OCCASIONAL REVIEW

Acute effects of cigarette smoke on inflammation and oxidative stress: a review

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Compared with the effects of chronic smoke exposure on lung function and airway inflammation, there are few data on the acute effects of smoking. A review of the literature identified 123 studies investigating the acute effects of cigarette smoking on inflammation and oxidative stress in human, animal, and in vitro models. An acute smoking model is a relatively easy and sensitive method of investigating the specific effects of cigarette smoke on oxidative stress and inflammation. Acute smoke exposure can result in tissue damage, as suggested by increased products of lipid peroxidation and degradation products of extracellular matrix proteins. Acute cigarette smoke has a suppressive effect on the number of eosinophils and several inflammatory cytokines, possibly due to the anti-inflammatory effect of carbon monoxide. An acute smoking model can supplement other ways of studying the effects of smoking and is an as yet underinvestigated method for intervention studies in smoking related diseases.

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Chronic obstructive pulmonary disease (COPD) is a worldwide leading cause of morbidity and mortality and its prevalence is still rising.[1] It is therefore important to understand the development of this disease in order to develop strategies of prevention, treatment, and cure. In the past decade research has focused on the pathophysiological mechanisms underlying the development of COPD, yet several questions remain unanswered.

Most studies investigating the role of smoking in the pathophysiology of COPD have been carried out in chronic smokers. The drawback of studying the effects of actual smoke exposure in persistent smokers is the likely effect of already developed structural changes in the airways on the response to smoke. It is therefore important to study the response to the first smoke exposure of a ''naïve'' lung in order to assess the relevant changes that may have a role in the first steps of COPD development. In addition, an acute smoking model could be attractive for future intervention studies. We hypothesise that an acute smoking model can give clear and more specific information about the pathophysiological mechanisms of smoking induced lung disease.

In this paper we review the literature on the acute effects of smoking. We focus on human, animal, and in vitro models and systematically describe the effects of acute smoke exposure on the cellular response, specifically on oxidative stress and inflammatory mediators. We also review similarities and discrepancies in the smoking response between the three model systems and discuss how these results relate to the current insights on the development of COPD.

METHODS

The Medline, OldMedline, Winspirs and Cochrane Library databases were searched from their inception until October 2003. The language used was limited to English. Firstly, a database including all articles on the effects of smoking on pulmonary status was composed (keywords ''cigarette smoke, tobacco smoke'' and all subheadings and ''lungs, pulmonary'' and all subheadings). Secondly, a selection was made of the articles describing the acute effects of smoking (keyword ''acute''). Thirdly, all articles describing the acute effects of smoking on oxidative stress, inflammatory mediators, and inflammatory cells in humans, animals, and in vitro models were selected. Fourthly, a specific search was done on oxidative stress (keywords ''oxidative stress'' and all subheadings). Acute smoking was defined as an effect measured during the 24 hours after smoke exposure. It is explicitly mentioned when articles on chronic smoking or COPD have been used. Only studies describing mainstream cigarette smoke were included, the number of cigarettes smoked not being a selection criterion.

RESULTS

Acute effects of cigarette smoke in humans

Twenty five studies examining the acute effects of cigarette smoking (ACS) in humans were identified (see table S1 available online at www.thoraxjnl.com/supplemental), 16 on inflammation and nine on oxidative stress.

All studies were performed in chronic smokers with normal lung function. In 13 studies smokers were instructed to refrain from smoking

Abbreviations: ACS, acute cigarette smoking; AMs, alveolar macrophages; BALF, bronchoalveolar lavage fluid; CO, carbon monoxide; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CSE, cigarette smoke extract; EIC, elastase inhibitory capacity; GSH, reduced glutathione; GSSG, oxidised glutathione; HO-1, heme oxygenase-1; IFN-γ, interferon-γ; IL, interleukin; NE, neutrophil elastase; NO, nitric oxide; PMNs, polymorphonuclear cells; TBARS, thiobarbituric acid reactive substances; TEAC, trolox equivalent antioxidant capacity; TNF-α, tumour necrosis factor α before the acute smoke exposure, varying between 7 and 24 hours. Ten studies did not provide information on this and in two studies the subjects were not instructed to refrain from smoking.

Inflammatory cells

In chronic smoking the numbers of neutrophils are increased in the blood and bronchoalveolar lavage fluid (BALF).[2–4] With ACS both increased[5] and unchanged numbers of neutrophils have been reported in BALF.[6] Acute smoke exposure had no effect on the number of monocytes or the total number of leucocytes in BALF.[6] Peripheral blood neutrophil granulocytes increased (fig 1),[7–9] whereas peripheral blood eosinophils decreased after ACS.[8] ACS has different effects on subsets of blood lymphocytes: the number of CD19 positive B cells[7] and the total number of lymphocytes were depressed by ACS,[8] while the number of CD3 positive cells and the CD4/CD8 ratio did not change.[7] In capillary blood (finger) the total number of basophils decreased 10 minutes after smoking two cigarettes[10] and the number of degranulated basophils increased.[11]

Neutrophil kinetics in the lungs can be examined by measuring the removal of radiolabelled neutrophils during the first passage through the pulmonary circulation. MacNee *et al* showed increased neutrophil retention in the lungs after ACS using this method.[12] This increased neutrophil retention was not due to differences in pulmonary haemodynamics,[13] but may result from decreased deformability of leucocytes[14] or the increased expression of the adhesion molecule L-selectin on blood neutrophils after ACS.[15]

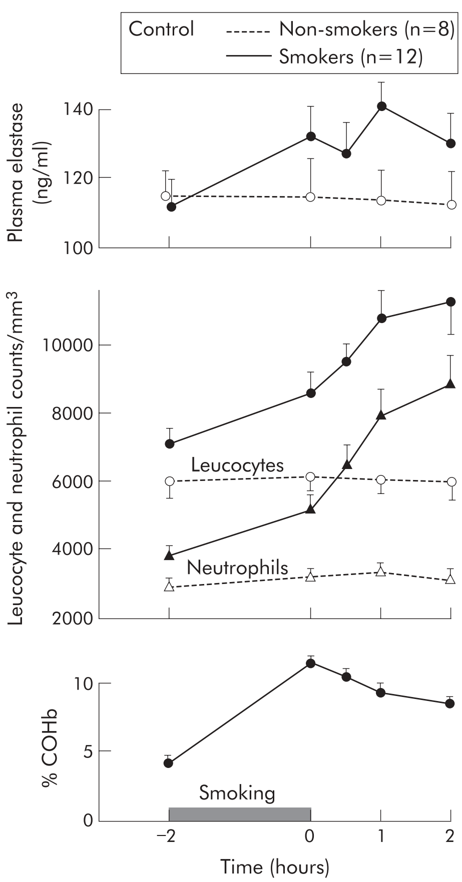

Figure 1 Increase in plasma elastase levels at 1 hour and blood neutrophil counts at 1 and 2 hours after smoking eight cigarettes in 2 hours compared with non-smoking. Reprinted from Abboud *et al*[9] with permission.

Epithelial permeability as measured by ^{99m}Tc-DTPA lung clearance[16] can be used to assess the disturbance of the airspace epithelial barrier. ACS increased epithelial permeability in chronic smokers after 1 hour to levels higher than in non-smokers.[5] However, Gil *et al*[17] showed no difference in epithelial permeability 15 minutes after ACS in chronic smokers. Endothelial permeability, as measured by radiolabelled urea, decreased after ACS[18] but no differences could be detected when measured by PET scanning using radiolabelled transferrin.[19]

Oxidative stress

The acute effects of cigarette smoking on markers of oxidative stress have been analysed in exhaled air, BALF, and blood. Most studies showed an immediate increase in oxidative stress after ACS, but in several studies smoking had no effect (table S1).

Five studies have described the effects of ACS on oxidative markers in breath condensate and exhaled air. In breath condensate 8-isoprostane, a lipid peroxidation product, increased 15 minutes after ACS (fig 2)[20] and hydrogen peroxide increased 30 minutes after smoke exposure.[21] Exhaled nitric oxide (eNO) increased at 1 and 10 minutes[22] but decreased 5 minutes after ACS in another study.[23] This inconsistency probably reflects differences in eNO measurements and subject characteristics. No difference in eNO was observed at 15,[23] 30 and 90 minutes[24] after smoking. Breath condensate levels of nitrate increased 30 minutes after ACS, but nitrite and nitrotyrosine levels did not change.[24]

One study[5] has investigated the effects of smoking on markers of oxidative stress in BALF, showing increased superoxide release from BALF leucocytes and an increased Trolox equivalent antioxidant capacity (TEAC). This latter surprising result can be explained by the fact that the subjects studied were all chronic smokers, associated with already high BALF levels of TEAC. No difference was seen in intracellular reduced glutathione (GSH) or oxidised glutathione (GSSG) in leucocytes or in thiobarbituric acid reactive substances (TBARS) in BALF and the epithelial lining fluid (ELF).

In peripheral blood, nitrate, nitrite and cysteine levels were depressed for a short time after smoking only one cigarette.[25] No difference was observed in the production of reactive oxygen intermediates from neutrophils.[7] In contrast to BALF, TBARS in plasma increased[26] and TEAC in plasma decreased 1 hour after smoking.[5 26] Levels of F_2-isoprostane, another lipid peroxidation product, did not change in plasma,[27] possibly because all subjects in this study were chronic smokers and already had high F_2-isoprostane levels.

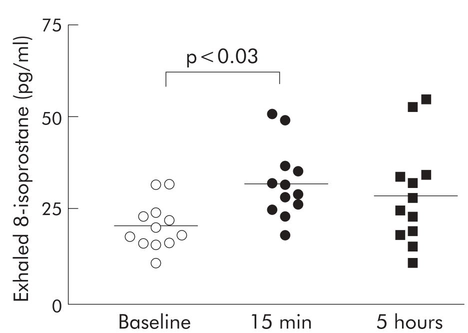

Figure 2 8-isoprostane concentrations in breath condensate in healthy smokers before smoking and 15 minutes and 5 hours after smoking. Reprinted from Montuschi *et al*[20] with permission from the American Thoracic Society.

Inflammatory mediators

Six studies have investigated the effects of ACS on inflammatory mediators and generally have found increased activity and recruitment of neutrophils and macrophages. In BALF, elastase activity increased[6] and leukotriene B_4 (LTB_4) release from alveolar macrophages (AMs) decreased 1 hour after smoking.[28]

In plasma, neutrophil elastase (NE) was increased immediately[14] and 1 hour after ACS (fig 1).[9] Leukotrienes B_4, D_4 (LTD_4), and E_4 (LTE_4) increased in peripheral blood immediately and 20 minutes after ACS, and their levels were positively correlated to C3a and C5a concentrations.[29] LTE_4 in urine increased twofold after smoking six cigarettes.[30]

Acute effects of cigarette smoke in animal models

We have identified 37 studies examining the acute effects of cigarette smoke in animal models (see table S2 available online at www.thoraxjnl.com/supplemental): 31 on inflammation and six on oxidative stress.

Most studies have been performed in guinea pigs (n = 11), mice (n = 10), and rats (n = 10). Five different methods of smoke exposure were used: nose only inhalation, nose and mouth inhalation, intratracheal inhalation, inhalation by anaesthesia mask, and inhalation via a smoking chamber. The cigarette brand differed between the studies as did the amount of smoke inhaled, ranging from 3 puffs to 30 cigarettes (table S2).

Inflammatory cells

ACS predominantly increases AMs and neutrophils in animal lung tissue and BALF (table S2). In lung tissue the volume fraction of AMs in the lung parenchyma[31] and the number of neutrophils in the airway wall (mucosa and outer adventitia) were increased 6 hours after ACS.[31–33] The number of mast cells in the airways was also higher 6 hours after ACS.[32] The opposite was true for the number of eosinophils which were decreased 6, 12, and 24 hours after smoking.[32]

In BALF most studies except three[34–36] showed increased numbers of AMs immediately,[37–39] 1 hour,[37 38 40] 6 hours,[40] 8 hours,[41] and 24 hours after ACS.[40 42 43] The phagocytic capacity of AMs, which is important for host defence, decreased immediately after ACS[38 44 45] and had returned to normal 12 hours later.[38] The viability of AMs in BALF also decreased after smoking.[46] The number and percentage of neutrophils in BALF were increased after 1 hour,[40 47] 6 hours,[40 48] 15 hours,[49] and 24 hours.[34 35 40–43 50] In contrast, four studies did not find an effect of smoke on polymorphonuclear cells (PMNs) either immediately[36 37 39] or at 1 hour[37 49] and 24 hours.[49] This discrepancy may be explained by differences in animal species, inhalation methods, or cigarette dose. Dhami *et al*[34] found that the number of neutrophils in mice had returned to normal after 48 hours. Both neutrophil and monocyte chemotaxis were reported to be higher 1 hour after smoke exposure than in sham exposed control animals.[48]

All studies but two[51 52] showed increased epithelial permeability after ACS within 30 minutes[32 39 53–56] and 6 hours.[40] In two studies[32 40] normalisation of epithelial permeability was observed after 24 hours. Two different explanations have been put forward for the enhanced permeability—damage to the epithelial cell membrane[32 53 54 57] or enlargement of the spaces between the epithelial cells.[54] Epithelial permeability was further increased after ibuprofen administration,[53] suggesting a role for arachidonic acid metabolism.

Oxidative stress

The acute effects of smoke inhalation on markers of oxidative stress in animals have been reported in lung tissue, BALF, and blood (table S2). Most studies showed a direct increase in oxidative stress after ACS.

In lung tissue of rats the amounts of GSH decreased immediately[35 58] and 1 hour after exposure to smoke.[40 59] After 2–6 hours GSH levels had either returned to normal[58 59] or were higher than baseline.[35] GSSG levels increased at 1 hour, decreased at 6 hours, and normalised at 24 hours after ACS.[40] ACS did not influence the amount of cysteine, an essential amino acid for the synthesis of GSH,[59] but it increased several other markers of oxidative stress in lung tissue including 8-OhdG, 4-HNE,[35 60] inducible nitric oxide synthase (iNOS) mRNA, and endothelial nitric oxide synthase (eNOS) mRNA.[61]

In BALF extracellular GSH was shown to be reduced immediately,[59] 1 hour, and 6 hours after smoke inhalation.[40] After 24 hours GSH concentrations returned to baseline levels.[40] ACS also depleted intracellular GSH concentrations.[59] It increased GSSG[36] and 8-OHdG levels[60] and decreased BALF levels of TEAC.[36]

In blood no effect from smoke inhalation has been observed on GSH.[59] However, ACS decreased the antioxidants methylumbelliferone glucuronide and ferroxidase[35 62] and increased lipid peroxide and 8-epi-$PGF_{2\alpha}$, markers of lipid peroxidation in blood.[36]

Inflammatory mediators

The acute effects of smoke inhalation on inflammatory mediators in animals have been described in lung tissue, BALF, and blood (table S2).

In lung tissue, tumour necrosis factor α (TNF-α), macrophage inflammatory protein (MIP), and macrophage chemoattractant protein 1 (MCP-1) gene expression increased 2 hours after smoke inhalation and normalised 6 hours thereafter.[42 50 63] Lung TNF-α was increased at 2, 6 and 24 hours, and E-selectin was increased at 6 and 24 hours.[63]

In BALF complement factor 3 increased 1 hour after ACS[48] and TNF-α release from AMs was augmented after 8 hours.[41] In contrast, LTB_4, another important chemoattractant, decreased directly after ACS.[53] Pessina *et al*[64] showed that interleukin (IL)-6 was partially degraded after ACS.

