Organic dust activates NF-κB in lung epithelial cells

Johan Lidén¹, Alexandra Ek¹, Lena Palmberg¹, Kjell Larsson*¹

Summary Exposure in swine confinement facilities induces airway inflammation in healthy subjects. The aim of the present study was to elucidate the role of nuclear factor (NF)-κB in the inflammatory response induced by organic dust. A human lung epithelial carcinoma cell line (A549) was transfected with reporter genes of the human IL-6 promoter or the NF-κB binding site fused to the luciferase reporter gene and stimulated with dust from a swine confinement building. Cytokine release in cell culture supernatants and luciferase activity was measured. The dust-induced the activities of the IL-6 promoter reporter gene and the NF-κB reporter gene in parallel with an increase in IL-6 and IL-8 release. The addition of pyrrolidinedithiocarbamate, a chemical NF-κB blocking agent, inhibited IL-6 and IL-8 secretion as well as the NF-κB reporter gene activity. Increasing the amount of IκBα led to inhibition of organic dust-induced IL-6 promoter and NF-κB reporter gene activities. Fluticasone inhibited the organic dust-induced NF-κB activation and IL-6 and IL-8 secretion. Finally, swine dust incubation of A549 cells resulted in a NF-κB DNA binding, which is composed of the NF-κB1 and RelA proteins. In conclusion, by interference at various levels we have shown that NF-κB plays a key role in the inflammatory response to organic dust.

Introduction

Farmers have a higher prevalence of respiratory symptoms and pulmonary diseases than the general population and swine farmers report higher prevalence of respiratory symptoms than other farmers and non-farming individuals.¹ It has previously been demonstrated that inhalation of organic dust from swine confinement facilities induces an intense airway inflammation and increased bronchial responsiveness to methacholine in healthy subjects.²,³ The airway cellular reaction to inhaled dust is dominated by neutrophils, but the number of alveolar macrophages² and activated T cells⁴ in bronchoalveolar lavage (BAL) fluid are also increased. In addition, the exposure leads to release of a number of pro-inflammatory cytokines in the lung as assessed by BAL⁵ and in vitro studies have shown that dust from swine houses induces cytokine release in epithelial cells.⁶,⁷ The dust-induced IL-6 and IL-8 release from epithelial cells is almost totally inhibited in vitro by the glucocorticoid fluticasone at low concentrations (10⁻¹⁰–10⁻⁹M).⁸

The in vivo and in vitro inflammatory responses to inhaled organic dust have been well characterized but the molecular mechanisms involved in the inflammatory reaction are unknown.

One key pathway in the cellular response to inflammatory signals leading to the cellular response...
is the intra-cellular NF-κB signalling cascade. A variety of agents including viral proteins, cytokerines, and bacterial lipopolysaccarides (LPS) activate the transcriptional activity of NF-κB. In mammalian cells the NF-κB family consists of RelA (p65), NF-κB1 (p50), c-Rel, p52 and RelB. RelA is the most potent transcriptional activator in the Rel homology protein family and the inducible NF-κB complex is usually composed of a heterodimer of RelA and NF-κB1. In the non-activated state, this heterodimer is sequestered in the cytoplasm through interaction with NF-κB-inhibiting proteins IκB, which masks the nuclear localization sequence in the heterodimer. During the activation process, IκB becomes phosphorylated and degrades. The phosphorylation of IκB is brought about by a protein complex, the I kappa B kinase complex (IKK), localized in the cytoplasm.9–13 This allows the NF-κB heterodimer to translocate into the nucleus, where it binds to specific NF-κB-binding sequences in promoter regions and subsequently activates genes involved in inflammation and immune responses.14–16

Glucocorticoids suppress inflammation in a wide variety of diseases and down regulation of a number of inflammatory genes has been demonstrated in vivo and in vitro.17 Glucocorticoids bind to and activate the intracellularly located glucocorticoid receptor (GR). The activated GR complex acts through one of several mechanisms that probably are not exclusive. The inhibitory effect of glucocorticoids has been described to be due to interaction between the activated GR and transcription factors such as activator protein (AP)-1, NF-κB and C/EBP (C/EBP)β. Both the IL-6 and the IL-8 promoter have binding sites for several nuclear factors including AP-1 and NF-κB.18,19 Mechanisms for NF-κB-dependent transcription include inhibition of NF-κB DNA binding, inhibition of activation via direct NF-κB–GR interactions and glucocorticoid-dependent up-regulation of IκBα expression.20 The activated GR complex can also reduce the half-life time and utility of cytokine mRNAs.21 The activated GR has been demonstrated to inactivate NF-κB-dependent transcription of many inflammatory genes.12,23

