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BACKGROUND

The International conference of the American Thoracic Society and the American Lung Association is an annual event with a basic clinical orientation but also strong sections on molecular biology of the lung, cell reactions and mediators.

This year 3,374 abstracts were presented in 88 thematic poster sessions. There were 33 scientific symposia and 20 sessions on clinical topics in pulmonary medicine.

The following presents a synthesis of the major presentations related to effects of tobacco smoke. Toxicological and epidemiological aspects were covered - due to time conflicts sessions on behaviourial aspects could not be attended.

Reference to the different studies will be made by the letter A, and a page number, which refers to the American Review of Respiratory Disease supplement, vol 145, number 4, April 1992.

IN VITRO STUDIES

• Wirtz *et al.*, from Wurzburg (A136) isolated type II cells from rats and bubbled smoke from 2R1 cigarettes through the culture medium. They measured phospatidylcholine and found a reduced secretion after exposure. The conclusions were that tobacco smoke in small doses could influence the secretion of surfactant.

Comment: Traditional *in vitro* model which emphasizes the role of the vapour phase.

• Lannan *et al.*, from Edinburgh (A136) also studied rat type II cells and they found that cigarette smoke administered *in vitro* caused a larger detachment of cells from the plate surface. Cells grown on type 1 collagen were more resistant.

Comment: Shows in essence that tobacco smoke is cytotoxic but relevance for pathology is unclear.

• Xiao Yang Li *et al.*, (from the same group - A136) studied epithelial permeability to serum albumin *in vitro* by measuring penetration through a cell culture layer and *in vivo* using intratracheal installations in rats. In the *in vivo* model, both whole smoke and vapour phase caused increased penetration - vapour phase slightly less. In the *in vitro* model, cigarette condensate was given intratracheally and they found an increased permeability and a glutathione depletion.

Comment: In essence, no new observations and doubtful *in vivo* model.

• Gosset *et al.*, from Lille, France (A651) exposed alveolar macrophages to different fractions of cigarette smoke. IL-1 and TNF-production were decreased by the exposure. They also concluded that this effect was a direct effect on the production of the cytokines and not an effect of inhibitors.

Comment: Results in accordance with others except for the inhibitors where in vivo models showed an increased secretion of IL-6 inhibitor.

• Drost *et al.*, from Edinburgh (A797) exposed PMN to cigarette smoke and measured the deformability. Exposed PMNs had a lower entry time through a 4.5 µm pipette opening and an increased F-actin content.

Comment: As PMNs don't get exposed directly to smoke *in vivo*, the relevance of the results is questionable.

ANIMAL STUDIES

• Nilsson *et al.*, from Sweden (A35) had studied fibrosis and smoking. Rats were exposed to a single dose of radiation. Some animals were given cigarette smoke (9 cig/day 60 min dose 4.1 mg) during 3 weeks before and 7 weeks after irradiation. The smoke exposed group had considerably less tissue damage and the elevated number of macrophages, mast cells and neutrophils found after radiation, was not present in the smoke exposed animals.

Comment: The mechanism behind this very clear effect could be tobacco smoke effects on macrophages, the major cells determining fibroblast chemotaxis and growth.

• Williams *et al.*, from Irvine Medical Center (A38) had exposed rats to smoke (10 min every hour 4 hours) followed by sacrification. The lungs were lavaged via the vasculature and cells collected. The number of neutrophils and lymphocytes was increased and zymosan stimulated oxygen radical production was increased. Transcription of mRNA for IL-1-ß and Il-6 was present in RNA isolated from lung tissue in smoked animals.

Comment: Nice study with results that correspond to our own results from LPS exposures. A chronic exposure experiment would be required before any conclusions concerning relation to emphysema could be drawn. Compare also to different results from study by Kwon *et al.*, below.

• Kwon *et al.*, (A47) from London had exposed guinea pigs to smoke through tracheotomy (50 puffs) and examined them 5 and 30 minutes later. Other animals received capsaicin. No neural endopeptidase (NEP) could be found in the tissue nor were NEP mRNA differences seen.

Comment: Entirely negative study. Why was it hypothesized that NEP could play a role? No positive control was present. Smoke dosing very crude. Not a very clever study.

• Evans *et al.*, from Albuquerque (A91) exposed rats to tobacco smoke in whole body chambers for two weeks (150 and later 250 mg/m^3, 6 h/day) and examined them 1 day or 2 weeks after exposure. Immediately after exposure, there was loss of intraepithelial mucosubstances in the mid-septum and an increase in the dorsal septum. Absence of mucus was related to increase in squamous metaplasia. At two weeks, there was no metaplasia but an increase in mucus.

Comment: Essentially, the same results that were produced at INBIFO several years ago and then with a much higher precision.