One study showed an increase in the elastase inhibitory capacity (EIC) in BALF after ACS,[49] but two other studies showed a decrease in the EIC in BALF[65] and plasma.[35] Furthermore, Churg *et al*[34 42 43 50] showed a consistent increase in desmosine and hydroxyproline, both degradation products of the extracellular matrix, in BALF of smoke exposed animals after 6 and 24 hours (fig 3). The above findings suggest that acute smoke exposure can result in damaging effects on lung tissue.

Only two studies have been published on the effects of smoke exposure on blood inflammatory mediators, showing an increase in myeloperoxidase (MPO)[66] but no changes in LTB_4 levels.[53]

Acute effects of cigarette smoke in in vitro models

Sixty two studies examining the acute effects of cigarette smoke in in vitro models were identified (see table S3 available online at www.thoraxjnl.com/supplemental): 50 on inflammation and 12 on oxidative stress.

Many different cells and cell lines have been used in acute smoke experiments (table S3). The following cells were most frequently described: AMs (n = 12), type II alveolar epithelial cell lines (A549, n = 10) and PMNs (n = 10). The methods of cigarette smoke exposure used were different between the studies. Fifty three studies used a cigarette smoke extract (CSE) and 14 used whole cigarette smoke (CS). The concentration of CSE and the time of exposure differed considerably between the studies with concentrations varying from 8×10^{-5} cigarette/ml to 4 cigarette/ml and exposure times varying between 1 second and 24 hours, respectively.

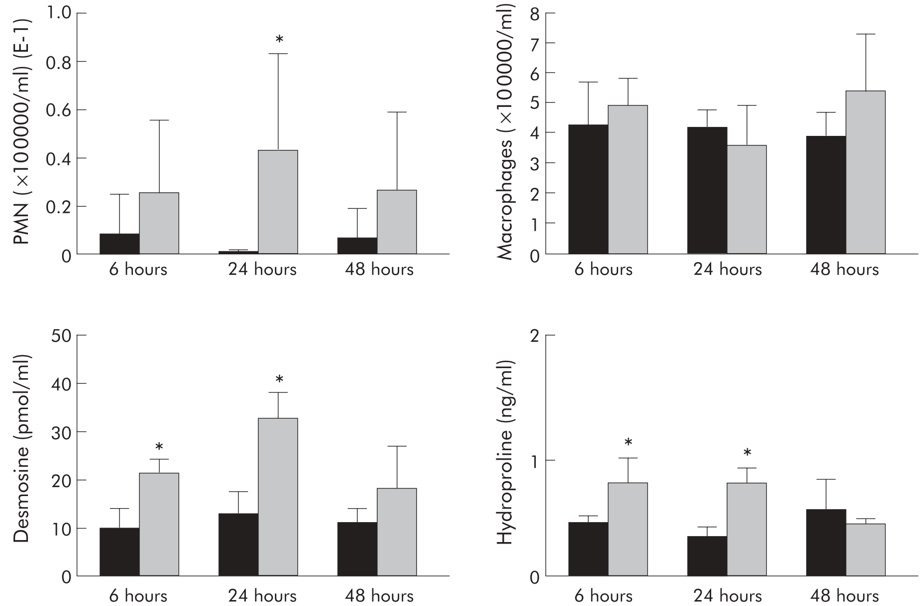

Figure 3 Desmosine and hydroxyproline increased in bronchoalveolar lavage fluid of mice 6 and 24 hours after acute cigarette smoking. Reprinted from Dhami *et al*[34] with permission from the American Thoracic Society.

Inflammatory cells

In vitro studies have shown various effects of CS and CSE on different cell characteristics which may provide useful information to enable a better understanding of the effects of smoking in vivo. Neutrophil and monocyte chemotactic activity of the supernatant of epithelial cells and fibroblasts incubated in CSE for 3–24 hours increased.[67–69] This increase diminished after lipoxygenase inhibitors and arachidonic acid metabolite inhibitors had been added.[67–69] In contrast, the chemotactic response of blood PMNs exposed directly to CS or CSE appeared to be decreased[70] or unchanged.[71] This suggests that CSE has an indirect effect on PMN chemotaxis.

Adhesion of human PMNs to a type II alveolar epithelial cell line decreased directly after exposure to CS,[71] but adhesion of human PMNs to a primary bovine bronchial epithelial cell line (BBEC) increased after incubation in CSE for 24 hours.[72] The adhesion of human monocytes to human umbilical vein endothelial cells (HUVEC) and human bronchial epithelial cells (HBEC) was also increased when incubated in CSE.[73 74] This might result from an increased expression of adhesion molecules CD11b, intercellular adhesion molecule 1 (ICAM-1), endothelial leucocyte adhesion molecule 1 (ELAM-1), and vascular cell adhesion molecule 1 (VCAM-1).[73 75] The expression of CD18 on human PMNs was increased in one study[76] but remained unchanged in another.[71] Surprisingly, ACS decreased the expression of L-selectin on PMNs.[76]

The phagocytic capacity of AMs, peritoneal macrophages (PMs), and PMNs was shown to decrease during CS exposure and 30 minutes, 2 and 24 hours after exposure to CS.[77–80] Increased phagocytic capacity of mice AMs was seen after exposure to only a low dose of CS.[77] The protein synthesis of rabbit AMs was depressed directly after CSE exposure and was restored after 24 hours.[81 82]

ACS can affect the function of fibroblasts in vitro. CSE inhibited the proliferation of human fetal lung fibroblasts (HFL1),[83] decreased fibronectin release,[84 85] viability and protein synthesis of fibroblasts,[81 86] and depressed fibroblast collagen mediated gel contraction, a model for wound repair.[84 85]

The viability of alveolar epithelial cells and AMs and PMs decreased after ACS in a concentration and time dependent manner.[46 77 79 87] Primary murine fibroblasts were less susceptible to cell death induced by CSE than murine AMs.[46] Six studies have shown that CSE resulted in apoptosis within 3–24 hours in different cell types.[86 88–92] However, Wickenden *et al*[93] showed that CSE exposure only induced necrosis. This might partly be explained by the fact that different cell types and CSE concentrations were used. Interestingly, two studies showed that exposing cells to low concentrations of CSE induced apoptosis while high concentrations resulted in necrosis.[91 92]

Two studies[94 95] on epithelial permeability in vitro showed an increase at 20 minutes and 1 hour after exposure to CS and CSE. Glutathione reduced this effect,[95] suggesting that oxidants contribute to the increase in epithelial permeability. Other interesting acute effects of CSE have been found. Firstly, CSE inhibited surfactant secretion of alveolar type II cells after 20 minutes of exposure.[96] Pinot *et al*[97] showed that surfactant can prevent oxidative stress induced by CSE in vitro. These results have clinical relevance since surfactant is important in maintaining alveolar stability and plays a role in alveolar and also (though less prominently) in bronchial clearance. Secondly, Takeyama *et al*[98] showed that CSE increased mucin synthesis by a pulmonary mucoepidermoid cell line already within 24 hours. This suggests the possibility of a rapid upregulatory mechanism of mucus production in vivo in chronic smokers. A decrease in mucus flow on ciliated epithelium was seen within minutes of exposure to CS.[99]

Oxidative stress

Twelve studies have investigated the effect of ACS on oxidative stress, all showing an increase in oxidative stress after exposure to CS. GSSG was released after 30 minutes[100] and intracellular GSH was decreased within 3 hours of ACS exposure.[86 95 101] When measurements were performed 24 hours after exposure, GSH and γ-GCS were in fact increased, suggesting a protective mechanism of cells against oxidative stress from smoke.[102] Immediately after six puffs of smoke, hydrogen peroxide and superoxide molecules from CS were detectable along the membranes of epithelial cells,[103] which were prevented by antioxidants. After 24 hours of incubation with CSE, nitric oxide (NO) was released from endothelial cells.[88] In contrast, iNOS expression and nitrate release from stimulated epithelial cells were decreased after CSE exposure.[104] The pentose phosphate pathway, the source of NADPH for the enzyme gluthatione reductase, was activated after incubation of endothelial cells with CSE.[100]

Inflammatory mediators

All studies but one[105] showed an increased release of IL-8 in various cell types after different exposure times to CSE (20 minutes in HBEC,[105] 4 and 8 hours in human endothelial cells,[106] 6 hours mRNA IL-8 in NCI-H292,[107] 12 hours in HBEC,[108] and 24 hours in HBEC and A549 cell line[108 109]). The results of the two negative studies might be explained by the low concentrations of CSE, the use of CS instead of CSE, or by the different cell types used.

Inconsistent results were also found for IL-1β, TNF-α, and soluble ICAM (sICAM): IL-1β and sICAM were increased in HBEC 20 minutes, 1 hour and 24 hours after exposure to CS[94 105] but were decreased when HBEC were exposed for 3 and 6 hours.[105] IL-1β and TNF-α release was increased when peripheral blood mononuclear cells (PBMCs) were exposed for 5 minutes[110] but decreased after 3 hours exposure.[111] TNF-α release from AMs was decreased when exposed for 1 hour at low concentrations[112] but increased when exposed for 18 hours with higher concentrations of CSE.[63] CSE had no effect on sICAM release from HUVEC at 24 hours.[113] mRNA expression of IL-8, IL-1β, and sICAM was increased after 30 minutes of incubation of HBEC in CSE.[114]

Cigarette smoke has been shown to have a depressive effect on some other inflammatory mediators in vitro. The release of LTB_4 from AMs[28] and interferon-γ (IFN-γ) and IL-2[111] from human PBMCs was less after incubation in CSE. The activity of both IL-6 and TNF-α secreted by AMs was diminished after exposure to CS.[115] CSE had no direct effect on the release of NE from human blood PMNs in vitro.[116]

DISCUSSION

Smoking is the main risk factor for the accelerated decline in lung function and development of COPD. Much is known of the effects of chronic smoke exposure on lung function and airway inflammation, but there is a paucity of data on the acute effects of smoking in this respect. It seems important to know these effects since repetitive acute smoke effects may constitute the underlying causal chain leading to the ultimate chronic effects.

We have identified 123 studies investigating the acute effects of CS on inflammatory cells, oxidative stress, and inflammatory mediators in humans, animals and in vitro models. Various cigarette brands with and without a filter and different doses have been studied, ranging from 1 puff to 30 cigarettes. Different time points and several body compartments in humans and animals have been investigated. An extensive collection of information has therefore been acquired, yet of various natures.

One of the problems in the comparison of the various studies is the difference in the way human, animal, and in vitro models have been exposed to smoke. Firstly, even though animals have a much smaller lung surface than humans, this review shows that animals are exposed to a higher number of cigarettes than humans (median 5 cigarettes (range 0.9–34) *v* median 2 cigarettes (range 1–24)). Secondly, in vitro studies mainly used CSE whereas all humans and almost all animals were exposed to CS. The composition of CSE and CS has important differences, especially regarding the water insoluble substances and free radicals.[117–119] Thus, the results of different models cannot therefore simply be compared.

In this review we have provided data that are of interest and importance to the damaging effects of smoke in diseases in general. We have shown that ACS is chemotactic to neutrophils and macrophages and activates these cells. Furthermore, acute smoke exposure results in tissue damage, as suggested by increased products of lipid peroxidation and matrix degradation products. A very intriguing finding was the suppressive effect of ACS on the number of eosinophils and several inflammatory cytokines. It may well be that this suppressive effect results from the anti-inflammatory carbon monoxide (CO) present in cigarette smoke or produced by inflammatory cells in the lung.[120]

Inflammatory cells

This review shows that neutrophils are already attracted and activated after the first puffs of CS in both human and animal studies. In line with this, increased neutrophil chemotactic activity of supernatant of epithelial cells exposed to CS was observed in vitro.

ACS induces increased numbers of AMs in animal lung tissue and BALF, but not in human BALF. This may be due to the short time interval or the low dose of smoke used. Furthermore, increased monocyte chemotactic activity of BALF and supernatant of epithelial cells exposed to CS was observed. Eosinophils seem to play a role in a subgroup of patients with stable COPD[121] and in those with COPD exacerbations.[122] ACS directly increased eosinophil numbers in animal BALF.[37] Intriguingly, two other studies[8 32] have shown a suppressive effect of smoke on the number of eosinophils in human blood and in animal tissue. This may be a reflection of local shifts in the Th1–Th2 type cytokine balance or an anti-inflammatory effect of substances in smoke such as CO.[123 124]

The effect of ACS on apoptosis and necrosis has mainly been investigated in in vitro studies. Interestingly, two studies showed that exposure of cells to low concentrations of CSE induced apoptosis but high concentrations of CSE resulted in necrosis.[91 92] Because apoptosis of (inflammatory) cells is associated with less damage of the extracellular matrix, one might even hypothesise that smokers who smoke intermittently or only a few cigarettes per day are less likely to develop lung damage than those who smoke many cigarettes in a chain.

ACS increased the air space epithelial permeability in human, animal, and in vitro studies. This increase was shown to occur within an hour after exposure to CS and returned to normal within 24 hours. Theoretically, impairment of the epithelial barrier may potentiate the damaging effects of noxious agents in the lung.

ACS also inhibits the function of fibroblasts which are important in repair processes in the lung. Injury and repair processes of the airway epithelium have been studied extensively in chronic airway disease. It is assumed that these repeated injury and repair processes may contribute to the development of airway pathology in chronic inflammatory airway diseases.[125] Repetition of acute smoke exposure may lead in this way to irreversible damage, especially if fibroblasts are not functioning normally. More studies on this subject should be performed to strengthen this hypothesis.

Summarising, ACS increases local inflammation as reflected by an increase in the number of neutrophils and macrophages in the lung. It reduces important qualitative cell characteristics, repair mechanisms, and the protection of the epithelial barrier. Furthermore, ACS results in a decrease in the number of eosinophils, indicating a possible local shift in the Th1–Th2 type cytokine balance or an anti-inflammatory effect of CO.

Oxidative stress

ACS increases markers of oxidative stress in all three models (human, animal, and in vitro). NO and GSH are the only two parameters that have been investigated in all models. NO and its related substances increase within 24 hours after smoke exposure. The GSH/GSSG ratio, reflecting the vital balance between oxidants and protecting antioxidants, decreased following acute smoke exposure in both animal and in vitro studies but not in the single study published in humans. This discrepancy can be explained by differences in species, smoke dose, or compartment (human BALF versus animal lung homogenate).

Interestingly, ACS even results in damage of fatty acids in cell membranes, as measured by an increase in degradation products of lipid peroxidation in humans (exhaled air and plasma)[20 26] and animals (BALF and lung tissue).[35 60] No in vitro studies investigating the acute smoke effects on lipid peroxidation products have been found.

Because different time points within 24 hours have been studied, it allowed us to observe a time response of oxidative stress. In humans all oxidative markers increase within the first hour after ACS and most markers returned to normal within 90 minutes. Exhaled air is the first compartment in which an increase in oxidative stress markers can be observed, followed by BALF and blood. In animals most markers of oxidative stress change in the first 6 hours after ACS and return to normal within 24 hours. In all compartments (lung tissue, BALF, and blood) GSH or its derivatives are depressed in the same time period, suggesting a generalised response to ACS. As in humans, only a few time points have been studied in in vitro models. The initial depletion of GSH after ACS appeared to be followed by an increase in GSH 24 hours later, suggesting a protective mechanism of cells against oxidative stress from smoke.[102] The importance of the GSH/GSSG balance was shown in several studies. When GSH was added to the experiment the oxidative stress and inflammatory response induced by cigarette smoke could be prevented.

