## Session 4

STIMULATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS BY DIFFERENT HEXAMETHYLENE DIISOCYANATE ANTIGENS IN HUMAN ISOCYANATE ASTHMA.
Wisnewski AV, Lemus R, Karol MH, Cullen MR, Redlich CA, Yale University School of Medicine, Occupational and Environmental Medicine Program and Pulmonary and Critical Care Section, New Haven, CT 06510, USA and University of Pittsburgh, Department of Environmental and Occupational Health, Pittsburgh, PA 115212, USA.

Isocyanates are highly reactive widely used low-molecular weight chemicals, and the most commonly reported cause of occupational asthma. We hypothesize that isocyanates act as foreign haptens that induce antigen specific T-cell responses. However, the Hexamethylene Diisocyanate (HDI) antigen(s) has not been well characterized. These studies describe the identification and initial characterization of candidate HDI antigens. HDI antigens were generated by reacting either liquid or vapor HDI with different human proteins and cells. Lymphoproliferative responses to the HDI antigens were determined using a $^{3}$H-thymidine-based *in vitro* assay with peripheral blood mononuclear cells (PBMCs) from isocyanate asthmatics and controls.

HDI was conjugated to the following: unfractionated plasma, human serum albumin (HSA), glutathione,
PBMCs from an HDI asthmatic but not to PMBCs from control individuals: HDI-plasma, HDI-HSA, HDI-epithelial cell proteins. Up to five-fold stimulation over baseline was obtained in a dose-dependent fashion, and was augmented by addition of the T-cell growth factor IL-2. The most potent stimulation was obtained using the human lung NCI-H292 epithelial cells and HDI-HSA with a 12:1 ration.

These studies demonstrate HDI binding to different human protein and cells, and specific lymphocyte proliferation in response to several different HDI antigens in isocyanate asthma. These HDI antigens will be better characterized and used to further investigate human cell-mediated immune responses to isocyanates. *In vitro* lymphocyte responses to HDI-antigens may serve as a useful peripheral blood marker to identify isocyanate asthmatics

## Session 6

TOLUENE DIISOCYANATE INHALATION ENHANCES SUBSTANCE P IMMUNOREACTIVITY AND PREPROTACHYKININ mRNA EXPRESSION IN TRIGEMINAL NEURONS INNERVATING THE NASAL EPITHELIUM.
R.D. Dey, D.D. Hunter, B.E. Satterfield, J. Huang, J. S. Fedan. Department of Anatomy, West Virginia University, Morgantown WV 26506 and Pathology and Physiology Research Branch, HELD, NIOSH, Morgantown, WV 26505, USA

Inhalation of irritants, like toluene diisocyanate (TDI), stimulates the release of substance P (SP) from peripheral processes of sensory neurons that innervate the airways. The purpose of this study was to determine if TDI inhalation affects intraneuronal levels of SP and preprotachykinin (PPT) mRNA in the sensory neurons of the trigeminal ganglion (TG) which innervate the respiratory epithelium of the nasal cavity. The nasal cavity of Fisher-344 male rats was instilled with rhodamine-labeled latex microspheres to identify neurons in the TG projecting to the nasal epithelium. Ten days after tracer application, the rats were exposed to 60 ppb of 2,4-2,6-TDI vapor for two hours. The TG were removed 1, 12, 24, 48, 72 and 96 hours after TDI-treatment and prepared for SP-immunocytochemistry and PPT *in situ* hybridization. SP nerve fiber density was measured in nasal mucosal tissues using immunocytochemistry. SP nerve fiber density (% nerve fiber area) was significantly increased 12, 24 and 48 hrs after TDI exposure. The proportion of microsphere-labeled cell bodies expressing high levels of SP immunoreactivity was decreased at 24 hrs but was increased above controls at 48 and 72 hrs. The proportion of microsphere-labeled cell bodies expressing high levels of PPT mRNA was increased above control levels at 24 and 48 hrs. All changes in SP immunoreactivity and PPT mRNA levels had recovered to control levels 96 hours after TDI inhalation. The percentage of leukocytes observed in nasal lavage fluid was significantly increased 1, 12, 24, 48 and 72 after inhalation and returned to control levels 96 hr after inhalation. These studies indicate that SP production in TG neurons projecting to the nasal epithelium is transiently increased after a single TDI exposure suggesting that TDI inhalation not only causes SP release but also increased intraneuronal neuropeptide levels. Increased neuronal SP levels may be involved in maintaining neurogenic inflammation or contribute to the development of airway hypreresponsiveness.
Supported by NHLBI HL 35812 and NIOSH.

