Eur Respir J 2011; 38: 1189–1199
DOI: 10.1183/09031936.00171110

TACE/TGF-α/EGFR regulates CXCL8 in bronchial epithelial cells exposed to particulate matter components

J. Øvrevik, M. Refsnes, A.I. Totlandsdal, J.A. Holme, P.E. Schwarze and M. Låg

ABSTRACT: Airborne particulate matter (PM) may induce or exacerbate neutrophilic airway disease by triggering the release of inflammatory mediators, such as CXC chemokine ligand (CXCL)8, from the airway epithelium. It is still unclear which PM components are driving CXCL8 responses, as most candidates occur at low concentrations in the dusts. We therefore hypothesised that different PM constituents may contribute through common mechanisms to induce CXCL8.

Human bronchial epithelial cells (BEAS-2B) were exposed to different PM components (Zn^{2+}/Fe^{2+} salts, 1-nitropyrene, lipopolysaccharide and diesel exhaust/mineral particles). Gene expression patterns were detected by real-time PCR array. CXCL8 responses were measured by real-time PCR and ELISA. CXCL8 regulation was assessed with a broad inhibitor panel and neutralising antibodies. Epidermal growth factor receptor (EGFR) phosphorylation was examined by immunoprecipitation and Western blotting.

Component-induced gene expression was mainly linked to nuclear factor-κB, Ca^{2+}/protein kinase C, phospholipase C, low-density lipoprotein and mitogenic signalling. Many inhibitors attenuated CXCL8 release induced by all PM components, but to varying extents. However, EGFR inhibition strongly reduced CXCL8 release induced by all test compounds and selected compounds increased EGFR phosphorylation. Interference with transforming growth factor (TGF)-α or tumour necrosis factor-α-converting enzyme (TACE), which mediates TGF-α ectodomain shedding, also attenuated CXCL8 release.

Different PM constituents induced CXCL8 partly through similar signalling pathways but the relative importance of the different pathways varied. However, TACE/TGF-α/EGFR signalling appears to be a convergent pathway regulating innate immune responses of airway epithelial cells upon exposure to multiple airborne pollutants.

KEYWORDS: Air pollution, chemokines, inflammation, interleukin-8, intracellular signal transduction, lung diseases

Airborne particulate matter (PM) may induce or exacerbate airway diseases through pulmonary inflammatory reactions. The airway epithelium plays a key role in these processes by regulating innate immune responses upon inhalation of noxious compounds [1–3]. Epithelial cells express a number of pattern-recognition receptors, including Toll-like receptors (TLRs), which recognise conserved pathogen motifs and trigger host responses mediated by various effectors, such as cytokines and chemokines [4]. We have recently shown that increased levels of the neutrophil-selective CXC chemokine ligand (CXCL)8/interleukin (IL)-8 is a dominating pro-inflammatory response to PM and PM components in bronchial epithelial BEAS-2B cells [5–7]. Markedly elevated CXCL8 levels, along with neutrophilia, is also characteristic of airway diseases such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and severe asthma [3, 8, 9]. The importance of neutrophils in pulmonary diseases has gained increased attention as neutrophilic inflammation appears to be resistant to corticosteroid treatment, and CXCL8 is therefore considered a potential therapeutic target [9, 10]. CXCL8 may also contribute to cancer development through its pro-inflammatory effects along with its potential role as a mitogenic, angiogenic and motogenic factor [11].

Multiple signalling pathways contribute in CXCL8 regulation. In most cell types, its transcription depends on nuclear factor (NF)-κB activation,

AFFILIATIONS
Dept of Air Pollution and Noise, Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway.

CORRESPONDENCE
J. Øvrevik
Dept of Air Pollution and Noise
Division of Environmental Medicine
Norwegian Institute of Public Health
P.O. Box 4404
Nydalen
N-0403 Oslo
Norway
E-mail: johan.ovrevik@fhi.no

Received:
Nov 03 2010
Accepted after revision:
April 03 2011
First published online:
May 03 2011

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

while activator protein (AP)-1 and CCAAT/enhancer-binding protein are required for maximal gene expression [12]. Maximal CXCL8 responses further require a combined activation of the three major mitogen-activated protein kinase (MAPK) cascades: extracellular signal-regulated kinase (ERK)1/2, c-Jun N-terminal kinase (JNK) and p38 [13, 14]. A range of studies also suggests the involvement of the epidermal growth factor receptor (EGFR) in CXCL8 responses induced by different agents [15–21], and EGFR and CXCL8 expression correlate in the airway epithelium of patients with CF and severe asthma [22, 23]. Accordingly, EGFR signalling has been suggested as a convergent pathway for regulation of CXCL8 and other immune responses [23]. Recent studies with endogenous and microbial ligands have shown that CXCL8 regulation by EGFR involves transforming growth factor (TGF)-α ectodomain shedding by the metalloprotease tumour necrosis factor-α-converting enzyme (TACE)/ADAM-17 [20, 21, 24, 25]. Whether organic or inorganic PM components regulate CXCL8 through similar mechanisms remains to be elucidated.

Airborne PM is a complex, variable mixture containing some minor components (by mass) with considerable gene-regulatory potential. These include endotoxins, metals, organic compounds such as polycyclic aromatic hydrocarbons, and ultrafine combustion-derived particles [26]. Clarifying which constituents of PM cause the adverse pulmonary effects is important in the development of more effective abatement strategies to improve outdoor air quality, while elucidating the underlying mechanisms, of these effects may improve treatment of sensitive groups. A particular puzzle is that PM from different locations has shown surprisingly little variation in risk estimates, despite considerable variation in the composition of the dust [26]. We hypothesised that different PM components contribute *via* common mechanisms to induce CXCL8 responses in airway epithelial cells. If several components act through a similar mechanism, this could explain why PM may elicit effects despite low concentrations of its individual active constituents, and also why variation in PM composition does not necessarily lead to considerable variation in effects. To assess this, we have investigated CXCL8 regulation in BEAS-2B cells. Crystalline silica, Zn^{2+} and Fe^{2+} salts, 1-nitropyrene (1-NP) and bacterial lipopolysaccharide (LPS) were chosen to model different components of PM [5]. Comparative experiments with diesel exhaust particles (DEPs) were also performed.