In summary, ACS immediately increases markers of oxidative stress in all models and even results in damage to the cell membrane. The GSH/GSSG balance plays an important role in the acute protection of the lung against oxidants in CS.

Inflammatory mediators

ACS induces a wide range of (pro)inflammatory responses. All three models (human, animal, and in vitro) studied the effect of ACS on NE, leukotrienes, and IL-6. Interestingly, NE was released only a few hours after a low dose of CS, both in animals and in humans. In contrast, direct exposure of human PMNs in vitro for 4 minutes did not affect the release of NE. This suggests that CS does not affect NE release by neutrophils directly, indicating that the local microenvironment may have a role in mounting this response. Another explanation might be that the in vitro exposure time was too short to activate these cells.

Inconsistent results have been shown for the effects of ACS on leukotrienes, with increased (human, in vitro), decreased (animal, in vitro), or no effects (animal). This could be due to differences in cigarette dose, cell type, or species under study.[53]

IL-6, which plays a role in innate and adaptive immunity, was also studied in all models. Alveolar macrophage IL-6 activity was decreased after in vitro smoke exposure and IL-6 degradation was increased in BALF of rats.[64 115] No effect of ACS was found on human blood levels of IL-6,[7] suggesting that ACS may have a depressive effect only locally in the bronchial tree or that is compensated for by IL-6 production by other cells.

In vitro, ACS increased the release of IL-8 from epithelial and endothelial cells and cell lines. This is in line with the observed increase in neutrophils after ACS in humans and animals, which suggests that IL-8 is a chemoattractant for neutrophils after exposure to ACS.

A suppressive effect of ACS was seen in some inflammatory mediators (TNF-α, IFN-γ, LTB_4, and IL-2) in vitro.[28 111 112 115] This suppressive effect may result from CO from CS or is produced by heme oxygenase-1 (HO-1) in inflammatory cells in the lung.[120]

In summary, ACS can disturb the balance between proteases such as NE and their inhibitors, possibly resulting in early tissue damage. In addition, it increases IL-8 which may contribute to chemotaxis of neutrophils as found after ACS. Interestingly, ACS has a suppressive effect on some inflammatory mediators, possibly due to the anti-inflammatory effect of CO.

Susceptible smoker

A vital question when investigating the development of COPD is how to pinpoint the susceptible smoker. Differences in smoke exposure and genetic factors do not give the complete answer. In this review we describe an acute decrease in the GSH/GSSG ratio after smoke exposure. This decrease puts the smoker at risk to oxidants of CS soon after the first exposure. The extent and velocity to which the GSH/GSSG balance is restored probably determines to some extent the degree of susceptibility. The balance between proteases and antiproteases may also have a role, but studies performed to date have shown contradictory results. One study showed that NE and EIC in animal BALF increase simultaneously after smoke exposure, suggesting a protective mechanism. Yet, acute smoke exposure in three other studies showed an increase in the matrix degradation products desmosine and hydroxyproline in animal BALF. This supports the hypothesis that the ability to maintain the balance between proteases and antiproteases is of vital importance for protecting the lung against proteolysis. Finally, a polymorphism in the HO-1 promoter region has been described in patients with COPD, resulting in a lower production of HO-1.[126] This review shows that ACS decreases the number of eosinophils and some inflammatory mediators which might be caused by the anti-inflammatory CO produced locally by HO-1 in the lung. One might hypothesise that, in smokers, HO-1 expression is important for the susceptibility to develop COPD. More

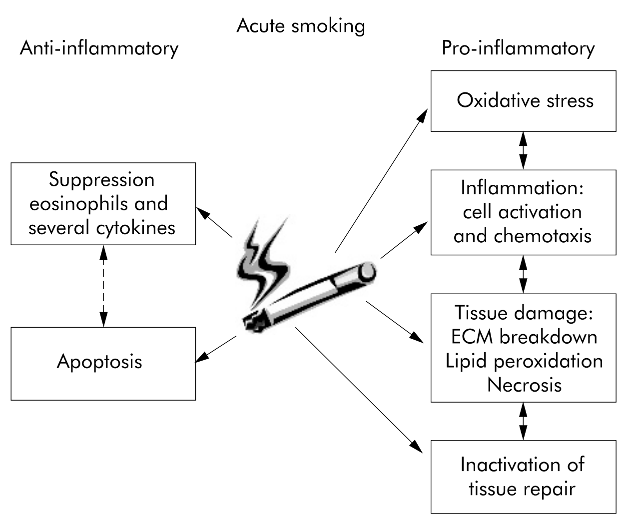

Figure 4 Summary of the acute effects of cigarette smoking. Data extracted from human, animal, and in vitro studies. ECM, extracellular matrix.

studies on acute smoking with larger groups should be performed to further unravel this complicated but very important issue.

CONCLUSIONS

This review shows that an acute smoking model is a relatively easy and sensitive method for investigating the specific effects of cigarette smoke on oxidative stress and inflammation. We have shown that ACS is chemotactic to neutrophils and macrophages and activates these cells. An intriguing finding was the suppressive effect of ACS on the number of eosinophils and several inflammatory cytokines, possibly explained by a local shift in the Th1–Th2 type cytokine balance or by the anti-inflammatory effect of CO. Importantly, even acute smoke exposure might result in tissue damage, as suggested by increased products of lipid peroxidation and degradation products of extracellular matrix proteins. This review supports the view that an imbalance between oxidants and antioxidants and between proteases and antiproteases may play an important role in the susceptible smoker, and it has become clear that disturbances in effective tissue repair also deserve attention (fig 4). It is, however, difficult to draw firm conclusions because of the small sample sizes studied, essential differences between human, animal and in vitro models, and other methodological divergences. An acute smoking model is a useful supplement to other methods of studying the effects of smoking, and is an as yet underinvestigated method for intervention studies in smoking related diseases such as COPD.

Tables S1, S2, and S3 are available online on the *Thorax* website (www.thoraxjnl.com/supplemental)

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REFERENCES

1 **Murray CJ**, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *Lancet* 1997;**349**:1498–504.
2 **Kuschner WG**, D'Alessandro A, Wong H, *et al.* Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. *Eur Respir J* 1996;**9**:1989–94.
3 **Hunninghake GW**, Crystal RG. Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983;**128**:833–8.
4 **van Eeden SF**, Hogg JC. The response of human bone marrow to chronic cigarette smoking. *Eur Respir J* 2000;**15**:915–21.
5 **Morrison D**, Rahman I, Lannan S, *et al.* Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. *Am J Respir Crit Care Med* 1999;**159**:473–9.
6 **Janoff A**, Raju L, Dearing R. Levels of elastase activity in bronchoalveolar lavage fluids of healthy smokers and nonsmokers. *Am Rev Respir Dis* 1983;**127**:540–4.
7 **Hockertz S**, Emmendorffer A, Scherer G, *et al.* Acute effects of smoking and high experimental exposure to environmental tobacco smoke (ETS) on the immune system. *Cell Biol Toxicol* 1994;**10**:177–90.
8 **Winkel P**, Statland BE. The acute effect of cigarette smoking on the concentrations of blood leukocyte types in healthy young women. *Am J Clin Pathol* 1981;**75**:781–5.
9 **Abboud RT**, Fera T, Johal S, *et al.* Effect of smoking on plasma neutrophil elastase levels. *J Lab Clin Med* 1986;**108**:294–300.
10 **Walter S**, Nancy NR. Basopenia following cigarette smoking. *Indian J Med Res* 1980;**72**:422–5.
11 **Walter S**, Walter A. Basophil degranulation induced by cigarette smoking in man. *Thorax* 1982;**37**:756–9.
12 **MacNee W**, Wiggs B, Belzberg AS, *et al.* The effect of cigarette smoking on neutrophil kinetics in human lungs. *N Engl J Med* 1989;**321**:924–8.
13 **Skwarski KM**, Gorecka D, Sliwinski P, *et al.* The effects of cigarette smoking on pulmonary hemodynamics. *Chest* 1993;**103**:1166–72.
14 **Drost EM**, Selby C, Bridgeman MME, *et al.* Decreased leukocyte deformability after acute cigarette smoking in humans. *Am Rev Respir Dis* 1993;**148**:1277–83.
15 **Patiar S**, Slade D, Kirkpatrick U, *et al.* Smoking causes a dose-dependent increase in granulocyte-bound L- selectin. *Thromb Res* 2002;**106**:1–6.
16 **Morrison D**, Skwarski K, Millar AM, *et al.* A comparison of three methods of measuring 99mTc-DTPA lung clearance and their repeatability. *Eur Respir J* 1998;**11**:1141–6.
17 **Gil E**, Chen B, Kleerup E, *et al.* Acute and chronic effects of marijuana smoking on pulmonary alveolar permeability. *Life Sci* 1995;**56**:2193–9.
18 **Ward C**. Bronchoalveolar lavage fluid urea as a marker of pulmonary permeability in healthy smokers. *Eur Respir J* 2000;**15**:285.
19 **Kaplan JD**, Calandrino FS, Schuster DP. Effect of smoking on pulmonary vascular permeability. A positron emission tomography study. *Am Rev Respir Dis* 1992;**145**:712–5.
20 **Montuschi P**, Collins JV, Ciabattoni G, *et al.* Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 2000;**162**:1175–7.
21 **Guatura SB**, Martinez JA, Santos Bueno PC, *et al.* Increased exhalation of hydrogen peroxide in healthy subjects following cigarette consumption. *Sao Paulo Med J* 2000;**118**:93–8.
22 **Chambers DC**, Tunnicliffe WS, Ayres JG. Acute inhalation of cigarette smoke increases lower respiratory tract nitric oxide concentrations. *Thorax* 1998;**53**:677–9.
23 **Kharitonov SA**, Robbins RA, Yates D, *et al.* Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;**152**:609–12.
24 **Balint B**, Donnelly LE, Hanazawa T, *et al.* Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke. *Thorax* 2001;**56**:456–61.
25 **Tsuchiya M**, Asada A, Kasahara E, *et al.* Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation* 2002;**105**:1155–7.
26 **Rahman I**, Morrison D, Donaldson K, *et al.* Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med* 1996;**154**:1055–60.
27 **Morrow JD**, Frei B, Longmire AW, *et al.* Increase in circulating products of lipid peroxidation (F2- isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med* 1995;**332**:1198–203.
28 **Tardif J**, Borgeat P, Laviolette M. Inhibition of human alveolar macrophage production of leukotriene B4 by acute in vitro and in vivo exposure to tobacco smoke. *Am J Respir Cell Mol Biol* 1990;**2**:155–61.
29 **Kobayashi J**, Kihira Y, Kitamura S. Effects of cigarette smoking on blood levels of leukotrienes and plasma levels of complements C3a and C5a in healthy volunteers. *Arch Environ Health* 1988;**43**:371–4.
30 **Fauler J**, Frolich JC. Cigarette smoking stimulates cysteinyl leukotriene production in man. *Eur J Clin Invest* 1997;**27**:43–7.
31 **Vitalis TZ**, Kern I, Croome A, *et al.* The effect of latent adenovirus 5 infection on cigarette smoke-induced lung inflammation. *Eur Respir J* 1998;**11**:664–9.
32 **Hulbert WC**, Walker DC, Jackson A, *et al.* Airway permeability to horseradish peroxidase in guinea pigs: the repair phase after injury by cigarette smoke. *Am Rev Respir Dis* 1981;**123**:320–6.
33 **Kilburn KH**, McKenzie W. Leukocyte recruitment to airways by cigarette smoke and particle phase in contrast to cytotoxicity of vapor. *Science* 1975;**189**:634–7.
34 **Dhami R**, Gilks B, Xie C, *et al.* Acute cigarette smoke-induced connective tissue breakdown is mediated by neutrophils and prevented by alpha1-antitrypsin. *Am J Respir Cell Mol Biol* 2000;**22**:244–52.
35 **Ishizaki T**, Kishi Y, Sasaki F, *et al.* Effect of probucol, an oral hypocholesterolaemic agent, on acute tobacco smoke inhalation in rats. *Clin Sci (Lond)* 1996;**90**:517–23.
36 **Cavarra E**, Lucattelli M, Gambelli F, *et al.* Human SLPI inactivation after cigarette smoke exposure in a new in vivo model of pulmonary oxidative stress. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L412–7.
37 **Daffonchio L**, Hernandez A, Omini C. Sensory neuropeptides are involved in cigarette smoke induced airway hyperreactivity in guinea-pig. *Agents Actions Suppl* 1990;**31**:215–22.
38 **Ortega E**, Hueso F, Collazos ME, *et al.* Phagocytosis of latex beads by alveolar macrophages from mice exposed to cigarette smoke. *Comp Immunol Microbiol Infect Dis* 1992;**15**:137–42.
39 **Mordelet-Dambrine M**, Leguern-Stanislas G, Chinet TC, *et al.* Effects of tobacco smoke on respiratory epithelial clearance of DTPA and on lung histology in rats. *Eur Respir J* 1991;**4**:839–44.
40 **Li XY**, Rahman I, Donaldson K, *et al.* Mechanisms of cigarette smoke induced increased airspace permeability. *Thorax* 1996;**51**:465–71.
41 **Pessina GP**, Paulesu L, Corradeschi F, *et al.* Production of tumor necrosis factor alpha by rat alveolar macrophages collected after acute cigarette smoking. *Arch Immunol Ther Exp (Warsz)* 1993;**41**:343–8.
42 **Churg A**, Dai J, Tai H, *et al.* Tumor necrosis factor-alpha is central to acute cigarette smoke-induced inflammation and connective tissue breakdown. *Am J Respir Crit Care Med* 2002;**166**:849–54.
43 **Churg A**, Zay K, Shay S, *et al.* Acute cigarette smoke-induced connective tissue breakdown requires both neutrophils and macrophage metalloelastase in mice. *Am J Respir Cell Mol Biol* 2002;**27**:368–74.
44 **Yamaya M**, Zayasu K, Sekizawa K, *et al.* Acute effect of cigarette smoke on cytoplasmic motility of alveolar macrophages in dogs. *J Appl Physiol* 1989;**66**:1172–8.