Handout

# Altered Reactivity-modulating Function of Epithelium in Models of Occupational Asthma

***Jeffrey S. Fedan, PhD***

Saturday, May 2, 1998

# Is asthma an epithelial disease?

Hogg and Eggleston, 1984

1

- Basic mechanisms of epithelial regulation of airway reactivity
- Asthma
  - High molecular weight: Ovalbumin
  - Low molecular weight: Toluene diisocyanate (TDI)
- Ozone

2

## Hypotheses

- The respiratory epithelium regulates airway reactivity by releasing factors which modulate the behavior of smooth muscle.
- The respiratory epithelium is involved in the etiology of occupationally- and environmentally-induced obstructive diseases.

3

Effect of epithelium removal on sensitivity of guinea-pig tracheal strips

| Agent | n | +Epithelium | -Epithelium |
|---|---|---|---|
| | | $-\log EC_{50}$ (M) | |
| Histamine | 14 | 5.37 ± 0.06 | 5.60 ± 0.05* |
| Methacholine | 19 | 5.94 ± 0.04 | 6.25 ± 0.05* |

*Significantly different from +Epithelium

From Hay *et al.*, 1986

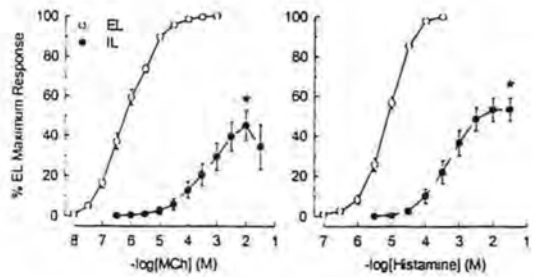
4

Perfused trachea apparatus

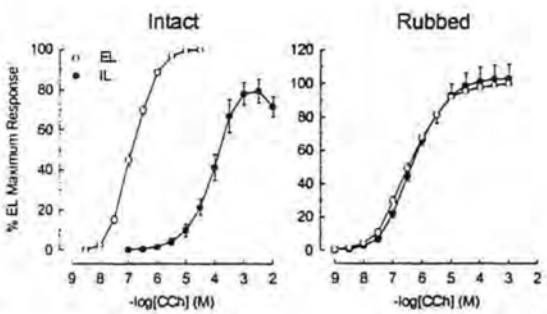
5

Innards of perfused trachea holder

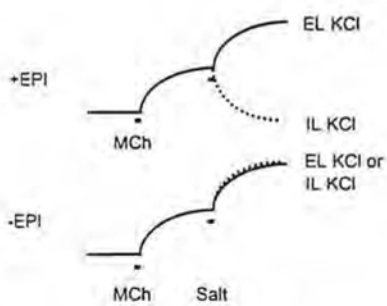
6


% EL Maximum Response
EL
IL
-log[MCh] (M)
-log[Histamine] (M)

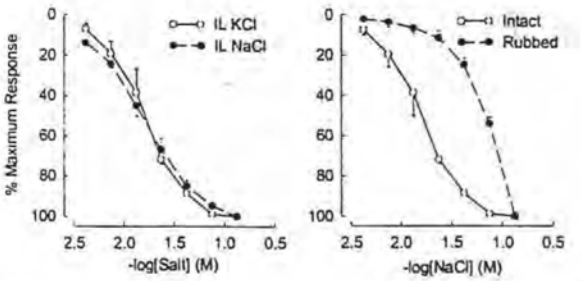
7


Intact
Rubbed
% EL Maximum Response
EL
IL
-log[CCh] (M)
-log[CCh] (M)