Since activation of the NF-κB pathway leads to transcriptional activation of genes involved in inflammation (reviewed in15,16), we hypothesized that this might be one molecular mechanism mediating the effects observed after exposure to organic dust. We addressed this question by interfering with the NF-κB signalling cascade at different levels in in vitro cultured lung epithelial cells exposed to organic dust from a swine house.

### Methods

#### Reagents

Dexamethasone (dex) was obtained from Sigma Chemical Company (Stockholm, Sweden). Tumor necrosis factor alpha (TNFα) was from Boehringer Mannheim (Indianapolis, IN, USA). Pyrrolidinedithiocarbamate (PDTC) was from Sigma-Aldrich Corporation (St. Louis, MO, USA). Cell culturing media, antibiotics, fetal bovine serum and LipofectamineTM reagent were all purchased from Life Technologies Ltd. (Paisley, Scotland, UK). GlaxoSmithKline (Stevenage, UK) generously provided fluticasone propionate (FP). Swine dust was collected approximately 1.2 m above the floor in a swine confinement building that contained 700–900 pigs. The dust was dissolved in a mixture of 50% Dulbecco’s Modified Eagle Medium and 50% Ham’s F12 Nutrient Mixture supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine and incubated in an ultrasound bath for 10 min. The concentration of the stock solution was 2 mg/ml.

#### Cell culture

The human lung epithelial cell line A549 was a generous gift from Dr. M. Nord, Karolinska Institutet. The cells were cultured in a mixture of 50% Dulbecco’s Modified Eagle Medium and 50% Ham’s F12 Nutrient Mixture supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% heat inactivated fetal calf serum (FCS). The cells were grown at 37°C 5% CO₂.

#### Reporter plasmids and expression vector

The reporter plasmids 1168hulL6P-LUC and 1168hulL6P-LUC NF-κBmut24,25 contain 1168 bp of the human IL-6 promoter fused to the luciferase (LUC) reporter gene. NF-κBmut indicates that the NF-κB binding site in the 1168hul IL-6 promoter is mutated. The NF-κB binding site in the hul-L6P-LUC reporter gene was mutated to 5’-CAAATGTGA-GATCTTTCCCATGAGTCTC-3’ (for more details see26). The RSV–LUC reporter drives LUC expression under the control of the Rous sarcoma virus promoter. The plasmid 3 × NF-κB(IIC)tk-LUC27 contains three copies of the NF-κB binding site from the human intercellular adhesion molecule-1 (ICAM-1) promoter in front of a minimal thymidine kinase promoter fused to the LUC reporter gene. CMV-IκBα28 harbors the human IκBα cDNA under the control of the CMV IκBα promoter.
Experimental design

The day before transfection the cells were plated in six-well plates in complete medium at a density of 40000–80000 cells/well. The cells in each well were transfected with 2 μg reporter plasmid and with 0, 5 or 20 ng expression vector as indicated in the figures. The Lipofectamine™ reagent was used according to manufacturer’s recommendations and the cells were transfected for 3 h, in serum-free Opti-Mem medium. After 3 h an equal amount of normal growth medium with 20% FCS was added to the cells and they were further incubated during 20 h. The reagents at concentrations indicated in the figure legends were then added to the cells in fresh growth medium containing 10% FCS.

After 24 h treatment, the supernatants were collected, centrifuged (1000 g, 10 min) and frozen until assayed for cytokine content, and the cells were harvested for LUC assay in 1 ml buffer containing 25 mM Tris phosphate pH 7.8, 2 mM EDTA, 10% glycerol 1% Triton X-100 and 1 mM DTT. Control experiments were performed in which the cells where detached by exposure to trypsin/EDTA solution and cell viability was determined by exclusion of Trypan blue 0.4% from Sera-Lab (Sussex, UK) in phosphate buffer saline. No difference in viability was detected due to the different treatments (data not shown). Each experiment was performed in quadruplicates. Control experiments were also performed in which the cells were transfected with 20 and 200 ng RSV-LUC and treated with the different agents used in this study. No significant difference in LUC activity depending on the different agents added to the medium was detected from the constitutive RSV-LUC reporter gene.