45 **Ortega E**, Barriga C, Rodriguez AB. Decline in the phagocytic function of alveolar macrophages from mice exposed to cigarette smoke. *Comp Immunol Microbiol Infect Dis* 1994;**17**:77–84.
46 **Holt PG**, Keast D. Acute effects of cigarette smoke on murine macrophages. *Arch Environ Health* 1973;**26**:300–4.
47 **Wright J**, Harrison N. Cardiopulmonary effects of a brief exposure to cigarette smoke in the guinea pig. *Respiration* 1990;**57**:70–6.
48 **Kew RR**, Ghebrehiwet B, Janoff A. The role of complement in cigarette smoke-induced chemotactic activity of lung fluids. *Am Rev Respir Dis* 1986;**133**:478–81.
49 **Abrams WR**, Kucich U, Kimbel P, *et al.* Acute cigarette smoke exposure in dogs: the inflammatory response. *Exp Lung Res* 1988;**14**:459–75.
50 **Wright JL**, Farmer SG, Churg A. Synthetic serine elastase inhibitor reduces cigarette smoke-induced emphysema in guinea pigs. *Am J Respir Crit Care Med* 2002;**166**:954–60.
51 **Nishikawa M**, Ikeda H, Fukuda T, *et al.* Acute exposure to cigarette smoke induces airway hyperresponsiveness without airway inflammation in guinea pigs. Dose-response characteristics. *Am Rev Respir Dis* 1990;**142**:177–83.
52 **Simani AS**, Inoue S, Hogg JC. Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. *Lab Invest* 1974;**31**:75–81.
53 **Witten ML**, Quan SF, Sobonya RE, *et al.* Acute cigarette smoke exposure alters lung eicosanoid and inflammatory cell concentrations in rabbits. *Exp Lung Res* 1988;**14**:727–42.
54 **Burns AR**, Hosford SP, Dunn LA, *et al.* Respiratory epithelial permeability after cigarette smoke exposure in guinea pigs. *J Appl Physiol* 1989;**66**:2109–16.
55 **Boucher RC**, Johnson J, Inoue S, *et al.* The effect of cigarette smoke on the permeability of guinea pig airways. *Lab Invest* 1980;**43**:94–100.
56 **Witten ML**, Lemen RJ, Quan SF, *et al.* Acute cigarette smoke exposure increases alveolar permeability in rabbits. *Am Rev Respir Dis* 1985;**132**:321–5.
57 **Reznik-Schuller HM**. Acute effects of cigarette smoke inhalation on the Syrian hamster lungs. *J Environ Pathol Toxicol* 1980;**4**:285–91.
58 **Bilimoria MH**, Ecobichon DJ. Protective antioxidant mechanisms in rat and guinea pig tissues challenged by acute exposure to cigarette smoke. *Toxicology* 1992;**72**:131–44.
59 **Cotgreave IA**, Johansson U, Moldeus P, *et al.* The effect of acute cigarette smoke inhalation on pulmonary and systemic cysteine and glutathione redox states in the rat. *Toxicology* 1987;**45**:203–12.
60 **Aoshiba K**, Koinuma M, Yokohori N, *et al.* Immunohistochemical evaluation of oxidative stress in murine lungs after cigarette smoke exposure. *Inhal Toxicol* 2003;**15**:1029–38.
61 **Wright JL**, Dai J, Zay K, *et al.* Effects of cigarette smoke on nitric oxide synthase expression in the rat lung. *Lab Invest* 1999;**79**:975–83.
62 **Uotila P**. Effect of cigarette smoke on glucuronide conjugation in hamster isolated lungs. *Res Commun Chem Pathol Pharmacol* 1982;**38**:173–6.
63 **Churg A**, Wang RD, Tai H, *et al.* Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. *Am J Respir Crit Care Med* 2003;**167**:1083–9.
64 **Pessina GP**, Paulesu L, Corradeschi F, *et al.* Pulmonary catabolism of interleukin 6 evaluated by lung perfusion of normal and smoker rats. *J Pharm Pharmacol* 1996;**48**:1063–7.
65 **Janoff A**, Carp H, Lee DK, *et al.* Cigarette smoke inhalation decreases alpha 1-antitrypsin activity in rat lung. *Science* 1979;**206**:1313–4.
66 **Bosken CH**, Doerschuk CM, English D, *et al.* Neutrophil kinetics during active cigarette smoking in rabbits. *J Appl Physiol* 1991;**71**:630–7.
67 **Sato E**, Koyama S, Takamizawa A, *et al.* Smoke extract stimulates lung fibroblasts to release neutrophil and monocyte chemotactic activities. *Am J Physiol* 1999;**277**:L1149–57.
68 **Koyama S**, Rennard SI, Leikauf GD, *et al.* Bronchial epithelial cells release monocyte chemotactic activity in response to smoke and endotoxin. *J Immunol* 1991;**147**:972–9.
69 **Shoji S**, Ertl RF, Koyama S, *et al.* Cigarette smoke stimulates release of neutrophil chemotactic activity from cultured bovine bronchial epithelial cells. *Clin Sci (Lond)* 1995;**88**:337–44.
70 **Bridges RB**, Kraal JH, Huang LJ, *et al.* Effects of tobacco smoke on chemotaxis and glucose metabolism of polymorphonuclear leukocytes. *Infect Immun* 1977;**15**:115–23.
71 **Selby C**, Drost E, Brown D, *et al.* Inhibition of neutrophil adherence and movement by acute cigarette smoke exposure. *Exp Lung Res* 1992;**18**:813–27.
72 **Robbins RA**, Koyama S, Spurzem JR, *et al.* Modulation of neutrophil and mononuclear cell adherence to bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1992;**7**:19–29.
73 **Kalra VK**, Ying Y, Deemer K, *et al.* Mechanism of cigarette smoke condensate induced adhesion of human monocytes to cultured endothelial cells. *J Cell Physiol* 1994;**160**:154–62.
74 **Floreani AA**, Wyatt TA, Stoner J, *et al.* Smoke and C5a induce airway epithelial intercellular adhesion molecule-1 and cell adhesion. *Am J Respir Cell Mol Biol* 2003;**29**:472–82.
75 **Shen Y**, Rattan V, Sultana C, *et al.* Cigarette smoke condensate-induced adhesion molecule expression and transendothelial migration of monocytes. *Am J Physiol* 1996;**270**:H1624–33.
76 **Ryder MI**, Fujitaki R, Lebus S, *et al.* Alterations of neutrophil L-selection and CD18 expression by tobacco smoke: implications for periodontal diseases. *J Periodontal Res* 1998;**33**:359–68.
77 **Thomas WR**, Holt PG, Keast D. Cigarette smoke and phagocyte function: effect of chronic exposure in vivo and acute exposure in vitro. *Infect Immun* 1978;**20**:468–75.
78 **Green GM**, Carolin D. The depressant effect of cigarette smoke on the in vitro antibacterial activity of alveolar macrophages. *N Engl J Med* 1967;**276**:421–7.
79 **Voisin C**, Aerts C, Fournier E, *et al.* Acute effects of tobacco smoke on alveolar macrophages cultured in gas phase. *Eur J Respir Dis Suppl* 1985;**139**:76–81.
80 **Zappacosta B**, Persichilli S, Minucci A, *et al.* Effect of aqueous cigarette smoke extract on the chemiluminescence kinetics of polymorphonuclear leukocytes and on their glycolytic and phagocytic activity. *Luminescence* 2001;**16**:315–9.
81 **Holt PG**, Keast D. The effect of tobacco smoke on protein synthesis in macrophages. *Proc Soc Exp Biol Med* 1973;**142**:1243–7.
82 **Yeager H Jr**. Alveolar cells: depression effect of cigarette smoke on protein synthesis. *Proc Soc Exp Biol Med* 1969;**131**:247–50.
83 **Nakamura Y**, Romberger DJ, Tate L, *et al.* Cigarette smoke inhibits lung fibroblast proliferation and chemotaxis. *Am J Respir Crit Care Med* 1995;**151**:1497–503.
84 **Carnevali S**, Nakamura Y, Mio T, *et al.* Cigarette smoke extract inhibits fibroblast-mediated collagen gel contraction. *Am J Physiol* 1998;**274**:L591–8.
85 **Kim HJ**, Liu X, Wang H, *et al.* Glutathione prevents inhibition of fibroblastmediated collagen gel contraction by cigarette smoke. *Am J Physiol Lung Cell Mol Physiol* 2002;**283**:L409–17.
86 **Carnevali S**, Petruzzelli S, Longoni B, *et al.* Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2003;**284**:L955–63.
87 **Hoshino Y**, Mio T, Nagai S, *et al.* Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L509–16.
88 **Tuder RM**, Wood K, Taraseviciene L, *et al.* Cigarette smoke extract decreases the expression of vascular endothelial growth factor by cultured cells and triggers apoptosis of pulmonary endothelial cells. *Chest* 2000;**117**:241–2S.
89 **Ishii T**, Matsuse T, Igarashi H, *et al.* Tobacco smoke reduces viability in human lung fibroblasts: protective effect of glutathione S-transferase P1. *Am J Physiol Lung Cell Mol Physiol* 2001;**280**:L1189–95.
90 **Aoshiba K**, Tamaoki J, Nagai A. Acute cigarette smoke exposure induces apoptosis of alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L1392–401.
91 **Vayssier-Taussat M**, Camilli T, Aron Y, *et al.* Effects of tobacco smoke and benzo[a]pyrene on human endothelial cell and monocyte stress responses. *Am J Physiol Heart Circ Physiol* 2001;**280**:H1293–300.
92 **Vayssier M**, Banzet N, Francois D, *et al.* Tobacco smoke induces both apoptosis and necrosis in mammalian cells: differential effects of HSP70. *Am J Physiol* 1998;**275**:L771–9.
93 **Wickenden JA**, Clarke MC, Rossi AG, *et al.* Cigarette smoke prevents apoptosis through inhibition of caspase activation and induces necrosis. *Am J Respir Cell Mol Biol* 2003;**29**:562–70.
94 **Rusznak C**, Mills PR, Devalia JL, *et al.* Effect of cigarette smoke on the permeability and IL-1beta and sICAM-1 release from cultured human bronchial epithelial cells of never- smokers, smokers, and patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2000;**23**:530–6.
95 **Li XY**, Donaldson K, Rahman I, *et al.* An investigation of the role of glutathione in increased epithelial permeability induced by cigarette smoke in vivo and in vitro. *Am J Respir Crit Care Med* 1994;**149**:1518–25.
96 **Wirtz HR**, Schmidt M. Acute influence of cigarette smoke on secretion of pulmonary surfactant in rat alveolar type II cells in culture. *Eur Respir J* 1996;**9**:24–32.
97 **Pinot F**, Bachelet M, Francois D, *et al.* Modified natural porcine surfactant modulates tobacco smoke-induced stress response in human monocytes. *Life Sci* 1999;**64**:125–34.
98 **Takeyama K**, Jung B, Shim JJ, *et al.* Activation of epidermal growth factor receptors is responsible for mucin synthesis induced by cigarette smoke. *Am J Physiol Lung Cell Mol Physiol* 2001;**280**:L165–72.
99 **Falk HL**, Tremer HM, Kotin P. Effect of cigarette smoke and its constituents on ciliated mucus-secreting epithelium. *J Natl Cancer Inst* 1959;**23**:999–1012.
100 **Noronha-Dutra AA**, Epperlein MM, Woolf N. Effect of cigarette smoking on cultured human endothelial cells. *Cardiovasc Res* 1993;**27**:774–8.
101 **Bridgeman MME**, Marsden M, Drost E, *et al.* The effect of cigarette smoke on lung cells. *Am Rev Respir Dis* 1991;**143**:A737.
102 **Rahman I**, Smith CA, Lawson MF, *et al.* Induction of gammaglutamylcysteine synthetase by cigarette smoke is associated with AP-1 in human alveolar epithelial cells. *FEBS Lett* 1996;**396**:21–5.
103 **Hobson J**, Wright J, Churg A. Histochemical evidence for generation of active oxygen species on the apical surface of cigarette-smoke-exposed tracheal explants. *Am J Pathol* 1991;**139**:573–80.
104 **Hoyt JC**, Robbins RA, Habib M, *et al.* Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. *Exp Lung Res* 2003;**29**:17–28.
105 **Rusznak C**, Sapsford RJ, Devalia JL, *et al.* Interaction of cigarette smoke and house dust mite allergens on inflammatory mediator release from primary cultures of human bronchial epithelial cells. *Clin Exp Allergy* 2001;**31**:226–38.
106 **Wang HY**, Ye YN, Zhu M, *et al.* Increased interleukin-8 expression by cigarette smoke extract in endothelial cells. *Environ Toxicol Pharmacol* 2000;**9**:19–23.
107 **Richter A**, O'Donnell RA, Powell RM, *et al.* Autocrine ligands for the epidermal growth factor receptor mediate interleukin-8 release from bronchial epithelial cells in response to cigarette smoke. *Am J Respir Cell Mol Biol* 2002;**27**:85–90.

108 **Mio T**, Romberger DJ, Thompson AB, *et al.* Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *Am J Respir Crit Care Med* 1997;**155**:1770–6.
109 **Witherden IR**, Goldstraw P, Pastorino U, *et al.* Interleukin-8 release by primary human alveolar type II cells in vitro: effect of neutrophil elastase and cigarette smoke. *Respir Med* 1997;**91**:A27.
110 **Ryder MI**, Saghizadeh M, Ding Y, *et al.* Effects of tobacco smoke on the secretion of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta from peripheral blood mononuclear cells. *Oral Microbiol Immunol* 2002;**17**:331–6.
111 **Ouyang Y**, Virasch N, Hao P, *et al.* Suppression of human IL-1beta, IL-2, IFN-gamma, and TNF-alpha production by cigarette smoke extracts. *J Allergy Clin Immunol* 2000;**106**:280–7.
112 **Higashimoto Y**, Shimada Y, Fukuchi Y, *et al.* Inhibition of mouse alveolar macrophage production of tumor necrosis factor alpha by acute in vivo and in vitro exposure to tobacco smoke. *Respiration* 1992;**59**:77–80.
113 **Zhang X**, Wang L, Zhang H, *et al.* The effects of cigarette smoke extract on the endothelial production of soluble intercellular adhesion molecule-1 are mediated through macrophages, possibly by inducing TNF-alpha release. *Methods Find Exp Clin Pharmacol* 2002;**24**:261–5.
114 **Hellermann GR**, Nagy SB, Kong X, *et al.* Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. *Respir Res* 2002;**3**:22.
115 **Dubar V**, Gosset P, Aerts C, *et al.* In vitro acute effects of tobacco smoke on tumor necrosis factor alpha and interleukin-6 production by alveolar macrophages. *Exp Lung Res* 1993;**19**:345–59.
116 **Brown GM**, Drost E, Donaldson K, *et al.* Reduction of the proteolytic activity of neutrophils by exposure to cigarette smoke in vitro. *Exp Lung Res* 1991;**17**:923–37.
117 **Johnson JD**, Houchens DP, Kluwe WM, *et al.* Effects of mainstream and environmental tobacco smoke on the immune system in animals and humans: a review. *Crit Rev Toxicol* 1990;**20**:369–95.
118 **Stedman RL**. The chemical composition of tobacco and tobacco smoke. *Chem Rev* 1968;**68**:153–207.
119 **Guerin MR**, Higgins CE, Griest WH. The analysis of the particulate and vapour phases of tobacco smoke. *IARC Sci Publ* 1987:115–39.
120 **Morse D**, Choi AM. Heme oxygenase-1: the ''emerging molecule'' has arrived. *Am J Respir Cell Mol Biol* 2002;**27**:8–16.
121 **Papi A**, Romagnoli M, Baraldo S, *et al.* Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;**162**:1773–7.
122 **Saetta M**, Di Stefano A, Maestrelli P, *et al.* Airway eosinophilia in chronic bronchitis during exacerbations. *Am J Respir Crit Care Med* 1994;**150**:1646–52.
123 **Chapman JT**, Otterbein LE, Elias JA, *et al.* Carbon monoxide attenuates aeroallergen-induced inflammation in mice. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L209–16.
124 **Melgert BN**, Postma DS, Geerlings M, *et al.* Short-term smoke exposure attenuates ovalbumin-induced bronchoconstriction, hyperresponsiveness, and airway inflammation in mice. *Am J Respir Crit Care Med* 2003;**167**:A64.
125 **Erjefalt JS**, Persson CG. Airway epithelial repair: breathtakingly quick and multipotentially pathogenic. *Thorax* 1997;**52**:1010–2.
126 **Yamada N**, Yamaya M, Okinaga S, *et al.* Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 2000;**66**:187–95.