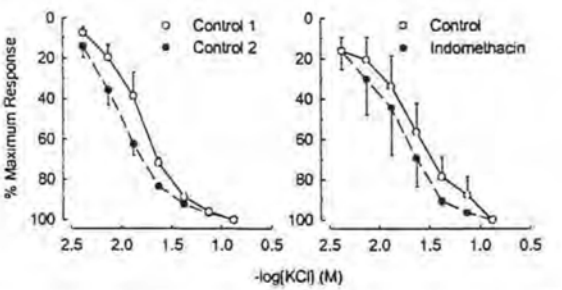
8


+EPI
EL KCl
IL KCl
MCh
EL KCl or
IL KCl
-EPI
MCh
Salt

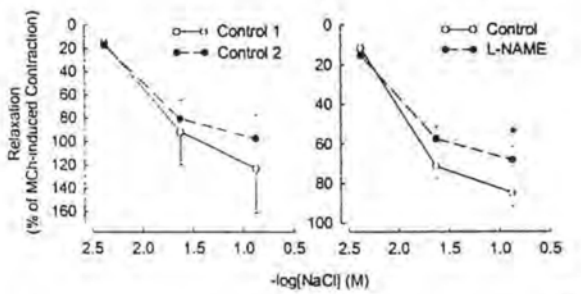
9


% Maximum Response
IL KCl
IL NaCl
-log[Salt] (M)
Intact
Rubbed
-log[NaCl] (M)

10


% Maximum Response
Control 1
Control 2
Control
Indomethacin
-log(KCl) (M)

11


Relaxation
(% of MCh-induced Contraction)
Control 1
Control 2
Control
L-NAME
-log[NaCl] (M)

12

## Hyperosmolar solution-induced release of EpDRF involves:

- Apical $Na^+$ channels (amiloride)
- Apical $Cl^-$ channels (DIDS)
- Basolateral $Na^+$-$K^+$-$2Cl^-$ co-transport (bumetanide)
- Basolateral $Na^+$-$K^+$ pumping (ouabain)



Bioelectric and mechanical events



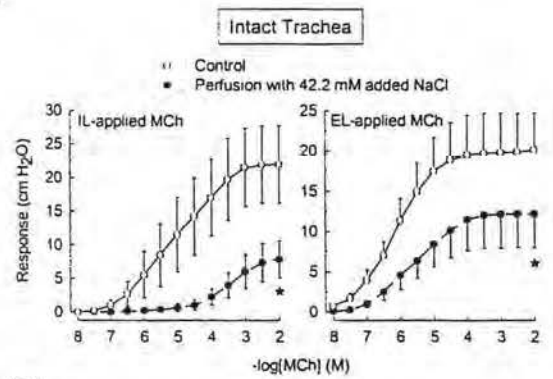
## Conclusion

Hyperosmolar solution releases EpDRF

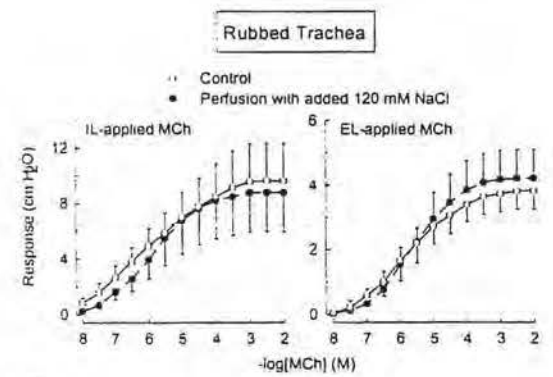


What is the effect of provoked EpDRF release on reactivity?