MATERIALS AND METHODS

Reagents

Crystalline silica (MIN-U-SIL® 5) was kindly provided by the US Silica Company (Frederick, MD, USA). DEPs were kindly provided by F.R. Cassee (National Institute for Public Health and the Environment, Bilthoven, the Netherlands). $ZnCl_2$, $FeSO_4$, 1-NP, LPS and 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM) were from Sigma-Aldrich (St Louis, MO, USA). SB202190, PD98059, SP600125, protein phosphatase 2, AG1478, Gö6976, rottlerin, GF109203, H-8 wortmannin, AG490, verapamil, W7, curcumin, tumour necrosis factor-α protease inhibitor (TAPI)-1 and TGF-α neutralising antibody were all from Calbiochem (La Jolla, CA, USA). EGFR neutralising antibody (LA1) was from Millipore (Billerica, MA, USA). All other chemicals used were purchased from commercial sources at the highest purity available.

Cell cultures and exposures

BEAS-2B cells, an adenovirus 12–simian virus 40 hybrid-transformed human bronchial epithelial cell line, were purchased from American Tissue Type Culture Collection (Rockville, MD, USA) and grown to near-confluence in serum-free LHC-9 medium (Biosource, Camarillo, CA, USA) at 37°C in a humidified atmosphere of 5% CO_2 in air, prior to exposure. Concentrations and incubation times for both the test compounds and inhibitors/neutralising antibodies were based on previous studies with BEAS-2B or other epithelial lung cells [5, 7, 18, 19, 27–31], or initial screenings in the BEAS-2B cells (data not shown).

Gene expression

Total RNA was isolated using Absolutely RNA Miniprep Kits (Stratagene, La Jolla, CA, USA), and reverse transcribed to cDNA on a PCR System 2400 (PerkinElmer, Waltham, MA, USA) using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). Gene expression was screened by use of the Signal Transduction PathwayFinder real-time PCR array (SABioscience, Frederick, MD, USA), run on an ABI 7500 Fast (Applied Biosystems) and normalised against average expression housekeeping genes (*B2M*, *HPRT1*, *RPL13A*, *GAPDH* and *ACTB*). Results were expressed as a fold change compared with the untreated control. Genes were grouped under signalling pathways according to suggestions by the manufacturer (SABioscience). DEP-induced CXCL8 expression was measured using pre-designed TaqMan Gene Expression Assays and the TaqMan Universal PCR Master Mix (Applied Biosystems). CXCL8 expression (Hs00174103_m1) was normalised against 18S ribosomal RNA (Hs99999901_s1).

CXCL8 release

CXCL8 protein levels in cell supernatants were determined by ELISA (Biosource) as described previously [5]. Absorbance was measured using a plate reader (TECAN Sunrise; Phoenix Research Products, Hayward, CA, USA) and Magellan software (version 1.10; Phoenix Research Products).

Immunoprecipitation

EGFR was immunoprecipitated as described previously [18]. Briefly, cells were washed twice in ice-cold PBS, once in ice-cold immunoprecipitation (IP) buffer and lysed in IP buffer with 1% NP-40. Cell lysates were centrifuged at $2,500 \times g$ for 10 min, incubated with anti-EGFR antibodies (Cell Signalling Technology, Beverly, MA, USA) for 1 h and incubated overnight with magnetic beads. The complexes were washed in IP buffer containing 0.5% NP-40 and immunoprecipitates were released by boiling in 2× Laemmli buffer for 5 min. EGFR phosphorylation was detected by Western blotting with anti-phosphotyrosine antibodies (P-Tyr-100; Cell Signalling Technology).

Statistical analysis

Statistical significance was evaluated using Prism (GraphPad Software Inc., San Diego, CA, USA), using ANOVA with a Bonferroni post-test.

RESULTS

Component-induced gene expression profiles

To compare effects of crystalline silica particles, Zn^{2+}, Fe^{2+}, 1-NP and LPS on cell signalling pathways in BEAS-2B cells, changes in

the expression of 84 genes were examined. As shown in figure 1, 26 genes were upregulated more than two-fold after exposure to at least one of the compounds, while 12 were downregulated more than two-fold, compared with controls. The results revealed a considerable quantitative variation, with 1-NP and silica generally inducing the strongest responses, and some qualitative variation in the types of genes activated. In agreement with previous findings [5], CXCL8 was among the most responsive genes. In addition, all compounds induced a marked upregulation of CC chemokine ligand (CCL)20 (macrophage inflammatory protein-3α), tumour necrosis factor (TNF)-α, colony-stimulating factor 2 (granulocyte-macrophage colony-stimulating factor (GM-CSF)), c-Jun (a major AP-1 subunit) and the antiapoptotic gene *BCL2A1* (less reliable due to low gene expression levels in most samples). Since the results are based on examination of effects of only one concentration at one time-point, some caution should be taken when interpreting the mRNA data. However, the relative effects on CXCL8, CCL20 and TNF-α are comparable to previous findings [5].

As a first step to identify intracellular signalling pathways induced by the test compounds, the 84 genes were clustered according to common upstream regulation mechanisms. As shown in table 1, the different exposures predominantly induced upregulation of genes linked to the NF-κB, Ca^{2+}/protein kinase (PK)C, phospholipase (PL)C, low-density lipoprotein (LDL) and mitogenic pathways.

Screening signalling pathways involved in CXCL8 regulation

All test compounds also induced a significant increase in CXCL8 protein release (fig. 2). To further examine the mechanisms of CXCL8 regulation, BEAS-2B cells were exposed to the different test compounds in combination with a battery of 15 inhibitors, targeting various signalling pathways (table 2).