Luciferase assay

In the presence of ATP and luciferin, LUC catalyzes a light-emitting reaction. For measurement of LUC activity 100 μl cell extract per sample was used together with the GeneGlow™ kit from Bio-Orbit (Turku, Finland). Luciferin and ATP, are added to the cellular extracts after transfection and the production of light was measured by a lumino-meter, Lucy 1 from Anthos Labtec Instruments (Salzburg, Austria). The luminometer quantified enzymatic activity in order to reveal the extent of expression of LUC by the LUC gene when NF-κB, or other transcription factors depending on the transfected reporter gene, activates transcription.

Cytokine assays

IL-6 and IL-8 content in cell culture supernatants were measured using enzyme-linked immunosorbent assay (ELISA) methods described by Larsson et al.29 The detection range in the IL-6 and IL-8 assays were 3–375 and 50–3200 pg/ml, respectively.

Electromobility shift assay (EMSA)

The EMSA was used to detect DNA-binding transcription factors. In this assay, the electrophoretic mobility of radiolabeled DNA fragment is determined in the presence and absence of a sequence-specific DNA-binding protein. Nuclear extracts (NE) were prepared from untreated and dust incubated (30 min with 200 μg dust/ml medium) A549 cells as previously described.30 For NF-κB detection a 20 μl binding reaction contained: 20 mM HEPES pH 7.8, 5 mM MgCl₂, 20% glycerol, 0.1% Triton X-100, 75 mM KCl, 2 μg poly dIdC, 27 pg (31 000 CPM) 32P-labeled oligonucleotide corresponding to the NF-κB-binding site from the human ICAM-1 gene promoter (upper strand 5’ GGA TTC GAA TTC CCT TT 3’) and 5 μg total protein NE. For C/EBPβ detection 20 μl binding reaction contained: 25 mM HEPES pH 7.8, 1 mM DTT, 0.1 mM EDTA, 2 μg poly dIdC, 75 mM KCl, 10% glycerol, 2.5 pg (39 000 CPM) 32P-labeled oligonucleotide corresponding to the CYP2B1 promoter (5’ CAT CTG AAG TTG CAT AAC TGA G 3’). Transfection and preparation of C/EBPβ containing from COS cells have previously been described.31 Supershifts were conducted by including 2 μl of the NF-κB1 antibody sc-114X or the RelA antibody sc-109X (Santa Cruz) in the binding reaction. Specific and non-specific competition experiments were performed by including a 50-fold excess of NF-κB-binding or C/EBPβ-binding oligonucleotide or NF-1 consensus oligonucleotide (5’ ATT TTG GCT TGA AGC CAA TAT G 3’), respectively, in the binding reactions. The reactions were incubated for 20 min at room temperature and then loaded onto a 4% (w/v) acrylamide electrophoresis gel containing 0.25 X TBE. After electrophoresis, the gel was dried and autoradiographed.

Statistical analysis

The results are presented as mean and standard deviation (±SD). Comparisons of cell culture results were assessed by using analysis of variance (ANOVA) with Fisher’s PLSD test when appropriate. A P value <0.05 was considered significant. Statistical analysis was performed using StatView® (SAS Institute; Cary, NC) version 5.0.1.
Results

Transcriptional activation of the human IL-6 gene by organic dust is mediated via the NF-κB response element

We investigated whether the IL-6 promoter required an intact NF-κB DNA binding site for the dust responsiveness. A549 cells were transfected with the 1168huIL6P-LUC reporter construct that contains an 1166 bp fragment from the human IL-6 promoter in front of the LUC reporter gene. As a positive control we used TNFα promoter in front of the LUC reporter gene. As a positive control we used TNFα (200 U/ml) administrated to the cells resulted in a 2.5-fold stimulation of reporter gene activity (Fig. 1) (P<0.001). Exposure of 1168huIL6P-LUC transfected cells to dust (100 and 200 μg/ml) yielded a significant (P<0.01 and P<0.001, respectively) induction of LUC activity similar to that observed after TNFα treatment (Fig. 1). When using the 1168huIL6P-LUC NF-κBmut vector in which the NF-κB DNA binding site of the IL-6 promoter is mutated, no stimulation of LUC activity was observed neither by swine dust, nor by TNFα (Fig. 1). This shows that an intact NF-κB DNA binding site is necessary for organic dust induced transcription of the IL-6 promoter.