16




17

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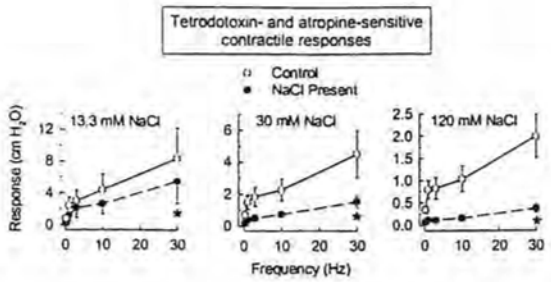


19

## Conclusion

- EpDRF released with hyperosmolar solution inhibits reactivity to exogenous and endogenous MCh.

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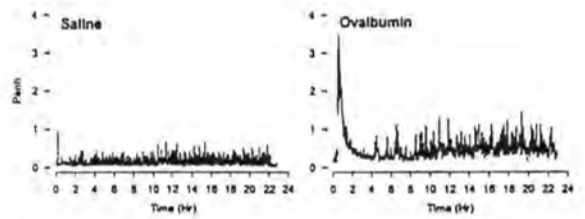
## Ovalbumin

21

## Ovalbumin (Ova) sensitization and challenge

- 10 µg Ova + 1 mg $Al(OH)_3$, s.c.
- Day 0 and day 14
- Inhalation challenge with Ova on day 21
- Pulmonary parameters measured before and after treatment

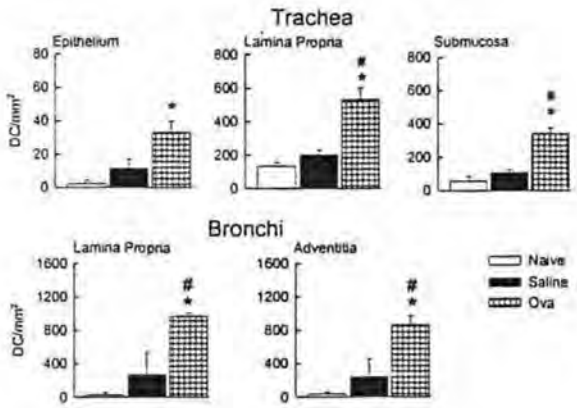
22




23

Histological section showing dendritic cells in guinea-pig airways

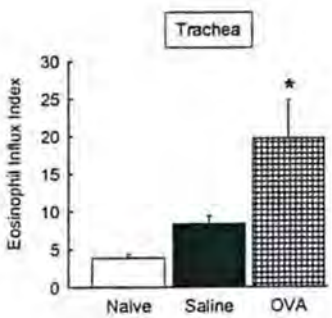
24



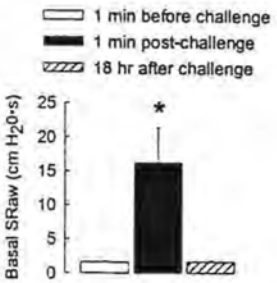

25

Histological section showing eosinophils in guinea-pig airways

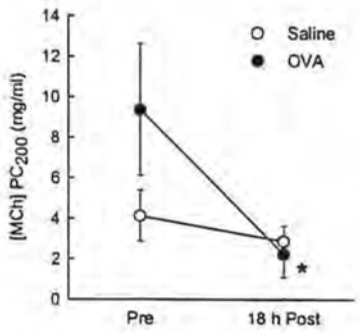
26



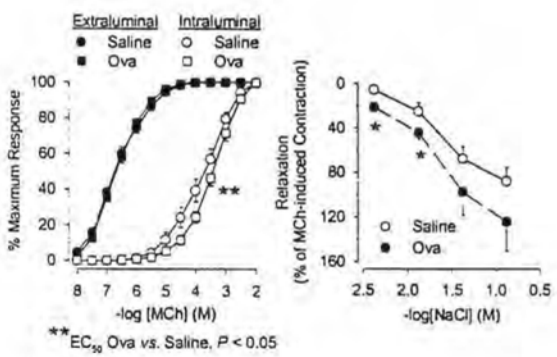

27


1 min before challenge
1 min post-challenge
18 hr after challenge
Basal SRaw (cm $H_2O$•s)
25
20
15
10
5
0
*