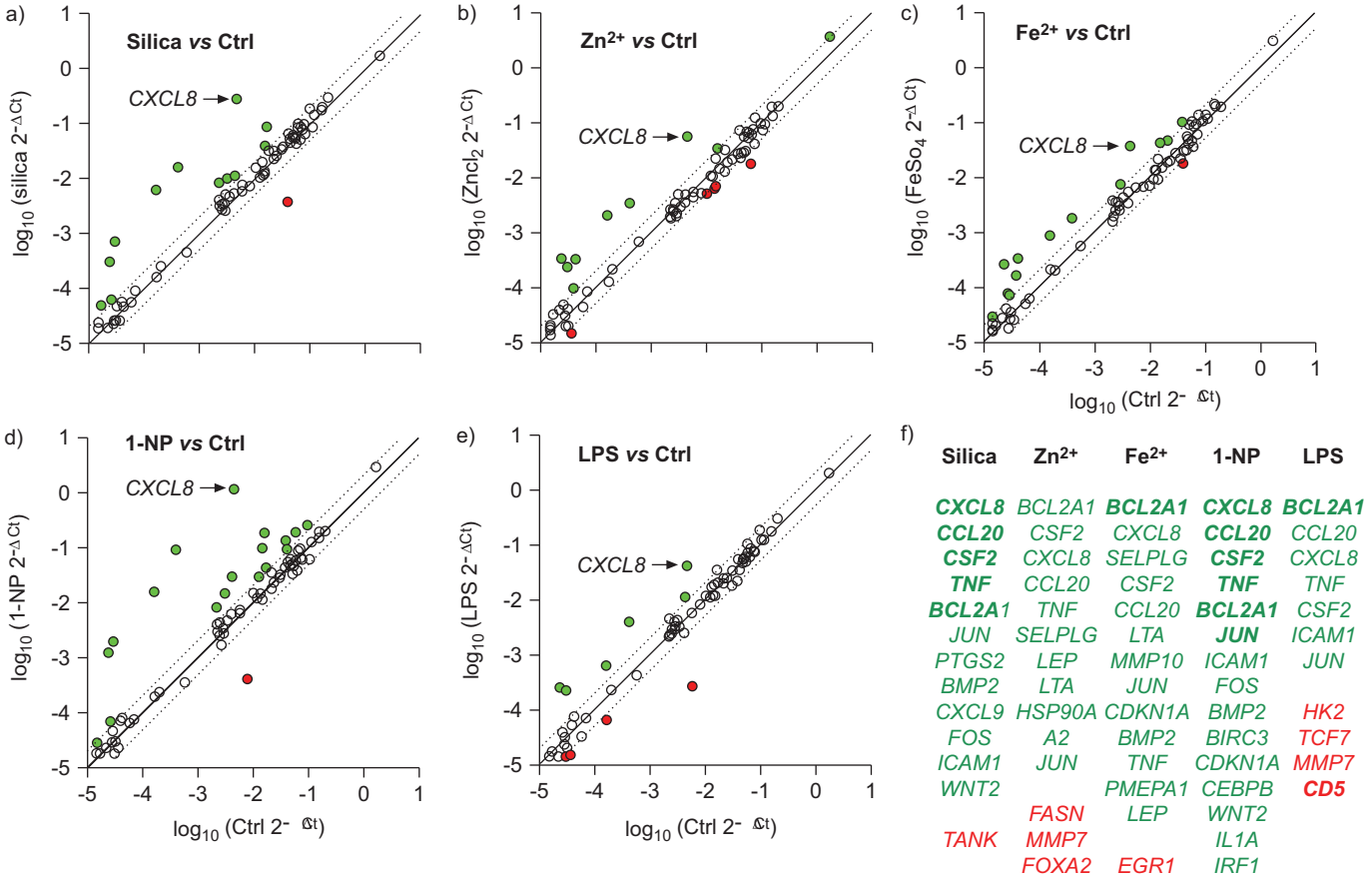

FIGURE 1. Scatter plots of gene expression induced by different particulate matter components in human bronchial epithelial cells (BEAS-2B). The cell cultures were incubated for 10 h with a) crystalline silica (40 μg·cm^{-2}), b) $ZnCl_2$ (90 μM), c) $FeSO_4$ (250 μM), d) 1-nitropyrene (1-NP) (30 μM) or e) lipopolysaccharide (LPS) (10 μg·mL^{-1}). Changes in mRNA levels of 84 genes were measured by real-time PCR array as described in the Materials and Methods section. The results are expressed as $\log_{10}(2^{-\Delta Ct})$ where -ΔCt is the cycle time for each gene after adjustment for the average cycle threshold (Ct) of housekeeping genes, and distance from the solid line represents the $\log_{10}$ value of fold-change compared with the untreated control (Ctrl). ·····: two-fold change; green: genes upregulated more than two-fold; red: genes downregulated more than two-fold. The data represent means of two independent experiments. f) Table of the genes up- (green) or downregulated (red) more than two-fold by the different test compounds. Bold indicates >10-fold change expression compared with control.

TABLE 1 Real-time PCR array of 84 genes representative of 18 different signalling pathways

Pathway	Genes	Exposure				
		Silica	$ZnCl_2$	$FeSO_4$	1-NP	LPS
Mitogenic	***EGR1***	-	-	↓	↑	-
	FOS	↑	-	-	↑	-
	JUN	↑	↑	↑	↑↑	↑
Wnt	*CND1C*	-	-	-	-	-
	JUN	↑	↑	↑	↑↑	↑
	LEF1	-	-	-	-	-
	MYC	-	-	-	-	-
	PPARG	-	-	-	-	-
	TCF7	-	-	-	-	↓#
	VEGFA	-	-	-	-	-
	WISP1	-	-	-	-	-
Hedgehog	*BMP2*	↑	-	-	↑	-
	BMP4	-	-	-	-	-
	EN1	-	-	-	-	-
	FOXA2	-	↓	-	-	-
	PTCH1	-	-	-	-	-
	WNT1	-	-	-	-	-
	WNT2	↑#	-	-	↑#	-
TGF-β	***CDKN1A***	-	-	↑	↑	-
	CDKN1B	-	↓	-	-	-
	CDKN2A	-	-	-	-	-
	CDKN2B	-	↓	-	-	-
PI3K/Akt	***BLC2***	-	-	-	-	-
	CCND1	-	-	-	-	-
	FN1	-	-	-	-	-
	JUN	↑	↑	↑	↑↑	↑
	MMP7	-	↓#	-	-	↓#
	MYC	-	-	-	-	-
JAK/Src	***BCL2***	-	-	-	-	-
	BCL2L1	-	-	-	-	-
JAK/STAT	*CXCL9*	↑#	-	-	-	-
	IL4	-	-	-	-	-
	IL4R	-	-	-	-	-
	IRF1	-	-	-	↑	-
	MMP10	-	-	-	-	-
	NOS2A	-	-	-	-	-
Ca^{2+}/PKC	***CSF2***	↑↑¶	↑↑¶	↑¶	↑↑¶	↑¶
	FOS	↑	-	-	↑	-
	ICAM1	↑	-	-	↑	↑
	JUN	↑	↑	↑	↑↑	↑
	MYC	-	-	-	-	-
	ODC1	-	-	-	-	-
	PRKCA	-	-	-	-	-
	PRKCE	-	-	-	-	-
	TERT	-	-	-	-	-
PLC	***BCL2***	-	-	-	-	-
	EGR1	-	-	↓	↑	-
	FOS	↑	-	-	↑	-
	ICAM1	↑	-	-	↑	↑
	JUN	↑	↑	↑	↑↑	↑
	NOS2A	-	-	-	-	-
	PTGS2	↑	-	-	-	-
	VCAM1	-	-	-	-	-