The dust induces reporter gene activity via NF-κB binding sites and induces IL-6 and IL-8 secretion from lung epithelial cells

The IL-6 reporter gene experiments indicated that a minimal NF-κB DNA binding site might be sufficient for transcriptional activation induced by organic dust. Cells transfected with a minimal heterologous promoter (tk) fused to the LUC reporter gene (tk-LUC) lacking the NF-κB binding site did not induce any LUC reporter activity following dust or TNFα stimulation (data not shown). In contrast, cells transfected with the 3xNF-κB(IC)tk-LUC gene reporter harboring minimal NF-κB DNA binding sites in front of the minimal tk promoter showed an increase in reporter gene activity following stimulation by both TNFα (data not shown) and swine dust. The dust induced a dose-dependent increase in NF-κB gene activity (Fig. 2a) in parallel with a dose-dependent increase of IL-6 and IL-8 release from A549 cells (Figs. 2b and c). At the highest dust concentration (400 μg/ml) IL-6 and IL-8 secretion was stimulated approximately 7-fold and the reporter gene activity was induced 3.5-fold.

Inhibition of the human IL-6 promoter gene or NF-κB activity prevents cellular responses to organic dust

In order to further investigate a NF-κB involvement in the inflammatory response induced by organic dust, we interfered with NF-κB activation by trapping NF-κB in the cytoplasm. Co-treatment of the A549 cells with PDTC, an agent which prevents NF-κB activation by inhibiting IκB degradation, inhibited swine dust-induced IL-6 and IL-8 secretion as well as the NF-κB reporter gene activity (Fig. 3). PDTC reduced IL-6 and IL-8 secretion and NF-κB reporter gene activity to levels even lower than observed in untreated cells. We also repressed the NF-κB signaling pathway by over-expressing the NF-κB inhibitory protein IκBα. The IL-6 promoter reporter gene activity and the NF-κB reporter gene activity was repressed in the TNFα stimulated cells following co-transfection with IκBα expression vector in a dose dependent way (Figs. 4 and 5, respectively). In cells stimulated with swine dust, increased levels of IκBα also abolished NF-κB-dependent reporter gene activities (Figs. 4 and 5).

Glucocorticoids repress swine dust-induced cytokine release and NF-κB reporter gene activity

Since glucocorticoids have been reported to repress NF-κB activated transcription, we tested this on dust stimulated expression of the NF-κB controlled reporter gene. The synthetic glucocorticoid FP repressed organic dust-induced NF-κB gene activity in a dose-dependent manner (Fig. 6). At 10⁻⁹ M,
fluticasone inhibited the dust-induced NF-κB reporter gene activity by 49% while dexamethasone at 10^{-6} M inhibited the NF-κB reporter gene activity by 40%. However, at the highest concentration of the glucocorticoid, dust-induced IL-6 and IL-8 release were inhibited to basal, pre-exposure levels. There was a relationship between the repression of NF-κB reporter gene activity and the repression of IL-6 and IL-8 secretion (Fig. 6). These experiments demonstrate that a minimal NF-κB DNA binding site is sufficient to drive transcriptional activation induced by organic dust in A549 cells and that glucocorticoids are partly able to repress this activity.

Swine dust induces robust NF-κB-binding and low levels of C/EBPβ-binding to their DNA response elements in vitro

The EMSA technique was used to test if swine dust induces DNA binding of NF-κB and C/EBPβ to their
enhancer elements. NE were prepared from untreated and dust incubated A549 cells (after 30 min of incubation with 200 μg swine dust/ml medium). As can be seen in Fig. 7A lane 2 a weak NF-κB binding is present in NE from untreated cells. This weak binding can be competed away by including a 50-fold excess of unlabeled NF-κB oligonucleotide (Fig. 7A lane 3) but not by 50 folds excess of unlabeled unrelated oligonucleotide (Fig. 7A lane 4). In contrast, robust NF-κB shift in NE from cells incubated with swine dust was observed (Fig. 7A lane 5). This shift is competed away by including a 50-fold excess of unlabeled NF-κB (Fig. 7A lane 6) but not by an unrelated unlabeled NF1
oligonucleotide (Fig. 7A lane 7). This demonstrates that the shifts observed are specific for the NF-κB oligonucleotide. To prove further that the observed shifts are composed of NF-κB family members antibodies directed against NF-κB1 (p50) and RelA (p65) were included in the binding reaction. Inclusion of the NF-κB1 and RelA antibodies resulted in super shifts (Fig. 7A lanes 8 and 9) that was not observed following inclusion of normal serum (Fig. 7A lane 10).