28


[MCh] $PC_{200}$ (mg/ml)
14
12
10
8
6
4
2
0
Saline
OVA
*
Pre
18 h Post

29


Extraluminal
Saline
Ova
Intraluminal
Saline
Ova
% Maximum Response
100
80
60
40
20
0
**
8 7 6 5 4 3 2
-log [MCh] (M)
Relaxation
(% of MCh-induced Contraction)
0
40
80
120
160
*
*
Saline
Ova
2.5 2.0 1.5 1.0 0.5
-log[NaCl] (M)
** $EC_{50}$ Ova vs. Saline, $P < 0.05$

30

There were no differences in the intraluminal $EC_{50}$ values in the absence of the epithelium.

31

## Summary

- Airway *hyper*reactivity to MCh *in vivo* is accompanied by *hypo*reactivity *in vitro* to intraluminally-applied MCh.
- Intraluminal hyporeactivity is epithelium-dependent.
- Intraluminal hyporeactivity is accompanied by an increased relaxation response to hyperosmolar solution, i.e., by enhanced EpDRF release.
- Is this a compensatory mechanism?

32

## TDI

33

## Experiments with TDI

*In vivo* treatments:

- TDI sensitization + TDI challenge
- TDI sensitization + TDI/guinea-pig serum albumin conjugate challenge

*In vitro* experiments:

- Reactivity of perfused trachea after treatment of animals
- TDI added to Krebs solution
- Exposure to TDI vapor



## TDI sensitization + TDI challenge (TDI-TDI)

- Sensitization: intranasal 5 µl of 10% TDI in ethyl acetate (EA) once/day x 7 days
- Challenge: 2 weeks later intranasal 0.2% TDI in EA
- Controls: intranasal EA



Time course of sRaw after TDI sensitization and challenge



Eosinophil response after TDI sensitization and challenge

37

Reactivity to inhaled MCh after TDI sensitization and challenge

38

Reactivity of perfused trachea to MCh after TDI sensitization and challenge

39

## TDI-TDI Summary

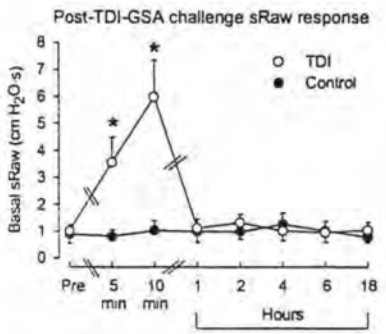
- Hyperreactivity to inhaled MCh is accompanied by eosinophilic inflammation but not by a change in reactivity *in vitro*

40

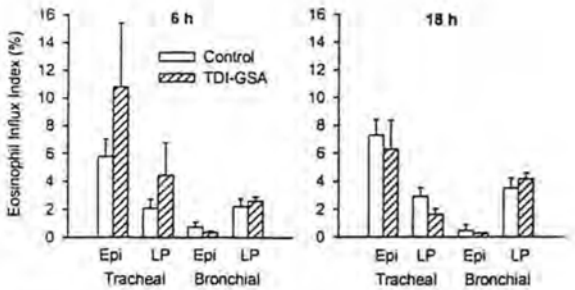
## TDI sensitization + TDI-GSA challenge (TDI-TDI/GSA)

- Sensitization: i.d. injection of 10 µl TDI on day 1 and day 6.
- Challenge: Two weeks later animals were challenged by exposure to 1% aerosol of TDI-GSA conjugates for 5 min.
- Controls: saline

41




42

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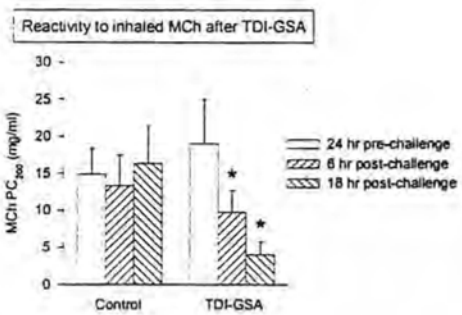