TABLE 1 Continued

Pathway	Genes	Exposure				
		Silica	$ZnCl_2$	$FeSO_4$	1-NP	LPS
NFAT	*CD5*	-	-	-	-	↓#
	FASLG	-	-	-	-	-
	IL2	-	-	-	-	-
NF-κB	*BCL2A1*	↑↑#	↑↑#	↑↑#	↑↑¶	↑↑#
	BIRC2	-	-	-	-	-
	BIRC3	-	-	-	↑	-
	NAIP	-	-	-	-	-
	TERT	-	-	-	-	-
	CCL20	↑↑¶	↑¶	↑¶	↑↑¶	↑¶
	ICAM1	↑	-	-	↑	↑
	IKBKB	-	↓	-	-	-
	IL1A	-	-	-	↑	-
	IL2	-	-	-	-	-
	IL8	↑↑	↑↑	↑	↑↑	↑
	LTA	-	↑¶	↑#	-	-
	NFKB1	-	-	-	↑	-
	NOS2A	-	-	-	-	-
	PECAM1	-	-	-	-	-
	TANK	↓	-	-	-	-
	TNF	↑↑¶	↑¶	↑#	↑↑¶	↑#
	VCAM1	-	-	-	-	-
p53	*BAX*	-	-	-	-	-
	CDKN1A	-	-	↑	↑	-
	FAS	-	-	-	-	-
	GADD45A	-	-	-	-	-
	IGFBP3	-	-	-	-	-
	MDM2	-	-	-	-	-
	TP53I3	-	-	-	-	-
CREB	*CYP19A1*	-	-	-	-	-
	EGR1	-	-	↓	↑	-
	FOS	↑	-	-	↑	-
Oestrogen	***BCL2***	-	-	-	-	-
	BRCA1	-	-	-	↓	-
	GREB1	-	-	-	-	-
	NRIP1	-	-	-	-	-
Androgen	*CDK2*	-	-	-	-	-
	CDKN1A	-	-	↑	↑	-
	KLK2	-	-	-	-	-
	PMEPA1	-	-	↑	-	-
Insulin	*CEBP*	-	-	-	↑	-
	FASN	-	↓	-	-	-
	GYS1	-	-	-	-	-
	HK2	-	-	-	-	↓
	LEP	-	↑#	↑#	-	-
LDL	*CCL2*	-	-	-	-	-
	CSF2	↑↑#	↑↑¶	↑¶	↑↑¶	↑¶
	SELE	-	-	-	↑#	-
	SELPG	-	↑#	↑#	-	-
	VCAM1	-	-	-	-	-
Retinoic acid	*EN1*	-	-	-	-	-
	HOXA1	-	-	-	-	-
	RBP1	-	-	-	-	-

TABLE 1 Continued

Pathway	Genes	Exposure				
		Silica	$ZnCl_2$	$FeSO_4$	1-NP	LPS
Stress	*ATF2*	-	-	-	-	-
	FOS	↑	-	-	↑	-
	HSF1	-	-	-	-	-
	HSPB1	-	-	-	-	-
	HSP90AA2	-	↑	-	-	-
	MYC	-	-	-	-	-
	TP53	-	-	-	-	-

Real-time PCR was performed on BEAS-2B cells exposed for 10 h to crystalline silica (40 μg·cm^{-2}), $ZnCl_2$ (90 μM), $FeSO_4$ (250 μM), 1-nitropyrene (1-NP) (30 μM) or lipopolysaccharide (LPS) (10 μg·mL^{-1}). Bold indicates genes that may be regulated through several signalling pathways and are therefore repeated in the table. TGF: transforming growth factor; PI3K: phosphoinositide 3-kinase; JAK: Janus kinase; STAT: signal transducer and activator of transcription; PK: protein kinase; PL: phospholipase; NFAT: nuclear factor of activated T-cells; NF: nuclear factor; CREB: cyclic adenosine monophosphate response binding element; LDL: low-density lipoprotein; -: unchanged; ↑: upregulated more than two-fold; ↑↑: upregulated >10-fold; ↓: downregulated more than two-fold. #: gene expression in control and exposed samples were low, suggesting that the estimated fold-change result may have greater variation; ¶: gene expression in control samples was low, suggesting that the actual fold-change value is at least as large as the calculated result.

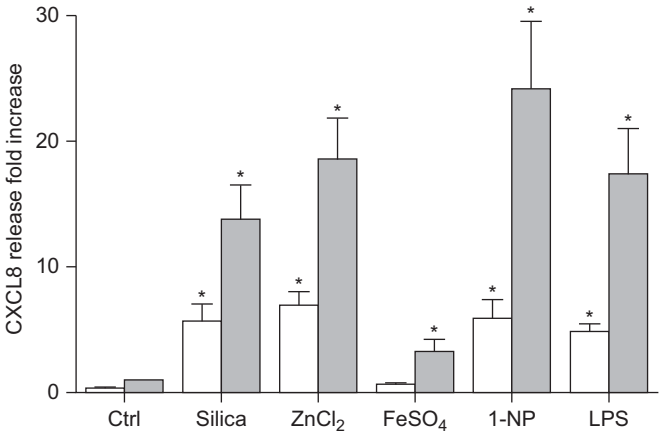

FIGURE 2. Induction of CXC chemokine ligand (CXCL)8 release after 10 h (□) and 20 h (■) exposure to different particulate matter components in human bronchial epithelial cells (BEAS-2B). The cell cultures were incubated with crystalline silica (40 μg·cm^{-2}), $ZnCl_2$ (90 μM), $FeSO_4$ (250 μM), 1-nitropyrene (1-NP) (30 μM) or lipopolysaccharide (LPS) (10 μg·mL^{-1}). Supernatants were harvested and analysed for CXCL8 release by ELISA, as described in the Materials and Methods section. Data are presented as relative increase (fold) compared with the untreated control (Ctrl). Data are presented as mean±SEM of three or more independent experiments. *: $p<0.05$ compared with unexposed control.

Despite some differences, the inhibitor screen also revealed apparent similarities: CXCL8 release induced by all compounds was significantly attenuated by a general PKC inhibitor. Moreover, the PKCδ/θ inhibitor rottlerin appeared to reduce

Generally, the inhibitors did not seem to affect cell viability, as judged by microscopy (decrease in cell density and increase in rounded/floating cells), except the Ca^{2+} chelator BAPTA-AM and the Janus kinase (JAK) inhibitor AG490, which had some toxicity (not quantified). Furthermore, since the screening was based on single concentrations and one incubation time, the results should be interpreted with some caution.