Table 1 Acute effects of smoking; human studies

First author; Year of study	***Subjects (no); Smoking status***	***Design R (hrs); S (no); T(hrs)***	***Effect of smoking***
		Inflammation	
Abboud RT (1986)[1]	8 CS	R: 8 S: 8 in 2 hrs T: 0.5, 1, 2	Blood: NE ↑ 1 hr, leukocytes and neutrophils ↑ 1 and 2 hrs
Drost EM (1993)[2]	8 CS	R: 12 S: 2 T: direct	Blood: Leukocyte deformability ↓, NE ↑
Fauler J (1997)[3]	12 NS	R; ? S: 6 T: 12	Urine: LTE4 ↑
Gil E (1995)[4]	9 CS	R: ? S: 1 T: 15 min	EP* = in CS
Hockertz S (1994)[5]	5 CS, 5 NS	R: ? S: 24 in 8 hrs T: during	Blood: Granulocytes ↑, CD19 cells ↓, CD3 cells =, CD4/CD8 ratio =, IL-1 =, IL-6 =, PGE2 =, sCD14 =, ROI =
Kaplan JD (1992)[6]	7 CS, 7 NS	R: no S: 1 T: 30 min	Endothelial permeability† =
Kobayashi J (1988)[7]	23 CS	R: 12 S: 5 T: 0, 20 min	Blood: LTB4, LTD4 and LTE4 ↑, correlated positively to C3a en C5a
Janoff A (1983)[8]	11 CS, 11 NS	R: ? S: 2 T: 0.5-1	BALF: Elastase ↑, Neutrophils =, monocytes =, leukocytes =
MacNee W (1989)[9]	24 CS, 6 NS	R: not S: 3-6 T: during	Out wash of neutrophils‡ ↓
Patiar S (2002)[10]	12 CS	R: 12 S:4 T: 10 and 30 min	Blood: Granulocyte L-selectin expression ↑ 10 and 30 min
Skwarski KM (1993)[11]	8 CS, 8 NS	R: 12 S: 1 T: 5 min	RBC transit time across the mid and lower part of the lung ↓
Tardif J (1990)[12]	8 CS, 4 NS	R: 7 S: 4 T:1	BALF: AM release of LTB4 ↓
Walter S (1980)[13]	25 CS	R: 12 S: 2 T: 10 min	Blood: Basophils ↓
Walter S (1982)[14]	27 CS	R: 12 S: 1-2 T: 10 min	Blood: Basophilic degranulation ↑

Ward C (2000)[15]	5 CS, 5 NS	R: ? S: 1 T: 10 min	Endothelial permeability[§] ↑
Winkel P (1980)[16]	4 Female CS	R: 24 S: 12 in 3-4 hrs T: during	Blood: Lymphocytes ↓, Eosinophils ↓, Neutrophils ↑ after 2.5 hrs
		Oxidative stress	
Balint B (2001)[17]	15 CS, 15 NS	R: ? S: 2 T: 0.5 and 1.5	Breath condensate: Nitrate + nitrite ↑ 30 minutes, 90 minutes =, nitrite =, peroxynitrite = Exhaled air: eNO =
Chambers DC (1998)[18]	24 CS	R: ? S: 1 T: 1, 10 min	Exhaled air: eNO↑
Guatura SB (2000)[19]	12 CS, 10 NS	R: 10 S: 1 T: 30 min	Breath condensate: H_2O_2↑
Kharitonov SA (1995)[20]	17 CS	R: 8 S: 1 T: 5, 15 min	Exhaled air: eNO↓ 5 min, = 15 min
Morrison D (1999)[21]	14 CS, 7 NS	R: 12 S: 2 T: 1	BALF: Leukocytes O^{2-} ↑, TBARS =, TEAC ↑, GSH =, GSSG =, Neutrophils ↑ Blood: TEAC ↓, TBARS↑ EP[‡]: ↑
Morrow JD (1995)[22]	10 CS, 10 NS	R: 10 S: 3 T: 0.5	Blood: F2-isoprostane =
Montuschi P (2000)[23]	12 CS	R: 12 S: 2 T: 15 min, 5	Breath condensate: 8-isoprostanes ↑ 15 min, = 5 hrs
Rahman I (1996)[24]	12 CS, 14 NS	R: ? S: 1 T: 1	Blood: TEAC↓, TBARS ↑
Tsuchiya M (2002)[25]	20 CS	R: ? S: 1 T: 5 and 30 min, 1	Blood: Nitrate, nitrite, ascorbic acid, cysteine, methionine, uric acid 5 min ↓, 30 min =

Definition of abbreviations:
CS: cigarette smokers NS: non-smokers R: time refrained from cigarette smoking (hrs) S: number of cigarettes smoked T: time between smoke inhalation and measurements (hrs)

AMs: alveolar macrophages; eNO: exhaled nitric oxide; EP: epithelial permeability: PGE2; prostaglandin E2; ROI: reactive oxygen intermediates; TEAC: trolox equivalent anti-oxidant capacity; TBARS: thiobarbituric acid reactive substances; LTB4: leukotrien B4; GSH: gluthatione; GSSG: oxidised gluthatione; NE: neutrophil elastase

*Tc-DTPA-scan
† PET
‡ ^{111}-In labelled neutrophils
§ Radioactive Urea

Table 2 Acute effects of cigarette smoking; animal studies

First author; Year of study	*Animals (no)*	*Smoke exposure S (no); E (hrs); T (hrs)*	*Route of administration*	*Effect of smoke exposure*
		Inflammation		
Abrams WR (1988)[26]	Beagle dogs N=12	S: 1, 3 or 6 E: ? T: 1, 4, 15, 24	AM	BALF: 3 and 6 cig: PMNs ↑ 15 hrs, = 1, 4 and 24 hrs 6 cigarettes: EIC ↑ 1 hr 3 cig: Elastase/PMN ↓ 4, 15 hrs, = 24 hrs
Bosken CH (1991)[27]	New Zealand White rabbits N= 5 CS, 5 NS	S: 12 puffs E: 12 min T: 4, 7 12 min	IT	Histology: PMNs retention in lungs ↑, PMNs retention in lowest lung slices ↑ Blood: MPO ↑ 4 and 7 min, = 12 min EP* =
Boucher RC (1980)[28]	Guinea Pigs N=10-15	S: 5 - 100 puffs E: ? T: direct	IT	EP† ↑ after 40 puffs and 100 puffs EM: 100 puffs ↑ intercellular spaces and structural changes in tight junctions of tracheal segments
Burns AR (1989)[29]	Guinea Pigs; N= 25	S: 15 E: ? T: direct	M&N	Lung tissue: Focal disruptions in type I pneumocytes, epithelial desquamation, trans epithelial FITC-D penetration, FITC-D intracellular in type I pneumocytes EP*↑
Churg A (2002)[30]	C57BL/6 mice N= 4 CS, 4 NS	S:4 E: ? T: 24	N	Lavage: PMNs, AMs, desmosine and hydroxyproline ↑ 24 hrs No effect of smoke in MME knock-out mice, except AMs↑ Lung tissue: α-1-antitrypsin ↑ 24 hrs
Churg A (2002)[31]	C57BL/6 mice N= 5 CS, 5 NS	S: 4 E: ? T: 2, 6, 24	N	Lung tissue: TNF-α, MIP-2 and MCP-1 gene expression ↑ 2 hrs, = 6, 24 hrs Lavage: Desmosine, hydroxyproline, PMNs and AMs ↑ 24 hrs No smoke effect in TNF-α receptor knock-out mice
Churg A	C57BL/6 mice	S: 4 E: 1 T: 2, 6,	N	Gene expression 2 hrs: TNF-α ↑, MCP-1 ↑, MIP-2

(2003)[32]	N=3 CS, 3 NS	24		↑, 6 hrs: TNF-α =, MCP-1 =, MIP-2 ↑, protein: TNF-α ↑ 2, 6, 24 hrs, E-selectin ↑ 6 and 24 hrs MMP-12 knock-out mice no effect on gene upregulation, but inhibits effect on TNF-α and E-selectin
Daffonchio L (1990)[33]	Dunkin-Hartley guinea pigs N= ?	S: ? E: 10 min T: 5, 50 min	IT	BALF: 5 min: total cells ↑, neutrophils =, eosinophils ↑, macrophages ↑ 50 min: total cells ↑, neutrophils =, eosinophils =, macrophages ↑
Dhami R (2000)[34]	C57-BL/6 mice N= 5 CS, 5 NS	S:2 E: ? T: 6, 24	N	BALF: PMNs ↑ 24 hrs, AMs = 24 hrs, desmosine ↑ 6, 24 hrs, hydroxyproline ↑ 6, 24 hrs, serine and metalloelastase activity↑ Anti-PMN and α-1 AT: inhibit smoke effect on PMNs, desmosine, hydroxyproline and serine elastase activity
Holt PG (1973)[35]	C57 black inbred mice N= 10	S: 30 E: 8 min T: direct	SM	Lavage: Viability AMs ↓
Hulbert WC (1981)[36]	Camm Hartley Guinea Pigs N= 30	S: 100 puffs E: ? T: 30 min, 1, 6, 12, 24	IT	EP[†]: ↑ 30 min, = 24 hrs Histology: Exudate↑ 0.5-1 hr, = 6, 12, 24 hrs, Cells expressed per mm epithelial cells: PMNs↑ 6 hrs, basal membrane ↓ 0.5-6 hrs, ↑ 24 hrs, goblet cells ↓ 0.5-12 hrs, plasma cells ↓, eosinophils ↓ 6, 12 and 24 hrs, mast cells ↑ 6 hrs, = 12 hrs
Ischizaki T (1996)[37]	Sprague-Dawley rats N=103, groups of 6-15 rats	S: 5 E: 20 min T: 2, 6, 24	SC	Plasma EIC ↓ 2 hrs, =6 and 24 hrs Plasma ferroxidase activity ↓ 2 hrs, = 6 and 24 hrs, plasma lipid peroxide ↑ 2 and 6 hrs, =24 hrs Lung tissue: lipid peroxide = 2 hrs, ↑ 6 hrs, = 24 hrs, GSH ↓ direct, ↑ 2 and 6 hrs, ↓ 24 hrs, GSH/GSSG ratio ↓ 2, 6 and 24 hrs BALF: total cell count and neutrophils↑ 24, AM and lymphocytes = 24 hrs
Janoff A	Sprague-	S: 3 or 6 puffs E:	SC	BALF: EIC per α-1 AT↓, after adding reducing

(1979)[38]	Dawley rats N= 21 CS, 16 NS	? T: direct		agent recovery of 75% of EIC
Kew RR (1985)[39]	Sprague-Dawley rats N= 4 CS, 4 NS	S: 12 puffs E: 4 min T: 1	SC	BALF: 1 hr: C3 ↑, PMNs ↑, monocytes↑, leukocyte chemotactic activity↑, prevented by depletion of complement
Kilburn KH (1975)[40]	Syrian hamsters N= 3	S: ? E: 4 T: 2, 8, 20	N	Histology: Ratio PMN/ 100 epithelial cells time dependent ↑ 6-24 hrs
Li XY (1996)[41]	Rats N=16	S: ? E: 0,2 ml CSC T: 1, 6, 24	IT	EP‡: ↑ 6 hrs, = 24 hrs BALF: AMs and PMNs ↑ 1, 6 and 24 hrs, GSH ↓ 1, 6 hrs, GSSG ↑ 1 hr, = 24 hrs Lung homogenate: GSH ↓ 1 hr, = 6 hrs, GSSG: ↑ 1 hr, ↓ 6 hrs, = 24 hrs
Mordelet-Dambrice M (1991)[42]	Wistar rats N= 28 CS, 28 NS	S: 32 puffs E: 8-9 min T:15 min	N	EP[§]: ↑ 15 min BALF: AMs ↑, PMNs =
Nishikawa M (1990)[43]	Hartley Guinea Pigs N= 46 CS, 18 NS	S: 5, 10 or 20 puffs E: ? T: direct, 5, 24	N&M	EP [II]: = Histology: Neutrophils and eosinophils in the trachea =
Ortega E (1992)[44]	Swiss mice IFFA CREDO N= ?	S: 1 E: 15 min T: direct, 1, 12, 24	SC	Histology: AMs ↑ direct and 1 hr, phagocytic index ↓ direct and 1 hr, = 12 hrs, % activated AMs ↑ direct, phagocytic efficiency ↓ direct and 1 hr
Ortega E (1993)[45]	Mice N= ?	S: 1 E: 15 min T: 1	SC	Adherence AMs =, chemotaxis AMs =, phagocytosis of Candida Albicans ↓
Pessina GP (1993)[46]	Out bred Wistar rats N= 12	S: 3 E: 1 T: 8, 24	SC	BALF: AMs ↑ 8 hrs, PMNs ↑ 24 hrs, TNF-α release from AMs ↑ 8 hrs, IFN release from AMs =
Pessina GP (1996)[47]	Wistar rats N= 6 CS, ? NS	S:3 E: ? T: direct	SC	BALF: Degradation of IL-6 ↑
Reznik-Schuller HM	Syrian Hamster	S: 20 puffs E: ? T: 1	SM	EM: Haemorrhages, swollen cytoplasm and protrusions in the lumen of type I pneumocytes and

(1980)[48]	N= 40 CS, 40 NS			endothelial cells. Occasionally the cell membrane was ruptured.
Simani AS (1974)[49]	Guinea Pigs N= 67	S:1 or 10 E: 24 hrs T: direct	M&N	EP[†] = after 1 and 10 cigarettes EM: Tight junctions ↑ after 10 cigarettes
Vitalis TZ (1998)[50]	Guinea Pigs N= 6 CS, 6 NS	S: 5 E: 40 min T: direct	N	Lung parenchyma: AMs↑ Airway wall: PMNs ↑
Walker DC (1988)[51]	Hartley Guinea Pigs N= 30 CS, 5 NS	S:15- 100 puffs E: ? T: direct	M&N	No increased HRP in epithelial tight junctions of tracheal segments
Witten ML (1985)[52]	New-Zealand white rabbits N =12 CS, 6 NS	S: 5-30 breaths E: ? T: during	IT	EP[§]:↑ from 20 breaths EM: Focal alveolar edema and haemorrhage, no alveolar-capillary membrane damage
Witten ML (1988)[53]	Rabbits N= 6 CS, 6 NS	S: 5-30 breaths E: ? T: direct	IT	EP[§]:↑ during smoking, Ibuprofen ↑ EP EM: Focal alveolar edema BALF: TxB2 ↑, 6keto PGF1α ↑, lymphocytes ↓, LTB4 ↓ Blood: LTB4 =, TxB2 =, 6keto PGF1α ↑
Wright J (1990)[54]	Guinea pigs N= 8 CS, 8 NS	S: ? E: 15 min T: 15 min, 1.5	IT	BALF: 1.5 hrs: leukocytes =, PMNs ↑ Blood: 15 min: leukocytes and PMNs ↑
Wright JL (2002)[55]	C57BL/6 mice N= 6 Guinea Pigs N= 5	S: 4 E: ? T: 2 S: 5 E: 3-4 T: 24	N	Mice: Lung homogenate: mRNA MIP-2, MCP-1, TNF-α ↑ 2 hrs Plasma: TNF-α ↑ 2 hrs Guinea Pigs: BALF: PMNs, desmosine, hydroxyproline ↑ 24 hrs Neutrophil elastase inhibitor: prevented smoke effects, except on TNF-α mRNA
Yamaya M (1989)[56]	Mongrel dogs N= 40	S: 1, 3, 5 or 9 E: ? T: direct, 7, 14	IT	BALF: Cytoplasmic motility AMs↑ direct, = 7 min after 1, 3 or 5 cigarettes

		min		
		Oxidative stress		
Aoshiba K (2003)[57]	C57-BL/6 mice N=6	S: 10 E: 1 T: 1, 3, 16, 24	SC	Lung tissue: 8-OHdG and 4-HNE ↑ 1 hr in bronchial epithelial cells and type II alveolar cells, cellularity ↑ 1-16 hrs, BALF: 8-OHdG levels ↑ 1 hr, = 24 hrs
Bilimoria MH (1992)[58]	Sprague-Dawley rats N=8 Hartley GP N=?	S: 40, 120, 240 puffs E: ? T: direct and 3	N	Lung homogenate: GSH ↓ direct, = 3 hrs, Ascorbic acid = direct
Cavarra E (2001)[59]	C57-BL/6J mice N=35 CS, 70 NS	S: 5 E: 20 min T: direct, 20, 60 min	SC	BALF: TEAC ↓ direct, = 20 min BALF, direct: GSSG ↑, ascorbic acid ↓, protein thiols ↓, total gluthatione =, vitamin E =, 8-epi-PGF2α ↑, all prevented by NAC Plasma: 8-epi-PGF2α ↑ direct, 20 and 60 min Total cell count, AMs, PMNs and lymphocytes = Inactivation of human SLPI
Cotgreave IA (1987)[60]	Sprague-Dawley rats N= ?	S:10 E: 1 T: direct	N	BALF: Intracellular GSH ↓, free GSH in lavage fluid ↓ Blood: GSH =, Cysteine ↑ Lung tissue: Cysteine =, Intracellular GSH
Uotila P (1982)[61]	Syrian hamsters N= ?	Experiment 1[¶]: S: 5 E:1 T: 20 Experiment 2[**]: S:12 E: 2 T: during	SC	Experiment 1: Blood: MUG ↑ 20 hrs Experiment 2: Blood: MUG ↓ during smoking
Wright JL (1999)[62]	Rats N= ?	S: 7 E:2 T: 24	N	Lung homogenate: 24 hrs: cNOS mRNA and protein =, iNOS mRNA ↑, protein =, eNOS mRNA ↑, protein =