Asbestos fibers induce DNA binding by C/EBPβ in A549 cells has been reported. Therefore we tested if this also was the case in A549 cells exposed to swine dust. In NE from non-stimulated A549 cells we observed a C/EBPβ shift using the EMSA (Fig. 7B lane 2). In NE from swine dust exposed A549 cells C/EBPβ shifts were not observed (Fig. 7B lane 1). Inclusion of antibodies directed to NFκB1 (p50) and RelA (p65) as well as inclusion of normal serum was also used when indicated. The bands of super-shifts and free probe in the autoradigram are indicated by arrows. (B) EMSA using 32P labeled C/EBPβ oligonucleotide as probe. A 50-fold excess of unlabeled C/EBPβ and a 50-fold excess of un-labeled non-related NF1 oligonucleotide was included in the binding reactions when indicated. Incubation of the labeled C/EBPβ probe with nuclear extracts from over-expressing COS cells are also indicated. Arrows to the right of the autoradigraph indicate the levels of the C/EBPβ shifts and free probe, respectively.
exposed cells, this C/EBPκ DNA binding was weakly increased (Fig. 7B lane 3). The induced C/EBPκ DNA binding was competed away by including a 50-fold excess of unlabeled C/EBPκ oligonucleotide, but not by inclusion of a non-related unlabeled oligonucleotide (Fig. 7B lanes 4 and 5). In order to demonstrate that the shifts observed was due to C/EBPκ binding NE from C/EBPκ over-expressing COS cells was analyzed in EMSA. Inclusion of these extracts in the binding reaction resulted in a shift with the same mobility as the complex seen in A549 cells (Fig. 7B lane 6). Again inclusion of a 50 folds excess of unlabeled C/EBPκ oligonucleotide competed away the shift but not by inclusion of the unrelated unlabeled NFκκB oligonucleotide (Fig. 7B lanes 7 and 8).

These results demonstrate that dust incubation of the A549 cells results in a robust NFκκB DNA binding, which is composed of the NFκκB1 and RelA proteins. The results also demonstrate C/EBPκ DNA binding which is only weakly induced following dust incubation of the A549 cells.

Discussion

The NFκκB signaling cascade is activated by many stimuli such as inflammatory cytokines, bacterial LPS and UV irradiation. The results demonstrate for the first time a central role of the NFκκB pathway in the inflammatory response to organic dust. It is well known that exposure to organic dust, e.g., from swine confinement houses, induces an intense inflammatory airway reaction in previously unexposed healthy subjects.2,3 It is likely that airway epithelial cells participate in the inflammatory response to inhaled dust and previous in vitro studies have shown that dust from swine houses induces cytokine release from human epithelial cells.7 In the present study, we demonstrate that NFκκB is involved in the dust-induced inflammatory response of airway epithelial cells and that activation of this transcription factor mediates secretion of the pro-inflammatory cytokines IL-6 and IL-8. In addition, it was shown that a glucocorticoid inhibition of dust-induced cytokine release from human epithelial cells is at least in part mediated by inhibition of NFκκB activation.