• Bascom *et al.*, from Baltimore (A91) reported an experiment where two different inbred strains of mice were exposed to particle-filtered main stream smoke through a tracheostomy. The animals were treated with 5-hydroxytryptamine and atropine. Lung compliance was the endpoint. They concluded that genetic differences was a major reason for differences in response to the vapour phase of the smoke.

Comment: Technically correct study but the results are hardly surprising. No marker for human studies was defined.

• Von Behren *et al.*, from Springfield (A92) exposed mice to cigarette smoke 20 min, three times/day for three weeks. The animals were then exposed to an intratracheal instillation of Histoplasma capsulatum and smoking continued. The mortality in the smoke exposed group was higher than in the nonexposed control.

Comment: Basic study of the type that was done by Green, and by ourselves, in the 1970s. In the conclusion, they related the results to "passive smoking" which they backed away from in our discussion.

• Joad *et al.*, from Davis (A92) exposed young rats to side stream smoke (CO 6.48 ppm, TPM 1.0 mg/m^3) 6 hours/day for the first 8 or 15 weeks of their life. Their lungs were isolated in a perfusion system. All parameters measured - lung weight, artery pressure, etc, were negative. They concluded that this was not a good model to study ETS-effects on childrens' lungs.

Comment: I could not agree more. Even if they had found changes, I would have said this is a bad model.

• Baluk *et al.*, from San Francisco (A188) exposed rats to smoke for two weeks and gave i.v. injections of capsaicin and counted intravascular blood vessel adherent neutrophils in tracheal preparations. The number of cells induced by capsaicin was three times higher in smoked rats, whereas rats exposed to smoke

only, had very few cells. The smoke exposure did not potentiate capsaicin-induced extravasation of plasma.

Comment: Logical finding in view of the priming of neutrophils to produce oxygen radicals after tobacco smoke exposure as reported below.

• Doerschuck *et al.*, from Vancouver (A563) exposed 5 rabbits to cigarette smoke and studied adhesion molecules on PMNs in the lung capillaries. Adhesion molecules were upregulated particularly between the plasma membrane of adherent PMN and the microvascular epithelium.

Comment: Proper study with results that are very reasonable.

• Rogers *et al.*, from London (A611) exposed guinea pigs and evaluated the extravasation of plasma using Evans blue. They found that nedocromil sodium and morphine inhibited the smoke induced extravasation, suggesting an effect mediated via capsaicin-sensitive sensory nerves.

Comment: Correct interpretation, but there are probably other mechanisms that could explain the extravasation such as activation of epithelial cells or macrophage secretion of cytokines. An adjacent poster described the role of NK-cells for the microvascular leakage.

STUDIES ON HUMANS

• Leonard *et al.*, from Baltimore (A87) studied "ETS-sensitive" subjects defined as those who had shown a decreased nasal air flow in previous exposures to side stream smoke. They exposed the subjects to substance P or saline and demonstrated that substance P caused an increased nasal irritation and increased secretion.

Comment: So what? As no ETS-nonsensitive subjects were included the only conclusion we can draw from the study is that substance P has an effect.

• Bascom *et al.*, (A92) from Baltimore (the same group - A92) challenged 29 subjects of whom 13 were ETS-sensitive to side stream smoke at 1, 5 and 15 ppm CO. Subjective symptoms of irritation were recorded and acoustic rhinometry demonstrated changes in mid-nasal volume at the lowest concentration.

Comment: This group has worked in this field for several years and is producing interesting data. In the end, they will probably come out with a better background for guidelines for ETS than was produced by Weber in Zürich in the 1980s.

• Frew *et al.*, from Vancouver (A197) and Xu *et al.*, from Harvard (A199) both studied decline in pulmonary function over time. It was reported that smoking asthmatics but not non-smoking asthmatics had an accelerated decline and increased airway reactivity, and that cigar and pipe smoking also caused an increased loss which was irreversible if the subjects stopped smoking after the age of 45 (see also Ross *et al.*, below).

Comment: Not unexpected - accelerated losses were quite small.

• McCrea *et al* from Baltimore (A269) studied alveolar macrophages from 3 non-smokers and 4 smokers. The LPS-induced production of TNFα was significantly decreased in smokers.

Comment: Small, but interesting and relevant study, confirmed by other groups (see below).

• Sauty *et al.*, from Lausanne (A275) also studied alveolar macrophages obtained by lavage. Smokers' macrophages produced less IL-1ß, IL-6 and TNFα but more oxygen radicals after stimulation with PMA.

Comment: Nice study with relevant findings.

• Ross *et al.*, from Aberdeen (A296) investigated smoking habits of 287 adults defined in a health survey in 1964. Lung function values were more closely related to a history of asthma in childhood than to smoking habits.