44

Reactivity of perfused trachea after TDI-GSA challenge

45

## TDI-TDI/GSA Summary

- Airway *hyper*reactivity *in vivo* to MCh is accompanied by *hypo*reactivity *in vitro* to intraluminally-applied MCh.
  - Analogous to Ova; different from TDI-TDI
- Not accompanied by eosinophilic inflammation
  - Different from Ova



## Effects of TDI *in vitro*

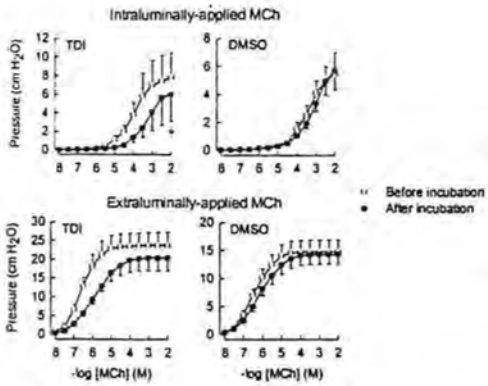
- What are the direct, non-immune, chemical effects of TDI on the airways?



## TDI in Krebs solution *in vitro*

- Isolated, perfused trachea preparation
- Control MCh concentration-response curve obtained
- Apply 1 mM TDI in DMSO to IL bath
- Control: 0.13% DMSO
- Repeat MCh concentration-response curve






49

Effects of TDI on reactivity to IL MCh in denuded trachea

| | $EC_{50}$ (95% C.I.) |
|---|---|
| Before TDI (6) | $7.7(4.2\text{-}13.9)\times10^{-7}$ |
| After TDI (6) | $1.7(1.1\text{-}2.7)\times10^{-6}$* |
| Before DMSO (5) | $4.4(3.1\text{-}3.3)\times10^{-7}$ |
| After DMSO (5) | $7.5(5.0\text{-}11.0)\times10^{-7}$ |

*Before *vs.* after, $P < 0.05$

50

## Conclusion: TDI in Krebs

- Exposure to TDI in Krebs solution causes *hypo*reactivity *in vitro*

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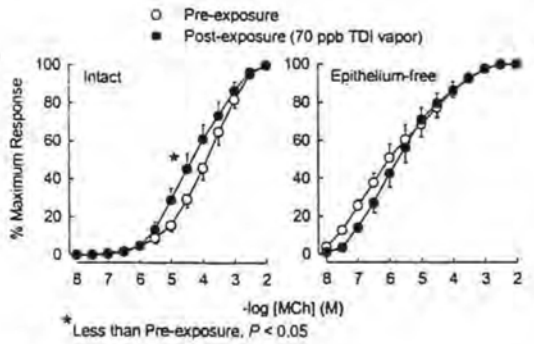
## Effects of TDI vapor *in vitro*

- Isolated, perfused trachea preparation
- Control MCh concentration-response curve obtained
- Deliver TDI to lumen of air-filled trachea (20 - 70 ppb) for 30 min
- Control: Air
- Repeat MCh concentration-response curve

52

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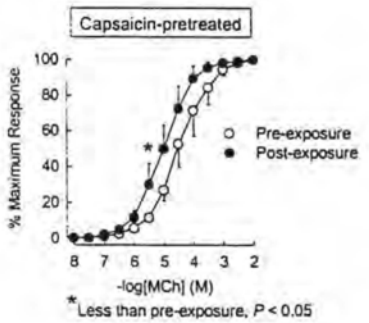


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## Conclusions: TDI vapor

- Exposure to TDI vapor causes *hyper*reactivity *in vitro*
- Substance P is not involved