Definition of abbreviations:
S: number of cigarettes exposed (no) E: exposure time (hrs) T: time between smoke exposure and measurement (hrs)
AM: anesthesia mask; IT: intra tracheal inhalation; N: nose-only inhalation; N&M: nose and mouth inhalation; SC: smoking chamber; SM: smoking machine

α-1 AT: α-1 antitrypsin; BALF: broncho-alveolar lavage fluid; CS: cigarette smoking animals; CSC: cigarette smoke condensate; EIC: elastase inhibitory capacity; EM: electron Microscopy; EP: Epithelial permeability; FITC-D: fluorescein isothiocyanate-dextran GSH: Gluthatione; HRP: Horseradish Peroxidase; ^{125}I-BSA= 125 Iodine labelled Bovine Serum Albumin; MME: macrophage metalloelastase; NOS: nitric oxide synthase; 6keto PGF1α: stable metabolite of prostacycline, prostaglandin I_2; MCP-1: macrophage chemoattractant protein-1; MIP-2: macrophage inhibitory protein-2; MPO: myeloperoxidase; MUG: 4- methylumbelliferone glucuronide; NS: non-smoking animals; PMNs: polymorphonuclear cells; SLPI: secretory leukoprotease inhibitor; TxB2: stable metabolite of tromboxane A_2

[*] Measured by FITC-D inhalation
[†] Measured by HRP
[‡] Measured by ^{125}I-BSA
[§] Measured by ^{99m}TcDTPA
[||] Measured by wash out of Evans Blue
[¶] Lungs were isolated, ventilated with cigarette smoke and thereafter perfused with MUG.
[**] Isolated lungs were simultaneously ventilated with cigarette smoke and perfused with MUG.

Table 3 Acute effects of cigarette smoke exposure; in vitro studies

First author; Year of study	*Cell types*	*Smoke exposure S (cig/ml); E (hrs); T (hrs)*	*Effect of smoke exposure*
		Inflammation	
Aoshiba K (2001)[63]	Murine, rat and human AMs	CSE: S: 0.1 E: 4-24 T: 4-24	24 hrs: 93% of AMs in apoptosis[*], inhibition by anti-oxidants
Brown GM (1991)[64]	Human PMNs	CSE: S: 1 cig E: 4 min T: 4 min	NE = 4 minutes PMNs: Extensive blebbing of cell membranes
Bridges RB (1977)[65]	Human PMNs	CSE: S: ? E: ? T: ?	Chemotaxis of PMNs ↓ concentration dependent
Cantral DE (1995)[66]	BBEC	CSE: S: 0.01 E: 2, 6, 24 T: 2, 6, 24	2 and 6 hrs exposure: attachment[†] of BBEC ↓, cell migration 2, 6 and 24 hrs = 24 hrs exposure: attachment[†] of BBEC ↑
Carnevali S (1998)[67]	HFL-1	CSE: 0.0016-0.0024 E: 24 T: 24	Fibroblast-mediated collagen gel contraction ↓ PGE2 release =, α2β1 integrin expression =, fibronectin release ↓
Carnevali S (2003)[68]	HFL-1	CSE: S: 0.002-0.004 E: 3 T: 3	Intracellular H_2DCFDA ↑ Apoptosis[‡] ↑, prevented by NAC Intracellular GSH ↓, inhibited by NAC DNA fragmentation ↑, inhibited by NAC
Churg A (2003)[32]	Mice AMs	CSE: S: 0.3 E: 18 T: 18	TNF-α release ↑
Drost EM (1992)[69]	Human PMNs	CS: S: 1, 3, 5 puffs E: 4 min T: 4 min	PMN filtration pressure ↑ after 1, 3, or 5 puffs, no effect of anti-CD18, inhibited by anti-oxidants and actin filaments cytoskeletal inhibitors Release H_2O_2 ↓
Dubar V (1993)[70]	Guinea pig and human AMs	CS: S: 2 E: ? T: ?	Activity of IL-6 and TNF-α ↓
Falk HL (1959)[71]	Ciliated epithelium from fogs, rat and rabbit trachea	CS: S: 50 ml E: 2 sec T: 1, 16, 46 min CSE: S:0.5 E: 2-30 sec T: 1, 16, 46 min	Mucus flow along epithelium ↓ 1-46 minutes
Floreani AA	Primary	CSE:	Adhesion THP-1 monocytes to HBEC ↑, inhibited by

(2003)[72]	HBECs BEAS-2B	S: 0.020 E: 1 T: 1	anti-TNF-α ICAM-1 expression HBEC ↑ Adhesion AMs, THP-1, peripheral blood monocytes to BEAS-2B↑
Green GM (1967)[73]	Rabbit AMs monolayer	CSE: S: 1-4 ml CS/ml E: ? T: 2	Phagocytosis staphylococcus albus ↓ dose dependent
Higashimoto Y (1991)[74]	Mice AMs	CSE: S: 0.04-0.001 E: 1 T: 1	TNF-α↓
Hellerman GR (2002)[75]	HBEC	CSE: S: ? E: 30 min T: 30 min	mRNA of IL-1β, IL-8, IL-6, GM-CSF, ICAM-1, RANTES ↑
Holt PG (1972)[76]	Rabbit AMs, murine PMs, secondary cultured murine embryonic fibroblasts	CS: S: 0.5- 2 puffs E: 4 sec T: 30 min-24 CSE: S: 35 ml smoke E: 70 min T: 70 min	CS: AMs and PMs: 30 min viability ↓ and ^{3}H-protein synthesis ↓, 24 hrs = Fibroblasts: 30 min viability =, ^{3}H-protein synthesis 30 min and 24 hrs ↓ CSE: macrophages and fibroblasts: ^{3}H-protein synthesis decreased dose dependent
Holt PG (1973)[35]	Primary cultured mice AMs, PMs, fibroblasts	CS: S: ? E: 60 sec T: 30 min, 24	30 minutes: viability AMs and PMs ↓, fibroblasts = 24 hrs: viability PMs and fibroblasts ↓ RNA synthesis of survivors ↑
Hoshino Y (2001)[77]	A549 cell line	CSE: S: 0.008 - 0.01 E: 3, 12, 24 T: 3, 12, 24	Cell viability ↓ time and dose dependent 3 hrs 0.8% CSE: ↑ oxidative activity in cells, inhibited by NAC
Ishii T (2001)[78]	HFL1	CSE: S: 1 E: 4, 8, 12, 16, 20, 24 T: 4, 8, 12, 16, 20, 24	10-25% CSE → Apoptosis[‡] 20, 24 hrs 50-100% CSE → Necrosis[§] 4-48 hrs GSTP1 sense vector ↓ necrosis 20, 24 hrs, apoptosis = GSTP1 anti-sense vector ↑ necrosis 4-48 hrs, apoptosis =
Kalra VKE (1994)[79]	Human monocytes, HUVEC	CSE: S: 10-60 µg/ml E: 5-90 min T: 5-90 min, 2-24 hrs	CD11b expression monocytes ↑ time dependent, optimum after 25 min exposure Adhesion monocytes to HUVEC ↑, 30 min exposure HUVEC: ICAM-1 and ELAM-1 ↑ 8, 24 hrs (60 min exposure)
Kim HJ (2002)[80]	HFL-1	CSE: S: 0.0004 E: 24 T: 24	Collagen gel contraction ↓ Fibronectin release ↓, prevented by NAC Intracellular GSH ↓, prevented and repleted by NAC EIIIA and B fibronectin mRNA ↓
Koyama S	Bronchial	CSE	MCA ↑ dose dependent

(1991)[81]	epithelial cell monolayers	S: 0.004 E: 2, 6, 24 T: 2, 6, 24	MCA ↑ after 2 hrs, time dependent Arachidonic metabolites inhibitors: ↓ MCA
Lannan S (1992)[82]	Human PMNs	CSE: 4 puffs E: 4 min T: 4 min	Diameter and circumference of neutrophils ↑ Surface membrane blebbing ↑
Lannan S (1994)[83]	A549 cell line	CS: S: 1 cig E: 5 min T: 5 min CSE: S: 1 E: during smoke exposure T: during smoke exposure	CS: Attachment[ll] ↓ 45 min- 24 hrs, prevented by GSH CSE: Detachment[ll] ↑, prevented by GSH, augmented by depleting GSH CSE: Proliferation ↓, prevented by GSH
Marwick JA (2002)[84]	A549 cell line	CSE: S: 10% E: 4, 24 T: 4, 20, 24	P21waf1/cip1 mRNA = 4 hrs, ↑ 24 hrs HDAC mRNA = 4, 24 hrs HDAC-2 protein 20 hrs ↓
Masubuchi T (1998)[85]	A549 cell-line	CSE S: 0.002 E: 12, 24 T: 12, 24	NCA = 12, 24 hrs
Mio TD (1997)[86]	HBEC	CSE: S: 0.004 E: 12-24 T: 12-24	IL-8 release ↑ concentration dependent IL-8 release ↑ time dependent, 12- 48 hrs. mRNA IL-8 ↑ 12 hrs
Nakamura Y (1995)[87]	HFL1	CSE: S: 0.002 E: 1-24 T: 1-24	Proliferation fibroblast: = 1 hr, ↓ 24 hrs
Niki E (1993)[88]	Rabbit erythrocytes	CSE: S: ? E: ? T: ?	Haemolysis rabbit erythrocytes, anti-oxidants no protection.
Ouyang Y (2002)[89]	HUVEC	CSE S: ? E: 24 T: 24	sICAM =
Richter A (2002)[90]	NCI-H292	CSE: S: 0.002 E: 6, 24 T: 6, 24	6 hrs: IL-8 mRNA ↑, TGF-α mRNA =, AR mRNA ↑, HB-EGF mRNA↑ 24 hrs: HB-EGF mRNA ↑
Robbins RA (1992)[91]	BBEC, human PMNs, mononuclear cells	CSE: S: 0.004 E: 24 T: 24	Adherence of PMNs to BBEC ↑
Rusznak C (2000)[92]	HBEC from HS, CS and COPD patients	CS: S: 4 E: 20 min T: 20 min	EP ↑ in all groups, COPD> HS> CS Il-1β and sICAM ↑ 24 hrs
Rusznak C (2001)[93]	HBEC	CS: S: ? E: 20 min, 1, 3, 6; T: 20 min, 1, 3, 6	20 min: IL-8 ↑, sICAM↑, IL-1β ↑ 1 hr: IL-8 ↓, IL-1β ↑ 3 and 6 hrs: IL-1 β ↓, IL-8 ↓, sICAM ↓

Ryder MI (1998)[94]	Human blood neutrophils	CS: S: ? E: 1-5 min T: 1-5 min	CD18 expression ↑ after 5 min L-selectin expression ↓ 1-5 min
Ryder MI (2002)[95]	PBMCs from 8 CS and 8 NS	CS: S:? E: 1, 2, 5 min T: 1, 2, 5 min	IL-1β ↑ 5 min in NS group TNF-α = TGF-β =
Sato E (1999)[96]	HFL1	CSE: S: 0.004 E: 6, 12, 24 T: 6, 24	MCA and NCA 24 hrs ↑, both inhibited by lipoxygenase inhibitors, anti-GM-CSF and anti-LTB4; NCA inhibited by anti-IL-8, MCA inhibited by, anti-MCP-1 mRNA IL-8, GM-CSF and MCP-1 ↑ 6 hrs
Selby C (1992)[97]	Human PMNs	CS: S: 1-4 cig E: 4 min T: 4 min	Basal adherence[¶] ↓ CD18 expression =, no effect of GSH Neutrophil chemokinesis ↓ Chemotaxis =
Shen Y (1996)[98]	HUVEC	CSE: S: 25 μg/ml E: 30 min-8 T: 30 min-8	ICAM-1, ELAM-1, VCAM-1 ↑
Shoij S (1995)[99]	Bovine epithelial cells	CSE: S: 0.04 E:3, 6, 12, 24 T: 3, 6, 12, 24	Dose and time dependent ↑ NCA from 3 hrs, inhibition by lipoxygenase inhibitors
Takeyama K (2001)[100]	NCI-H292	CSE: S: 0,5 puff/ml E: 15 T: 6, 12, 24	EGFR mRNA and MUC5AC mRNA ↑ 6-12 hrs MUC5AC protein ↑ 24 hrs dose dependent, inhibition by anti-oxidants
Tardiff J (1990)[12]	Human AMs	CSE: S: 0.04 E: 1 T: 1	Unstimulated AMs: LTB4 = PMA stimulated AMs: LTB4 ↓
Thomas WR (1977)[101]	Murine PMs	CS: S: 0.5-2 puffs E:? T: 30 min, 2, 3, 5, 24	Dose dependent ↓ of viability and phagocytosis of Pseudomonas aeruginosa 2 puffs: phagocytic activity ↓ 30 minutes and 24 hrs 1 puff: phagocytic activity ↓ at 2 hrs,↑ 5 hrs
Vayssier M (1998)[102]	U-937 cell line	CSE: S: 0.003-0.1 E: 4-16 T: direct	Low CSE: apoptosis[‡] 16 hrs High CSE: necrosis 16 hrs HSP70 expression ↑ dose dependent BCL-2 expression ↑ dose dependent
Vayssier-Taussat M (2001)[103]	Human PBMCs TrHBMECs	CSE: S: 0.3-2.4 E: 4, 16 T: 4, 16	Low CSE: at 4h: HSP70 and HO-1 expression ↑, at 16 hrs apoptosis[‡], inhibited by NAC High CSE: HO-1 expression ↓, at 16 h: necrosis, inhibited by NAC
Voisin C	Guinea pig	CS:	Viability AMs ↓ dose dependent, inhibited by NAC