The transcriptional activation of the IL-6 and IL-8 promoters by NFκκB has been described earlier.34,35 The activation of NFκκB and its coupling to IL-6 and IL-8 secretion in the inflammatory response to organic dust has in the present study been established by interfering in NFκκB signaling at several levels. First, the involvement of NFκκB was shown following transfection with an IL-6 promoter reporter gene. In contrast to the intact IL-6 promoter reporter gene a reporter gene construct lacking the IL-6 NFκκB site was unable to mediate organic dust-induced expression. Second, in cells transfected with a minimal heterologous promoter (tk) lacking the NFκκB binding site, dust did not induce the reporter gene. In contrast, in the reporter gene harboring minimal NFκκB DNA binding sites, there was an increase in gene activity in parallel with a increase of cytokine release following stimulation with dust. Third, co-incubation with PDTC concomitantly inhibited dust induced NFκκB reporter gene activity and cytokine release. The repression of IL-6 and IL-8 secretion following PDTC administration to lower levels than the basal secretion observed in non-stimulated cells can be explained by agents present in the medium/sera inducing NFκκB or due to endogenous constitutive active NFκκB in the A549 cells. It should be noted that PDTC is not a 100% specific NFκκB inhibitor although it has been commonly used for this purpose in a number of publications. Fourth, increasing the levels of 1α,25d-hydroxyvitamin D3 inhibited organic dust-induced IL-6 promoter reporter gene activity and NFκκB reporter gene activity. FP and dexamethasone inhibited NFκκB reporter gene activity in parallel with the inhibition of cytokine release. Fifth and finally, using the EMSA technique we demonstrated that swine dust incubation of the A549 cells resulted in induction of DNA binding of NFκκB to its response element in vitro. We also showed that this induced complex was composed of a heterodimer of RelA and NFκκB1. By using the EMSA technique we showed that the non-stimulated A549 contains high levels of constitutive DNA binding C/EBPκ. When the cells were incubated with swine dust this binding was marginally increased.

We have also shown that the synthetic glucocorticoid fluticasone could suppress, but not completely inhibit the organic dust-induced NFκκB reporter gene activity. With regard to NFκκB repression by the GR, this has been demonstrated to involve protein–protein interactions between the GR and NFκκB-complex.36,37 The current hypothesis is that NFκκB and GR interact on the DNA forming a transcriptional inert complex. The level of repression by glucocorticoids differs between experiments, depending on cell lines used and the expressed amount of GR. We have earlier shown repression levels by glucocorticoids from 50% to 95% of NFκκB activated transcription. In the present study we demonstrate that fluticasone at 10⁻⁹M inhibited the dust induced IL-6 and IL-8 release to basal levels which is consistent with our previous
studies. In another study we found that two weeks of fluticasone treatment of healthy individuals partially inhibited the IL-8 release in the upper airways following exposure to organic dust in a swine confinement building. However, in contrast to LPS, causes a massive influx of neutrophils into the LPS, exposure to organic dust in swine houses swine confinement houses. Similar to inhalation of response following inhalation of dust from inhalation of the most important factor causing the inflammatory observations contradicting endotoxin being the inflammatory stimulus and as a causative agent for dust is often claimed to be of importance as a pro-inflammatory stimulus of IL-8 release from A549 cells. These Dust at the same weight basis was much more dust is often claimed to be of importance as a pro-inflammatory stimulus and as a causative agent for dust is often claimed to be of importance as a pro-inflammatory stimulus and as a causative agent for the inflammatory responses following dust exposure. In addition, in contrast to swine house dust, LPS does not activate NF-κB in the lung epithelial A549 cells. In summary we have demonstrated that transcriptional activation of the IL-6 promoter by organic dust is dependent on an intact NF-κB binding site. When a minimal NF-κB binding site in a reporter gene construct was tested it was demonstrated to be both necessary and sufficient for organic dust-induced transcription. The addition of the chemical NF-κB inhibitory protein IkBz led to inhibition of organic dust-induced IL-6 promoter reporter gene activity and NF-κB reporter gene activity. The two synthetic glucocorticoids FP and dexamethasone inhibited organic dust-induced IL-6 and IL-8 secretion and organic dust-induced NF-κB reporter gene activity. Finally, we show that swine dust incubation of A549 cells results in a NF-κB DNA binding, which is composed of the NFκB1 and RelA proteins. All taken together, these results show a central role for NF-κB in the inflammatory response to organic dust and that the effect of glucocorticosteroids used in the treatment of inflammatory respiratory disorders, partly can be explained by their capability to inhibit the transcription factor NF-κB.

Acknowledgements

The IL-6 reporter gene constructs were a generous gift from Dr. G. Haegeman (Gent, Belgium). We thank Gary Faulds for suggestions and critical reading of this manuscript. We also wish to thank Siw Siljerud and Ingalill Rafter for excellent technical assistance. This work was supported by the Swedish Cancer Society, the Swedish Medical Research Council (No. 13X-2819), Alex and Eva Wallströms Foundation, Robert Lundbergs, Lars Hiertas Memorial Founds, GlaxoSmithKline and Swedish Heart and Lung Foundation.

References

Organic dust activates NF-κB in lung cells