Whereas the gene expression data indicated activation of Ca^{2+}/PKC and mitogenic signalling, the inhibitor screen further suggested that these pathways were involved in CXCL8 regulation (fig. 3). Overall, the results showed that many of the same inhibitors reduced CXCL8 release induced by different test compounds, but that the relative effects varied considerably (fig. 3). Inhibitors of the three main MAPK pathways were generally effective inhibitors of CXCL8 release. However, whereas p38 inhibition significantly attenuated CXCL8 expression induced by silica, Zn^{2+}, Fe^{2+} and 1-NP, only marginal effects were observed for LPS. Moreover, ERK and JNK inhibition significantly reduced the effects of Fe^{2+}, 1-NP and LPS, but did not affect silica- and Zn^{2+}-induced responses significantly. The PKCα/β-inhibitor increased background levels of CXCL8 in controls and boosted the effects of Zn^{2+} and LPS. Similarly, the JAK2/3 inhibitor increased background levels of CXCL8, suggesting that both PKCα/β and JAK2/3 under normal conditions suppress CXCL8-release in BEAS-2B cells. However, both PKCα/β and JAK2/3 inhibitors attenuated CXCL8-induction by 1-NP. Whether this may be due to nonspecific effects of the inhibitors or linked to the ability of 1-NP to induce CXCL8 remains to be clarified.

TABLE 2 Pharmacological inhibitors used to screen for mechanisms of CXC chemokine ligand 8 regulation

Inhibitor	Concentration μM	Target
SB202190	5	p38
PD98059	10	MEK1/2 (ERK1/2)
SP600125	10	JNK
PP2	5	Src-family kinases
AG1478	5	EGFR
Gö6976	3	PKCα/β_1
Rottlerin	3	PKCδ/θ
GF109203X	3	PKC (general)
H-8	50	PKA
Wortmannin	0.1	PI3K
AG490	10	JAK2/3
Verapamil	100	Ca^{2+} channels
BAPTA-AM	20	Ca^{2+} chelator
W-7	25	Calmodulin (Ca^{2+})
Curcumin	10	Multiple targets including NF-κB and AP-1

PP: protein phosphatase; BAPTA-AM: 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′*N*′-tetraacetic acid tetrakis (acetoxymethyl ester); ERK: extracellular signal regulated-kinase; JNK: c-Jun N-terminal kinase; EGFR: epidermal growth factor receptor; PK: protein kinase; PI3K: phosphoinositide 3-kinase; JAK: Janus kinase; NF: nuclear factor; AP: activator protein.

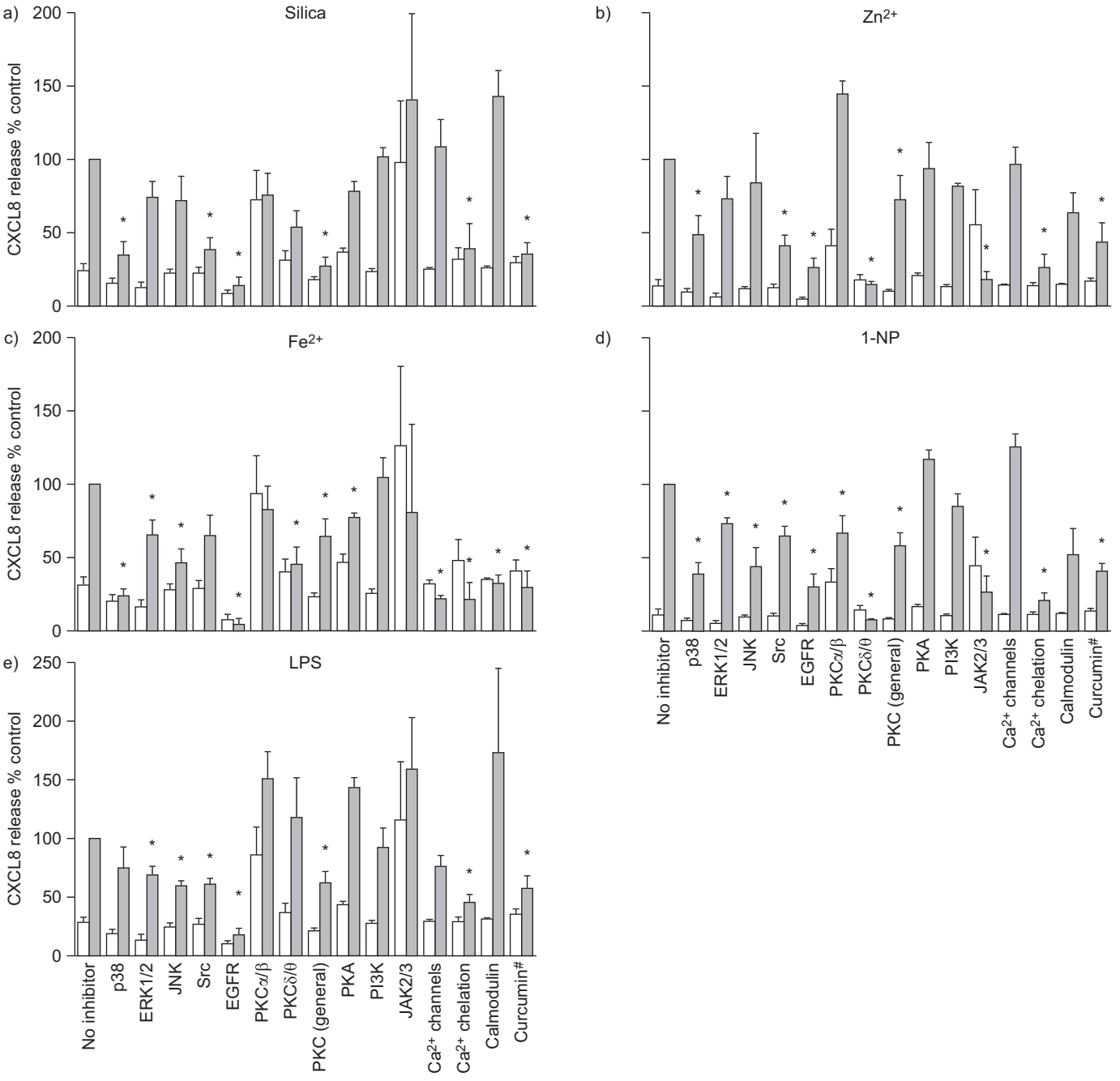

FIGURE 3. Inhibitor screen to identify pathways involved in CXC chemokine ligand (CXCL)8 release induced by different particulate matter (PM) components in human bronchial epithelial cells (BEAS-2B). The cell cultures were pre-treated with a panel of 15 different inhibitors targeting different signalling pathways for 1 h prior to 20 h incubation with a) crystalline silica (40 μg·cm^{-2}), b) $ZnCl_2$ (90 μM), c) $FeSO_4$ (250 μM), d) 1-nitropyrene (1-NP) (30 μM) or e) lipopolysaccharide (LPS) (10 μg·mL^{-1}). The inhibitors used are given in table 2. Supernatants were harvested and analysed for CXCL8 release by ELISA, as described in the Materials and Methods section. The results are expressed as relative CXCL8 release compared with PM component-exposed cells in the absence of the inhibitor (100% response). Data are presented as mean±SEM (n=3–6). □: control; ■: exposed to PM component. ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; EGFR: epidermal growth factor receptor; PK: protein kinase; PI3K: phosphoinositide 3-kinase; JAK: Janus kinase. #: targets multiple pathways. *: $p<0.05$ compared with control.