(1985)[104]	AMs	S: 1-5 cig E: ? T: direct-3	Bactericidal activity of AMs ↓, inhibited by NAC
Wang HY (2000)[105]	ECV304	CSE: S: ? E: 2, 4, 8 T: 2, 4, 8	Time and dose dependent ↑ IL-8 secretion 4-8 hrs
Witherden IR (1997)[106]	Primary human alveolar type II cells	CSE: S: 0.01- 0.05 E: 24 T: 24	0.01 Cig/ml: IL-8 release ↑ 0.05 Cig/ml: cytotoxity
Wirtz HR (1996)[107]	Rat alveolar type II cells	CSE: S: 0.04 E: 0-1 T: 0-5	Surfactant secretion dose and time dependent ↓ from 20 min, no effect anti-oxidants
Yeager H (1968)[108]	Rabbit AMs	CSE: S: ? E: 20-120 min T: 20-120 min	Protein synthesis ↓ dose and time dependent
York GK (1973)[109]	Sheep AMs	CSE: S: 0.1 E: ? T: ?	Dose dependent ↓ of O_2 consumption Cell viability ↓
Zappacosta B (2001)[110]	Human PMNs	CSE S: ? E: 40 min T: during exposure	Phagocytic capacity ↓
Zhang X (2000)[111]	Human PBMCs	CSE: S: 1 E: 3 T: 3	TNF-α, IL-1β, IL-2 and IFN-γ ↓ dose dependent
		Oxidative stress	
Bridgeman M (1991)[112]	Erythrocytes, neutrophils, A549 cell line	CS: S: 1, 3, 5 puffs E: ? T: ?	Intracellular GSH ↓, no effect reducing agents
Hobson J (1991)[113]	Rat tracheal explants	CS: S: 1, 3, 6 puffs E: 10 min T: 40 min	H_2O_2 and O^{2-} ↑ along epithelial cell-membranes, prevented by SOD 3 and 6 puffs: cell separation, focal membrane blebbing, loss of cilia, cell disintegration.
Hoyt JC (2003)[114]	LA-4 A549 cell line HBEC	CSE: S: 0.0004-0.00008 E: 4, 24 T: 4, 24	Cells were stimulated for increased iNOS expression: CSE: nitrate ↓ 4 and 24 hrs in all cell types CSE: iNOS positive LA-4 cells ↓ 24 hrs CSE: iNOS mRNA ↓, eNOS and nNOS mRNA = in LA-4 cells 24 hrs, eNOS in A549 cells = 24 hrs
Kayyali US (2003)[115]	RPEMC	CSE: S: 20 µg/ml E: 4, 24 T: 4,24	XO activity ↑ 4 and 24 hrs mRNA XO ↑ 6 hrs
Li XY (1994)[116]	A549 cell line	CSE: S: 1 E: 1-6 T: 1, 4, 6 and 24 hrs after wash	EP**:↑ 1 hr, prevented by GSH, = 24 hrs after wash GSH intracellular ↓, = 24 hrs after wash

Noronha-Dutra A (1993)[117]	HUVEC	CSE: S: 0.5 E: 30 min T: 30 min	Pentose phosphate pathway activated GSSG release ↑
Pinot F (1999)[118]	Human peripheral blood monocytes	CSE: S:0.006-0.024 E: overnight T: direct after	O^{2-} production =, HSP 70 ↑ Membrane pseudopodes ↓, submembrane vacuoles ↑ Surfactant prevents effects CSE
Powell GM (1971)[119]	Rabbit AMs	CSE: S: ? E: ? T: ?	G3PD activity in AMs ↓, prevented by cysteine G6PD and LDH in AMs =
Rahman I (1996)[120]	A549 cell line	CSE: S: 1 puff/3 ml E: 4 T: 16-28	24 hrs after CSE intracellular: GSH ↑, GSSG =, γGCS activity ↑, γGCS-HS mRNA ↑
Tsuchiya MD (1992)[121]	Rat PMNs	CSE: S: 1 cig E: 20 min T: 20 min	ROS production from PMNs ↓, prevented by SOD O_2 consumption from PMNs ↑
Tuder RM (2000)[122]	Bovine artery endothelial cells, Monocytic U937, Hep G2, A549 cell line	CSE: S: 10% E: 24 T: 24	All cells: NO production ↑ All cells, except A549 cell line: VEGF ↓ protein and mRNA Apoptosis ↑ bovine artery endothelial cells
Wickenden JA (2003)[123]	A549 cell line HUVEC Jurkat cell	CSE: S: 0.05-0.1 E: 24 T: 24	Necrosis ↑, no apoptosis* GSH inhibits necrosis and apoptosis (Jurkat cell) GSH/GSSG ↓ intracellularly Inhibition caspase-3 activation (Jurkat cell)

Definition of abbreviations:
S: dose of smoke exposure (cig/ml). When possible, in order to compare cigarette smoke exposure between studies, the number of cigarettes per ml was calculated. E: time of smoke exposure (hrs) T: time between start of smoke exposure and measurement (hrs)

AMs: Alveolar Macrophages; AP-1: activator protein-1; AR: amphiregulin; BBEC: bovine bronchial epithelial cells; CS: cigarette smoke (gas phase); CSE cigarette smoke extract; ECV304: Human endothelial cell line; EGFR: epidermal growth factor receptor; ELAM-1: endothelial leukocyte adhesion molecule; EP: epithelial permeability; G3PD: glyceraldehydes 3-phosphate dehydrogenase; G6PD: glucose-6 phosphate dehydrogenase; γ-GCS-HS: γ-glutamylcysteine synthetase heavy subunit; GM-CSF: granulocyte-macrophage colony-stimulating factor; GSH: gluthatione; GSSG: oxidised gluthatione; GSTP1: gluthatione S-transferase P1; HBEC: human bronchial epithelial cell; HB-EGF: heparin-binding EGF like growth factor; trHBMECs: transfected human bone marrow endothelial cells; HFL1: human fetal lung fibroblasts; HSP 70: heat shock protein 70; HUVEC: human umbilical vein endothelial cells; ICAM-1: intercellular adhesion molecule 1; IFN-γ: interferon gamma; LA-4: murine lung epithelial cell line; LDH: lactate dehydrogenase; LTB4: leukotrien B4; NAC: N-acetylcysteine; NCI:H292: Human pulmonary mucoepidermoid carcinoma cell-line; NE: neutrophil elastase; NO: nitric oxide; PBMCs: peripheral blood mononuclear cells; PMs: peritoneal macrophages; PMNs: polymorphonuclear cells; RANTES: regulated on activation normal T-cell expressed and presumably secreted; ROS: radical oxidant scavengers; RPMEC: rat pulmonary micro vascular endothelial cells; sICAM: soluble intercellular adhesion molecule; SOD: superoxide dismutase; TGF-α: transforming growth factor α; TNF-α: tumor necrosis factor α; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling; U-937: premonocyte cell line; VCAM-1: vascular cell adhesion molecule 1; VEGF: vascular endothelial growth factor; XO: xanthine oxidase.

[*] Light microscopy, TUNEL and EM
[†] Attachment and migration to fibronectin-coated dishes
[‡] Annexin V
[§] 7-AAD uptake
[ll] Attachment/detachment to plastic
[¶] Functional adherence to A549 cell line

1 Abboud RT, Fera T, Johal S, *et al*. Effect of smoking on plasma neutrophil elastase levels. *J Lab Clin Med* 1986;108:294-300.

2 Drost EM, Selby C, Bridgeman MME, *et al*. Decreased leukocyte deformability after acute cigarette smoking in humans. *Am Rev Respir Dis* 1993;148:1277-83.

3 Fauler J, Frolich JC. Cigarette smoking stimulates cysteinyl leukotriene production in man. *Eur J Clin Invest* 1997;27:43-7.

4 Gil E, Chen B, Kleerup E, *et al*. Acute and chronic effects of marijuana smoking on pulmonary alveolar permeability. *Life Sci* 1995;56:2193-9.

5 Hockertz S, Emmendorffer A, Scherer G, *et al*. Acute effects of smoking and high experimental exposure to environmental tobacco smoke (ETS) on the immune system. *Cell Biol Toxicol* 1994;10:177-90.

6 Kaplan JD, Calandrino FS, Schuster DP. Effect of smoking on pulmonary vascular permeability. A positron emission tomography study. *Am Rev Respir Dis* 1992;145:712-5.

7 Kobayashi J, Kihira Y, Kitamura S. Effects of cigarette smoking on blood levels of leukotrienes and plasma levels of complements C3a and C5a in healthy volunteers. *Arch Environ Health* 1988;43:371-4.

8 Janoff A, Raju L, Dearing R. Levels of elastase activity in bronchoalveolar lavage fluids of healthy smokers and nonsmokers. *Am Rev Respir Dis* 1983;127:540-4.

9 MacNee W, Wiggs B, Belzberg AS, *et al*. The effect of cigarette smoking on neutrophil kinetics in human lungs. *N Engl J Med* 1989;321:924-8.

10 Patiar S, Slade D, Kirkpatrick U, *et al*. Smoking causes a dose-dependent increase in granulocyte-bound L- selectin. *Thromb Res* 2002;106:1-6.

11 Skwarski KM, Gorecka D, Sliwinski P, *et al*. The effects of cigarette smoking on pulmonary hemodynamics. *Chest* 1993;103:1166-72.

12 Tardif J, Borgeat P, Laviolette M. Inhibition of human alveolar macrophage production of leukotriene B4 by acute in vitro and in vivo exposure to tobacco smoke. *Am J Respir Cell Mol Biol* 1990;2:155-61.

13 Walter S, Nancy NR. Basopenia following cigarette smoking. *Indian J Med Res* 1980;72:422-5.

14 Walter S, Walter A. Basophil degranulation induced by cigarette smoking in man. *Thorax* 1982;37:756-9.

15 Ward C. Bronchoalveolar lavage fluid urea as a marker of pulmonary permeability in healthy smokers. *Eur Respir J* 2000;15:285.

16 Winkel P, Statland BE. The acute effect of cigarette smoking on the concentrations of blood leukocyte types in healthy young women. *Am J Clin Pathol* 1981;75:781-5.

17 Balint B, Donnelly LE, Hanazawa T, *et al*. Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke. *Thorax* 2001;56:456-61.

18 Chambers DC, Tunnicliffe WS, Ayres JG. Acute inhalation of cigarette smoke increases lower respiratory tract nitric oxide concentrations. *Thorax* 1998;53:677-9.

19 Guatura SB, Martinez JA, Santos Bueno PC, *et al*. Increased exhalation of hydrogen peroxide in healthy subjects following cigarette consumption. *Sao Paulo Med J* 2000;118:93-8.

20 Kharitonov SA, Robbins RA, Yates D, *et al*. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;152:609-12.

21 Morrison D, Rahman I, Lannan S, *et al*. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. *Am J Respir Crit Care Med* 1999;159:473-9.

22 Morrow JD, Frei B, Longmire AW, *et al*. Increase in circulating products of lipid peroxidation (F2- isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med* 1995;332:1198-203.

23 Montuschi P, Collins JV, Ciabattoni G, *et al*. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 2000;162:1175-7.

24 Rahman I, Morrison D, Donaldson K, *et al*. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med* 1996;154:1055-60.

25 Tsuchiya M, Asada A, Kasahara E, *et al*. Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation* 2002;105:1155-7.

26 Abrams WR, Kucich U, Kimbel P, *et al*. Acute cigarette smoke exposure in dogs: the inflammatory response. *Exp Lung Res* 1988;14:459-75.

27 Bosken CH, Doerschuk CM, English D, *et al*. Neutrophil kinetics during active cigarette smoking in rabbits. *J Appl Physiol* 1991;71:630-7.

28 Boucher RC, Johnson J, Inoue S, *et al*. The effect of cigarette smoke on the permeability of guinea pig airways. *Lab Invest* 1980;43:94-100.

29 Burns AR, Hosford SP, Dunn LA, *et al*. Respiratory epithelial permeability after cigarette smoke exposure in guinea pigs. *J Appl Physiol* 1989;66:2109-16.

30 Churg A, Zay K, Shay S, *et al*. Acute cigarette smoke-induced connective tissue breakdown requires both neutrophils and macrophage metalloelastase in mice. *Am J Respir Cell Mol Biol* 2002;27:368-74.

31 Churg A, Dai J, Tai H, *et al*. Tumor necrosis factor-alpha is central to acute cigarette smoke-induced inflammation and connective tissue breakdown. *Am J Respir Crit Care Med* 2002;166:849-54.

32 Churg A, Wang RD, Tai H, *et al*. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. *Am J Respir Crit Care Med* 2003;167:1083-9.

33 Daffonchio L, Hernandez A, Omini C. Sensory neuropeptides are involved in cigarette smoke induced airway hyperreactivity in guinea-pig. *Agents Actions Suppl* 1990;31:215-22.

34 Dhami R, Gilks B, Xie C, *et al*. Acute cigarette smoke-induced connective tissue breakdown is mediated by neutrophils and prevented by alpha1-antitrypsin. *Am J Respir Cell Mol Biol* 2000;22:244-52.

35 Holt PG, Keast D. Acute effects of cigarette smoke on murine macrophages. *Arch Environ Health* 1973;26:300-4.

36 Hulbert WC, Walker DC, Jackson A, *et al*. Airway permeability to horseradish peroxidase in guinea pigs: the repair phase after injury by cigarette smoke. *Am Rev Respir Dis* 1981;123:320-6.

37 Ishizaki T, Kishi Y, Sasaki F, *et al*. Effect of probucol, an oral hypocholesterolaemic agent, on acute tobacco smoke inhalation in rats. *Clin Sci (Lond)* 1996;90:517-23.

38 Janoff A, Carp H, Lee DK, *et al*. Cigarette smoke inhalation decreases alpha 1-antitrypsin activity in rat lung. *Science* 1979;206:1313-4.

39 Kew RR, Ghebrehiwet B, Janoff A. The role of complement in cigarette smoke-induced chemotactic activity of lung fluids. *Am Rev Respir Dis* 1986;133:478-81.

40 Kilburn KH, McKenzie W. Leukocyte recruitment to airways by cigarette smoke and particle phase in contrast to cytotoxicity of vapor. *Science* 1975;189:634-7.

41 Li XY, Rahman I, Donaldson K, *et al*. Mechanisms of cigarette smoke induced increased airspace permeability. *Thorax* 1996;51:465-71.

42 Mordelet-Dambrine M, Leguern-Stanislas G, Chinet TC, *et al*. Effects of tobacco smoke on respiratory epithelial clearance of DTPA and on lung histology in rats. *Eur Respir J* 1991;4:839-44.

43 Nishikawa M, Ikeda H, Fukuda T, *et al*. Acute exposure to cigarette smoke induces airway hyperresponsiveness without airway inflammation in guinea pigs. Dose-response characteristics. *Am Rev Respir Dis* 1990;142:177-83.

44 Ortega E, Hueso F, Collazos ME, *et al*. Phagocytosis of latex beads by alveolar macrophages from mice exposed to cigarette smoke. *Comp Immunol Microbiol Infect Dis* 1992;15:137-42.

45 Ortega E, Barriga C, Rodriguez AB. Decline in the phagocytic function of alveolar macrophages from mice exposed to cigarette smoke. *Comp Immunol Microbiol Infect Dis* 1994;17:77-84.