## Conclusions from TDI

- The effects of TDI are protocol-specific
- *In vivo*:
  - TDI-TDI: hyperreactivity and inflammation
    - no effect on reactivity of perfused trachea
  - TDI-TDI/GSA: hyperreactivity without inflammation
    - intraluminal reactivity is decreased
- *In vitro*:
  - TDI in Krebs: hyporeactivity
  - TDI vapor: hyperreactivity
  - The epithelium is a target in both cases



## Ozone

## $O_3$-Treatment

- 3 ppm $O_3$ or filtered air for 1 hr
- Pulmonary function before and after
- Reactivity to MCh challenge before and after
- *In vitro* reactivity to MCh

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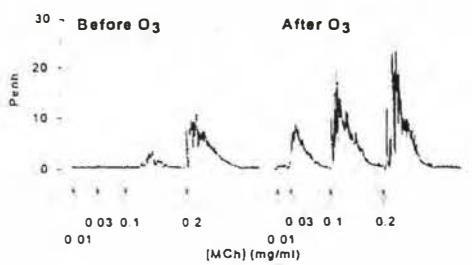
## Histological sections

59

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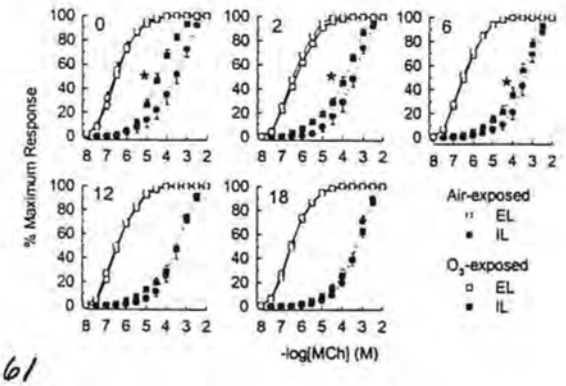
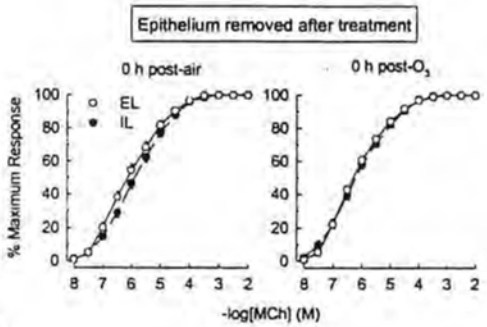
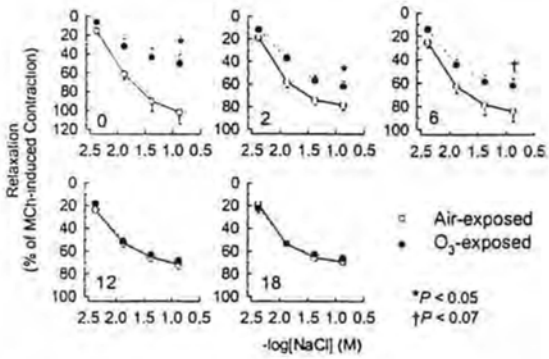

0
2
6
12
18
% Maximum Response
-log[MCh] (M)
Air-exposed
EL
IL
O3-exposed
EL
IL

61


Epithelium removed after treatment
0 h post-air
0 h post-O3
EL
IL
% Maximum Response
-log[MCh] (M)

62


Relaxation
(% of MCh-induced Contraction)
0
2
6
12
18
Air-exposed
O3-exposed
*P < 0.05
†P < 0.07
-log[NaCl] (M)

63

## Conclusions from $O_3$

- Airway hyperreactivity to MCh *in vivo* after $O_3$-treatment is accompanied by:
  - an increase in intraluminal reactivity to MCh *in vitro*
  - a decrease in EpDRF release
  - these two changes occur in parallel
- The airway epithelium is involved, at least in part, in $O_3$-induced airway hyperreactivity to MCh



We have never observed an effect of an agent, following treatment of the animal, directly on the smooth muscle.