CXCL8 levels induced by all test compounds except LPS. Another general observation was that the intracellular Ca^{2+} chelator BAPTA-AM more or less blocked CXCL8 release by all compounds, although only Fe^{2+}-induced CXCL8 was reduced by inhibitors of Ca^{2+} channels and calmodulin (fig. 3). Some caution should, however, be taken regarding the effect of BAPTA-AM, since it seemed to increase cytotoxicity (data not shown). The anti-inflammatory compound curcumin, which affects multiple intracellular targets, including NF-κB and AP-1 [12, 32, 33], also displayed a broad CXCL8-inhibitory potential. Notably, the EGFR inhibitor AG1478 more or less completely blocked the effects of silica, Fe^{2+} and LPS on CXCL8, and strongly downregulated the

effects of Zn^{2+} and 1-NP (fig. 3), suggesting that EGFR is a central regulator of CXCL8 in BEAS-2B cells.

Role of the TACE/TGF-α/EGFR pathway in CXCL8 regulation

The role of EGFR in CXCL8 regulation was further assessed by use of an EGFR-neutralising antibody and EGFR phosphorylation was examined by immunoprecipitation (fig. 4). Blocking EGFR ligand binding reduced CXCL8 induction by all PM components. Moreover, selected test compounds (silica and Zn^{2+}) also induced EGFR phosphorylation (fig. 4). Thus, the role of EGFR in CXCL8 regulation appeared to involve ligand binding and receptor activation. Based on this, we hypothesised that the TACE-mediated cleavage of pro-TGF-α could be a common mechanism of CXCL8 regulation by PM components. Accordingly, the TACE inhibitor TAPI-1 and a TGF-α-neutralising antibody both attenuated CXCL8 release induced by all test compounds, except Fe^{2+} (fig. 5). The lack of effect of TGF-α neutralisation on Fe^{2+}-induced CXCL8 is surprising, given the effects of AG1478, TAPI-1 and EGFR neutralisation. It is possible that Fe^{2+} may elicit effects through TACE-mediated cleavage of other EGFR ligands, such as amphiregulin.

These results suggest that multiple model compounds of common PM constituents stimulate CXCL8 release in BEAS-2B cells through activation of the TACE/TGF-α/EGFR cascade. To explore whether more complex particles could elicit effects through similar mechanisms, cells were treated with DEPs in absence or presence of AG1478 and TAPI-1. Since DEPs may bind CXCL8 protein and, thereby, interfere with ELISA assays [34], CXCL8 responses were measured at the mRNA level. In accordance with the effects on responses to the model compounds, both EGFR and TACE inhibitors attenuated DEP-induced CXCL8 expression significantly (fig. 6).

DISCUSSION

Innate immune responses of the airway epithelium are important in host defence against inhaled pollutants and pathogens, but inflammatory mediators from epithelial cells may also promote development and exacerbation of airway disease [1, 2, 35]. Previous exploration of cytokine/chemokine expression induced by the different PM components in BEAS-2B cells revealed that crystalline silica, Zn^{2+}, Fe^{2+}, 1-NP and LPS induced expression of at least one of the neutrophil-specific chemokines, CXCL1,