46 Pessina GP, Paulesu L, Corradeschi F, *et al*. Production of tumor necrosis factor alpha by rat alveolar macrophages collected after acute cigarette smoking. *Arch Immunol Ther Exp (Warsz)* 1993;41:343-8.

47 Pessina GP, Paulesu L, Corradeschi F, *et al*. Pulmonary catabolism of interleukin 6 evaluated by lung perfusion of normal and smoker rats. *J Pharm Pharmacol* 1996;48:1063-7.

48 Reznik-Schuller HM. Acute effects of cigarette smoke inhalation on the Syrian hamster lungs. *J Environ Pathol Toxicol* 1980;4:285-91.

49 Simani AS, Inoue S, Hogg JC. Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. *Lab Invest* 1974;31:75-81.

50 Vitalis TZ, Kern I, Croome A, *et al*. The effect of latent adenovirus 5 infection on cigarette smoke-induced lung inflammation. *Eur Respir J* 1998;11:664-9.

51 Walker DC, Burns AR. The mechanism of cigarette smoke induced increased epithelial permeability in guinea pig airways. *Prog Clin Biol Res* 1988;263:25-34.

52 Witten ML, Lemen RJ, Quan SF, *et al*. Acute cigarette smoke exposure increases alveolar permeability in rabbits. *Am Rev Respir Dis* 1985;132:321-5.

53 Witten ML, Quan SF, Sobonya RE, *et al*. Acute cigarette smoke exposure alters lung eicosanoid and inflammatory cell concentrations in rabbits. *Exp Lung Res* 1988;14:727-42.

54 Wright J, Harrison N. Cardiopulmonary effects of a brief exposure to cigarette smoke in the guinea pig. *Respiration* 1990;57:70-6.

55 Wright JL, Farmer SG, Churg A. Synthetic serine elastase inhibitor reduces cigarette smoke-induced emphysema in guinea pigs. *Am J Respir Crit Care Med* 2002;166:954-60.

56 Yamaya M, Zayasu K, Sekizawa K, *et al*. Acute effect of cigarette smoke on cytoplasmic motility of alveolar macrophages in dogs. *J Appl Physiol* 1989;66:1172-8.

57 Aoshiba K, Koinuma M, Yokohori N, *et al*. Immunohistochemical evaluation of oxidative stress in murine lungs after cigarette smoke exposure. *Inhal Toxicol* 2003;15:1029-38.

58 Bilimoria MH, Ecobichon DJ. Protective antioxidant mechanisms in rat and guinea pig tissues challenged by acute exposure to cigarette smoke. *Toxicology* 1992;72:131-44.

59 Cavarra E, Lucattelli M, Gambelli F, *et al*. Human SLPI inactivation after cigarette smoke exposure in a new in vivo model of pulmonary oxidative stress. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L412-L417.

60 Cotgreave IA, Johansson U, Moldeus P, *et al*. The effect of acute cigarette smoke inhalation on pulmonary and systemic cysteine and glutathione redox states in the rat. *Toxicology* 1987;45:203-12.

61 Uotila P. Effect of cigarette smoke on glucuronide conjugation in hamster isolated lungs. *Res Commun Chem Pathol Pharmacol* 1982;38:173-6.

62 Wright JL, Dai J, Zay K, *et al*. Effects of cigarette smoke on nitric oxide synthase expression in the rat lung. *Lab Invest* 1999;79:975-83.

63 Aoshiba K, Tamaoki J, Nagai A. Acute cigarette smoke exposure induces apoptosis of alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L1392-L1401.

64 Brown GM, Drost E, Donaldson K, *et al*. Reduction of the proteolytic activity of neutrophils by exposure to cigarette smoke in vitro. *Exp Lung Res* 1991;17:923-37.

65 Bridges RB, Kraal JH, Huang LJ, *et al*. Effects of tobacco smoke on chemotaxis and glucose metabolism of polymorphonuclear leukocytes. *Infect Immun* 1977;15:115-23.

66 Cantral DE, Sisson JH, Veys T, *et al*. Effects of cigarette smoke extract on bovine bronchial epithelial cell attachment and migration. *Am J Physiol* 1995;268:L723-L728.

67 Carnevali S, Nakamura Y, Mio T, *et al*. Cigarette smoke extract inhibits fibroblast-mediated collagen gel contraction. *Am J Physiol* 1998;274:L591-L598.

68 Carnevali S, Petruzzelli S, Longoni B, *et al*. Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L955-L963.

69 Drost EM, Selby C, Lannan S, *et al*. Changes in neutrophil deformability following in vitro smoke exposure: mechanism and protection. *Am J Respir Cell Mol Biol* 1992;6:287-95.

70 Dubar V, Gosset P, Aerts C, *et al*. In vitro acute effects of tobacco smoke on tumor necrosis factor alpha and interleukin-6 production by alveolar macrophages. *Exp Lung Res* 1993;19:345-59.

71 Falk HL, Tremer HM, Kotin P. Effect of cigarette smoke and its constituents on ciliated mucus-secreting epithelium. *J Natl Cancer Inst* 1959;23:999-1012.

72 Floreani AA, Wyatt TA, Stoner J, *et al*. Smoke and C5a induce airway epithelial intercellular adhesion molecule-1 and cell adhesion. *Am J Respir Cell Mol Biol* 2003;29:472-82.

73 Green GM, Carolin D. The depressant effect of cigarette smoke on the in vitro antibacterial activity of alveolar macrophages. *N Engl J Med* 1967;276:421-7.

74 Higashimoto Y, Shimada Y, Fukuchi Y, *et al*. Inhibition of mouse alveolar macrophage production of tumor necrosis factor alpha by acute in vivo and in vitro exposure to tobacco smoke. *Respiration* 1992;59:77-80.

75 Hellermann GR, Nagy SB, Kong X, *et al*. Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. *Respir Res* 2002;3:22.

76 Holt PG, Keast D. The effect of tobacco smoke on protein synthesis in macrophages. *Proc Soc Exp Biol Med* 1973;142:1243-7.

77 Hoshino Y, Mio T, Nagai S, *et al*. Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L509-L516.

78 Ishii T, Matsuse T, Igarashi H, *et al*. Tobacco smoke reduces viability in human lung fibroblasts: protective effect of glutathione S-transferase P1. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1189-L1195.

79 Kalra VK, Ying Y, Deemer K, *et al*. Mechanism of cigarette smoke condensate induced adhesion of human monocytes to cultured endothelial cells. *J Cell Physiol* 1994;160:154-62.

80 Kim HJ, Liu X, Wang H, *et al*. Glutathione prevents inhibition of fibroblast-mediated collagen gel contraction by cigarette smoke. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L409-L417.

81 Koyama S, Rennard SI, Leikauf GD, *et al*. Bronchial epithelial cells release monocyte chemotactic activity in response to smoke and endotoxin. *J Immunol* 1991;147:972-9.

82 Lannan S, McLean A, Drost E, *et al*. Changes in neutrophil morphology and morphometry following exposure to cigarette smoke. *Int J Exp Pathol* 1992;73:183-91.

83 Lannan S, Donaldson K, Brown D, *et al*. Effect of cigarette smoke and its condensates on alveolar epithelial cell injury in vitro. *Am J Physiol* 1994;266:L92-100.

84 Marwick JA, Kirkham P, Gilmour PS, *et al*. Cigarette smoke-induced oxidative stress and TGF-beta1 increase p21waf1/cip1 expression in alveolar epithelial cells. *Ann N Y Acad Sci* 2002;973:278-83.

85 Masubuchi T, Koyama S, Sato E, *et al*. Smoke extract stimulates lung epithelial cells to release neutrophil and monocyte chemotactic activity. *Am J Pathol* 1998;153:1903-12.

86 Mio T, Romberger DJ, Thompson AB, *et al*. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *Am J Respir Crit Care Med* 1997;155:1770-6.

87 Nakamura Y, Romberger DJ, Tate L, *et al*. Cigarette smoke inhibits lung fibroblast proliferation and chemotaxis. *Am J Respir Crit Care Med* 1995;151:1497-503.

88 Niki E, Minamisawa S, Oikawa M, *et al*. Membrane damage from lipid oxidation induced by free radicals and cigarette smoke. *Ann N Y Acad Sci* 1993;686:29-37.

89 Zhang X, Wang L, Zhang H, *et al*. The effects of cigarette smoke extract on the endothelial production of soluble intercellular adhesion molecule-1 are mediated through macrophages, possibly by inducing TNF-alpha release. *Methods Find Exp Clin Pharmacol* 2002;24:261-5.

90 Richter A, O'Donnell RA, Powell RM, *et al*. Autocrine ligands for the epidermal growth factor receptor mediate interleukin-8 release from bronchial epithelial cells in response to cigarette smoke. *Am J Respir Cell Mol Biol* 2002;27:85-90.

91 Robbins RA, Koyama S, Spurzem JR, *et al*. Modulation of neutrophil and mononuclear cell adherence to bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1992;7:19-29.

92 Rusznak C, Mills PR, Devalia JL, *et al*. Effect of cigarette smoke on the permeability and IL-1beta and sICAM-1 release from cultured human bronchial epithelial cells of never- smokers, smokers, and patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2000;23:530-6.

93 Rusznak C, Sapsford RJ, Devalia JL, *et al*. Interaction of cigarette smoke and house dust mite allergens on inflammatory mediator release from primary cultures of human bronchial epithelial cells. *Clin Exp Allergy* 2001;31:226-38.

94 Ryder MI, Fujitaki R, Lebus S, *et al*. Alterations of neutrophil L-selection and CD18 expression by tobacco smoke: implications for periodontal diseases. *J Periodontal Res* 1998;33:359-68.

95 Ryder MI, Saghizadeh M, Ding Y, *et al*. Effects of tobacco smoke on the secretion of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta from peripheral blood mononuclear cells. *Oral Microbiol Immunol* 2002;17:331-6.

96 Sato E, Koyama S, Takamizawa A, *et al*. Smoke extract stimulates lung fibroblasts to release neutrophil and monocyte chemotactic activities. *Am J Physiol* 1999;277:L1149-L1157.

97 Selby C, Drost E, Brown D, *et al*. Inhibition of neutrophil adherence and movement by acute cigarette smoke exposure. *Exp Lung Res* 1992;18:813-27.

98 Shen Y, Rattan V, Sultana C, *et al*. Cigarette smoke condensate-induced adhesion molecule expression and transendothelial migration of monocytes. *Am J Physiol* 1996;270:H1624-H1633.

99 Shoji S, Ertl RF, Koyama S, *et al*. Cigarette smoke stimulates release of neutrophil chemotactic activity from cultured bovine bronchial epithelial cells. *Clin Sci (Lond)* 1995;88:337-44.

100 Takeyama K, Jung B, Shim JJ, *et al*. Activation of epidermal growth factor receptors is responsible for mucin synthesis induced by cigarette smoke. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L165-L172.

101 Thomas WR, Holt PG, Keast D. Cigarette smoke and phagocyte function: effect of chronic exposure in vivo and acute exposure in vitro. *Infect Immun* 1978;20:468-75.

102 Vayssier M, Banzet N, Francois D, *et al*. Tobacco smoke induces both apoptosis and necrosis in mammalian cells: differential effects of HSP70. *Am J Physiol* 1998;275:L771-L779.

103 Vayssier-Taussat M, Camilli T, Aron Y, *et al*. Effects of tobacco smoke and benzo[a]pyrene on human endothelial cell and monocyte stress responses. *Am J Physiol Heart Circ Physiol* 2001;280:H1293-H1300.

104 Voisin C, Aerts C, Fournier E, *et al*. Acute effects of tobacco smoke on alveolar macrophages cultured in gas phase. *Eur J Respir Dis Suppl* 1985;139:76-81.

105 Wang HY, Ye YN, Zhu M, *et al*. Increased interleukin-8 expression by cigarettesmoke extract in endothelial cells. *Environmental Toxicology and Pharmacology* 2000;9:19-23.

106 Witherden IR, Goldstraw P, Pastorino U, *et al*. Interleukin-8 release by primary human alveolar type II cells in vitro: effect of neutrophil elastase and cigarette smoke[abstract]. *Respir Med* 1997;91:A27.

107 Wirtz HR, Schmidt M. Acute influence of cigarette smoke on secretion of pulmonary surfactant in rat alveolar type II cells in culture. *Eur Respir J* 1996;9:24-32.

108 Yeager H, Jr. Alveolar cells: depression effect of cigarette smoke on protein synthesis. *Proc Soc Exp Biol Med* 1969;131:247-50.

109 York GK, Arth C, Stumbo JA, *et al*. Pulmonary macrophage respiration as affected by cigarette smoke and tobacco extract. *Arch Environ Health* 1973;27:96-8.

110 Zappacosta B, Persichilli S, Minucci A, *et al*. Effect of aqueous cigarette smoke extract on the chemiluminescence kinetics of polymorphonuclear leukocytes and on their glycolytic and phagocytic activity. *Luminescence* 2001;16:315-9.

111 Ouyang Y, Virasch N, Hao P, *et al*. Suppression of human IL-1beta, IL-2, IFN-gamma, and TNF-alpha production by cigarette smoke extracts. *J Allergy Clin Immunol* 2000;106:280-7.

112 Bridgeman MME, Marsden M, Drost E, *et al*. The effect of cigarette smoke on lung cells[abstract]. *Am Rev Respir Dis* 1991;143:A737.

113 Hobson J, Wright J, Churg A. Histochemical evidence for generation of active oxygen species on the apical surface of cigarette-smoke-exposed tracheal explants. *Am J Pathol* 1991;139:573-80.

114 Hoyt JC, Robbins RA, Habib M, *et al*. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. *Exp Lung Res* 2003;29:17-28.

115 Kayyali US, Budhiraja R, Pennella CM, *et al*. Upregulation of xanthine oxidase by tobacco smoke condensate in pulmonary endothelial cells. *Toxicol Appl Pharmacol* 2003;188:59-68.

116 Li XY, Donaldson K, Rahman I, *et al*. An investigation of the role of glutathione in increased epithelial permeability induced by cigarette smoke in vivo and in vitro. *Am J Respir Crit Care Med* 1994;149:1518-25.

117 Noronha-Dutra AA, Epperlein MM, Woolf N. Effect of cigarette smoking on cultured human endothelial cells. *Cardiovasc Res* 1993;27:774-8.

118 Pinot F, Bachelet M, Francois D, *et al*. Modified natural porcine surfactant modulates tobacco smoke-induced stress response in human monocytes. *Life Sci* 1999;64:125-34.

119 Powell GM, Green GM. Investigation on the effects of cigarette smoke on rabbit alveolar macrophages. *Biochem J* 1971;124:26P-7P.

120 Rahman I, Smith CA, Lawson MF, *et al*. Induction of gamma-glutamylcysteine synthetase by cigarette smoke is associated with AP-1 in human alveolar epithelial cells. *FEBS Lett* 1996;396:21-5.

121 Tsuchiya M, Thompson DF, Suzuki YJ, *et al*. Superoxide formed from cigarette smoke impairs polymorphonuclear leukocyte active oxygen generation activity. *Arch Biochem Biophys* 1992;299:30-7.

122 Tuder RM, Wood K, Taraseviciene L, *et al*. Cigarette smoke extract decreases the expression of vascular endothelial growth factor by cultured cells and triggers apoptosis of pulmonary endothelial cells. *Chest* 2000;117:241S-2S.

123 Wickenden JA, Clarke MC, Rossi AG, *et al*. Cigarette smoke prevents apoptosis through inhibition of caspase activation and induces necrosis. *Am J Respir Cell Mol Biol* 2003.