Comment: The results demonstrate the need to control for atopy and history of asthma (which have strong genetic components as reported in A299) in all studies on lung function and its relation to smoking.

• Twigg *et al.*, from Indianapolis (A468) studied alveolar macrophages from 6 smokers and 6 non-smokers. IL-6 secretion was significantly associated with IL-1 secretion in nonsmokers but not in smokers. The authors suggest that this was due to secretion of an IL-1-inhibitor.

Comment: Relevant study and interesting finding on inhibitor.

• Gebremichael *et al.*, at Davis, California (A798) used conditioned side stream smoke and exposed rat pups from birth to 100 days age (6 h/d, 5 d/w,TPM 1 mg, CO 6.6 ppm) and evaluated effects on the CP450 activity. Subgroup IA1 was increased at 50 and 100 days. They concluded that ETS may alter the lungs' capacity to metabolize proximate carcinogens.

Comment: In the discussion with the poster presentor, I pointed out the absence of relevance of their data for ETS. He agreed but thought that they were going to follow this up with lower concentrations in future experiments. Questionable model with doubtful endpoint.

STUDIES ON ETS

• Dales from Ottawa (A532) reviewed several studies on the relation between asthma and exposure to ETS, and discussed the reasons for discrepancy in results from different studies. He suggested that a major methodological error was misclassification, particularly due to the use of questionnaires for diagnosing asthma. In studies where the diagnos was made using bronchial reactivity testing, there was a better relationship.

Comment: Correct, but a major error is still confounding variables, not controlled for in the different studies.

• Fisher *et al.*, from the Netherlands (A532) analyzed data from a cross sectional study with 2101 children. ETS exposure was associated with a higher prevalence of acute lower and with a lower prevalence of acute upper respiratory symptoms. No significant relationships were found with damp housing conditions.

Comment: Important confounders such as pets, day care center attendance and diet were not controlled for.

• Collier *et al.*, from EPA, Chapel Hill (A532) measured cotinine in urine of 39 children up to 36 months of age, where the mother smoked and determined half-times. They found that the excretion was faster in older children.

Comment: No data on absolute levels were reported.

• O'Connor *et al.*, from Harvard (A532) studied 13975 children from 24 communities and determined the relation between emergency room visits and exposure to ETS. A relationship was found as well as for social class. ETS was responsible for 15% of the etiological fraction.

Comment: As above several important confounders were not controlled for.

• Vedal *et al.*, from Vancouver (A533) made a questionnaire study on 2199 children. Maternal smoking was associated with increased reported wheeze but not with prevalence of respiratory disease. Children of smoking mothers has lower lung function values. Wood burning in homes was not related to respiratory symptoms.

Comment: An ambitious study but several risk factors are not controlled for.

• Agudo *et al.*, from Barcelona (A533) studied 2216 school children 9-14 years old. Exercise induced bronchial reactivity was determined and a questionnaire was applied. Smoking habits of the mother but not the father was associated with increased reactivity as was cooking with butane gas.

Comment: Same as above.

• Hanrahan *et al.*, from Boston (A560) studied 99 healthy infants between birth and 18 months of age. Advanced lung function measurements were made. ETS exposure was not related to lung development parameters.

Comment: Well done study which gives a definite answer in contrast to results from previous cross-sectional studies.

• Rylander *et al.*, from Geneva/Gothenburg (A558) had studied 90 children 4-5 years of age. No relation was found between ETS exposure and respiratory infections but a strong, negative correlation was found for the diet factors egg and chicken meat.

Comment: Study earlier reported at INBIFO.

• Frischer *et al.*, from Freiburg (A659) followed diurnal variation of expiratory flow rates in 1,237 children aged 7-8 years. A higher diurnal variation was found for non-atopic children exposed to ETS but not for the atopic. They suggested that this absence of an effect was due to avoidance of smoking in families with atopic children but no data to support this was presented.

Comment: The significance of these findings is difficult to evaluate. No apparent relation is present between diurnal variation and risk for lung disease and the mechanism whereby an aggravation of the variation could account for an increased risk for asthma remains obscure.

GENERAL CONCLUSIONS

The *in vivo* experiments were of low quality and seemed more to be designed to support a previous concept than bringing new information. Several of the studies on lavage cells from humans yielded important information, particularly for the understanding of smoke induced inflammation. A relatively consistent picture emerges, indicating effects on the function of macrophages with altered cytokine and cytokine inhibitor secretion. The reduced risk for immune related diseases, e.g. hypersensitivity pneumonitis among smokers, now seems to be explained.

Generally, the epidemiological studies were of a poor quality and important confounders were not controlled for. There were no studies on mechanisms for cancer and tobacco smoke. Overall, this year's conference showed an increased interest in the inhalation toxicity of tobacco smoke but apart from data on lung lavage cells, relatively little new or original information emerged.