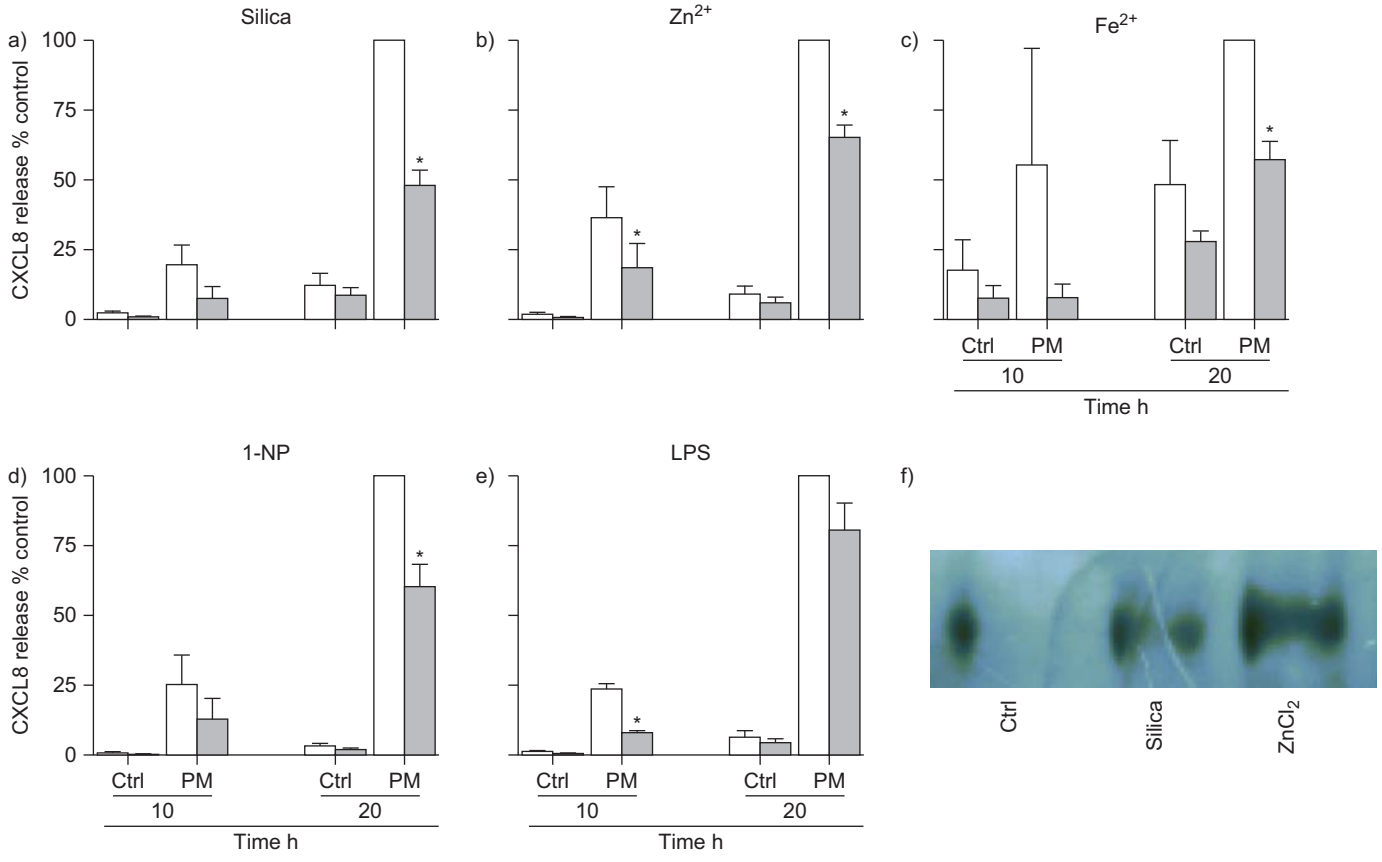

FIGURE 4. Involvement of epidermal growth factor receptor (EGFR) ligand binding in particulate matter (PM) component-induced CXC chemokine ligand (CXCL)8 release from human bronchial epithelial cells (BEAS-2B). The cell cultures were pre-treated with an anti-EGFR neutralising antibody for 1 h prior to 20 h incubation with a) crystalline silica (40 μg·cm^{-2}), b) $ZnCl_2$ (90 μM), c) $FeSO_4$ (250 μM), d) 1-nitropyrene (1-NP) (30 μM) or e) lipopolysaccharide (LPS) (10 μg·mL^{-1}). Supernatants were harvested and analysed for CXCL8 release by ELISA, as described in the Materials and Methods section. The results are expressed as relative CXCL8 induction compared with exposed cells in the absence of neutralising antibody at 20 h (100% response). Data are presented as mean±SEM (n=3). □: no inhibitor; ■: anti-EGFR (10 μg·mL^{-1}). *: $p<0.05$ compared with control (Ctrl). f) Effect of two selected test compounds on EGFR phosphorylation. BEAS-2B cells were incubated with crystalline silica (40 μg·cm^{-2}) or $ZnCl_2$ (90 μM) for 2 h prior to immunoprecipitation of the EGFR, as described in the Materials and Methods section. The precipitated proteins were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis and Western blots were incubated with an antibody against phosphotyrosine to investigate EGFR activation (n=2).

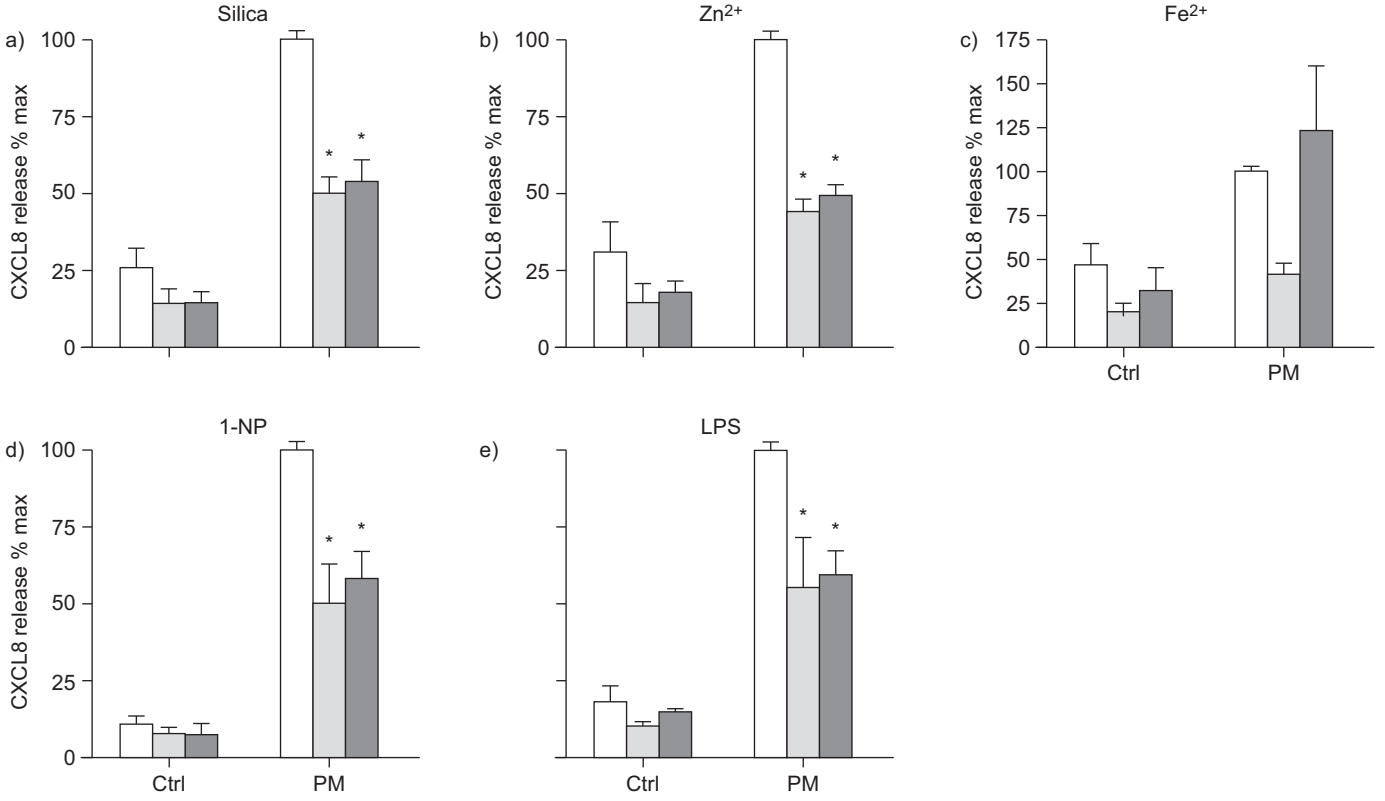

FIGURE 5. Involvement of tumour necrosis factor-α-converting enzyme (TACE) and transforming growth factor (TGF)-α in particulate matter (PM) component-induced CXC chemokine ligand (CXCL)8-release from human bronchial epithelial cells (BEAS-2B). The cell cultures were pre-treated with the TACE inhibitor tumour necrosis factor-α protease inhibitor (TAPI)-1 or a TGF-α-neutralising antibody for 1 h prior to 20 h incubation with a) crystalline silica (40 μg·cm^{-2}), b) $ZnCl_2$ (90 μM), c) $FeSO_4$ (250 μM), d) 1-nitropyrene (1-NP) (30 μM) or e) lipopolysaccharide (LPS) (10 μg·mL^{-1}). Supernatants were harvested and analysed for CXCL8 release by ELISA, as described in the Materials and Methods section. The results are expressed as relative CXCL8 induction compared with exposed cells in the absence of inhibitor or neutralising antibody (100% response). Data are presented as mean±SEM (n=3). □: no inhibitor; ▫: TAPI-1; ■: TGF-α-neutralising antibody. *: $p<0.05$ compared with control (Ctrl).