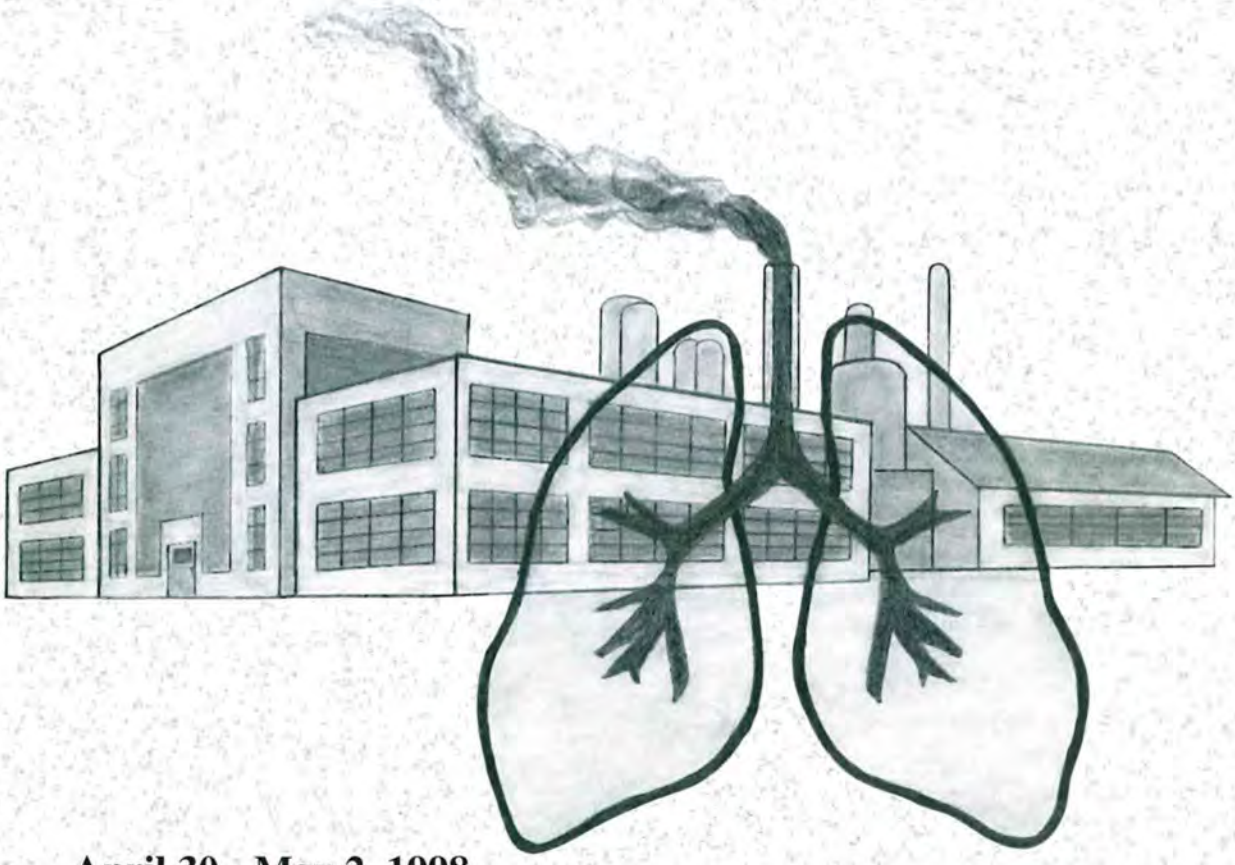


PROGRAM

# Occupational Asthma: In and Out of the Workplace

National Institute for Occupational Safety and Health
West Virginia University School of Medicine
Office of Continuing Medical Education

In cooperation with
American Academy of Allergy, Asthma, and Immunology
American Thoracic Society
National Institute of Allergy and Infectious Diseases
National Institute of Environmental Health Sciences
National Heart, Lung, and Blood Institute
United States Environmental Protection Agency



**April 30 - May 2, 1998**

National Institute for Occupational Safety and Health, Morgantown, WV