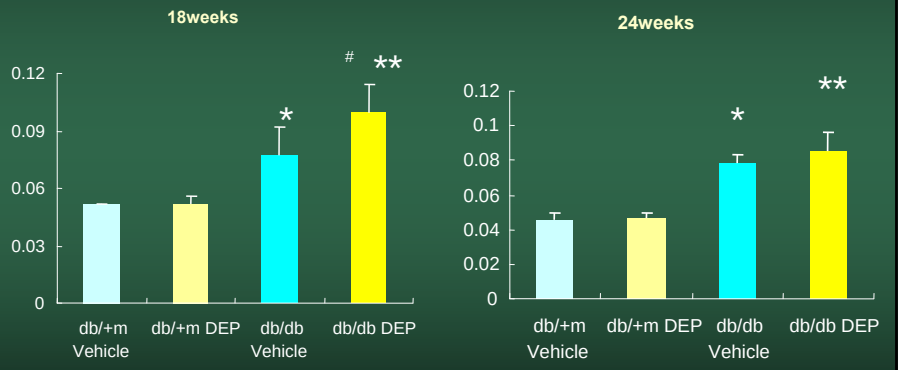
* P<0.05 versus db/+m mice receiving identical exposure
** P<0.01 versus db/+m mice receiving identical exposure
# P<0.05 versus vehicle exposure in the identical strains

Alanine aminotransferase (ALT)



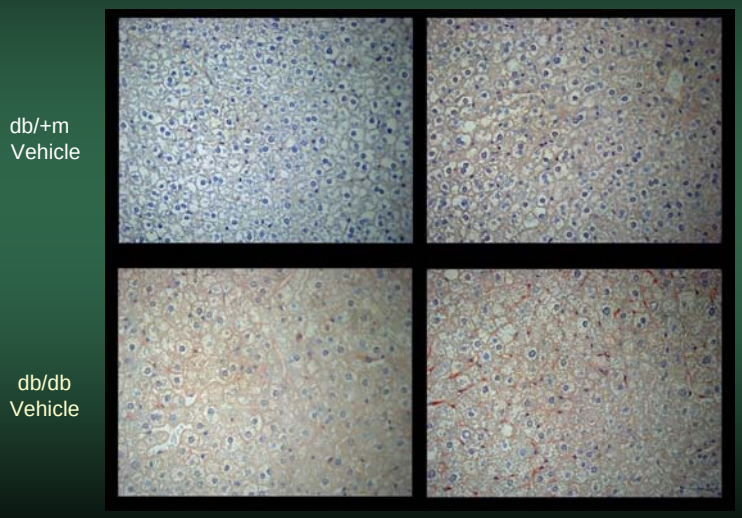
** P<0.01 versus db/+m mice receiving identical exposure

Effects of pulmonary exposure to DEP on liver weight



* 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

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-ichro Inoue, Rie Yanagisawa, Eiko Koike, Miho Sakurai, Naoko Ueki (NIES), Takamichi Ichionose, Kaori Sadakane, Kyoko Hiyoshi (Oita), Akinori Shimada (Tottori)