CXCL3, CXCL5 and CXCL8. Of these, only CXCL8 was induced by all agents and was among the most strongly upregulated on average [5]. Given its central role in neutrophilic airway disease [9, 10, 36], the present study was initiated to explore the regulation of CXCL8 in BEAS-2B cells upon exposure to PM components.

The selected PM components mainly induced gene expression linked to the NF-κB, Ca^{2+}/PKC, PLC, LDL and/or mitogenic signalling pathways. The generally strong effects on CXCL8 corroborated the available data suggesting that CXCL8 expression is a highly important epithelial response to inhaled

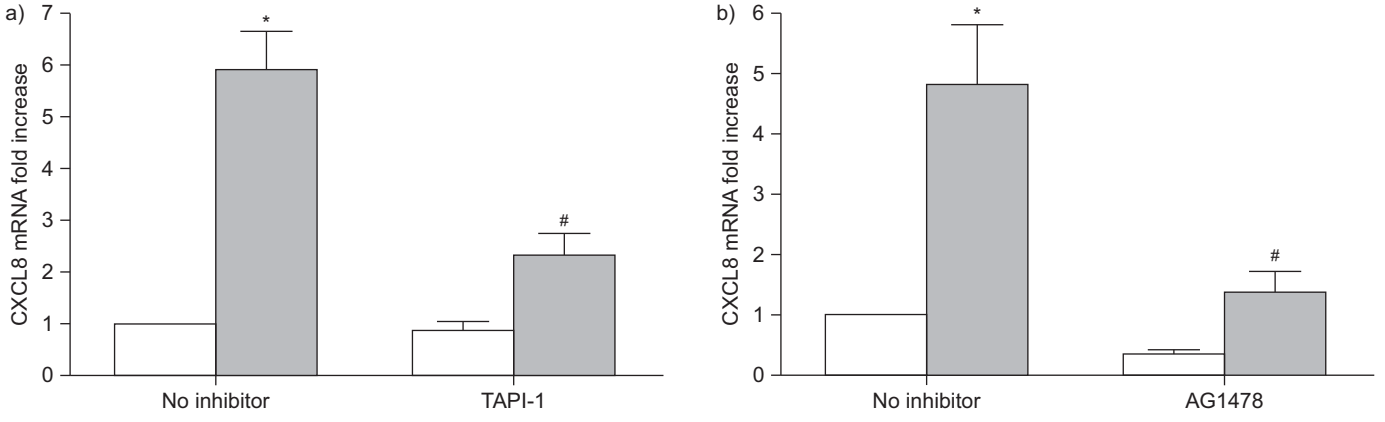

FIGURE 6. Involvement of tumour necrosis factor-α-converting enzyme (TACE) and epidermal growth factor receptor (EGFR) in diesel exhaust particle (DEP)-induced CXC chemokine ligand (CXCL)8 expression in human bronchial epithelial cells (BEAS-2B). The cell cultures were pre-treated with a) the TACE inhibitor tumour necrosis factor-α protease inhibitor-1 (TAPI-1) or b) the EGFR inhibitor AG1478 for 1 h prior to 4 h incubation with DEPs (100 μg·mL^{-1}). Changes in CXCL8 mRNA levels were measured by real-time PCR as described in the Materials and Methods section. The results are expressed as relative CXCL8 induction (fold) compared with unexposed controls after normalisation against 18S ribosomal RNA. Data are presented as mean±SEM (n=3–5). □: control; ■: DEPs. *: $p<0.05$ compared with control; #: $p<0.05$ compared with no inhibitor.

air pollutants, such as PM. We also found marked effects on GM-CSF, which prevents neutrophil apoptosis [37, 38]. Thus, PM components may trigger expression of not only epithelium-derived factors activating and recruiting neutrophils, but also of factors promoting sustained neutrophil survival in the lung.

By applying a panel of 15 inhibitors interfering with separate signalling pathways, we found that each component most probably activates several signalling pathways, which presumably act in concert on CXCL8 regulation. Different pathways may be triggered through separate mechanisms, possibly at different concentrations of the compound in question. Polycyclic aromatic hydrocarbons, for instance, may initiate their effects through interactions with the aryl hydrocarbon receptor, or formation of reactive metabolites and oxygen and nitrogen radicals (reactive oxygen species (ROS)/reactive nitrogen species) [39, 40]. Interestingly, LPS, which triggers its effects through TLR2/4 activation [41], a relatively specific mechanism, induced changes in the lowest number of genes. Variations in the relative effects of different inhibitors suggest that although different compounds may induce CXCL8 through similar pathways, the relative importance of the different signalling pathways varies.

We have previously observed that EGFR inhibition greatly reduces CXCL8 release induced by both F^- and crystalline silica in A549 alveolar epithelial cells [18, 19]. In line with this, the inhibitor screen identified EGFR signalling as a common mechanism of CXCL8 regulation in BEAS-2B cells. Use of EGFR-neutralising antibodies further supported this and implicated EGFR ligation in the regulation of CXCL8. Silica exposure seems to trigger an immediate phosphorylation of Src but not EGFR [18]. Here, we show that the receptor is activated at later time-points, which is strikingly similar to the effects of asbestos on epithelial cells [42], suggesting that EGFR activation, at least in the case of mineral particles, is a downstream event rather than a triggering mechanism.

EGFR ligands, such as TGF-α and amphiregulin, can induce CXCL8 in bronchial epithelial cells, and appear to mediate cigarette smoke-induced CXCL8 through an autocrine loop [43–45]. These growth factors are produced as membrane-bound pro-forms and released through ectodomain shedding by metalloproteases, primarily TACE/ADAM-17 [46]. TLR ligands (including LPS), rhinovirus and neutrophil elastase induce CXCL8 release from airway epithelial cells through activation of TACE, cleavage of pro-TGF-α and activation of EGFR [20, 21, 26, 47]. Our present data confirm that LPS-induced CXCL8 responses involve TACE/TGF-α/EGFR signalling in BEAS-2B cells. Furthermore, the study extends the list of compounds using this pathway of CXCL8 regulation in airway epithelial cells from endogenous and microbial molecules to organic and inorganic airborne pollutants. We also observed that inhibitors of TACE and EGFR attenuated DEP-induced CXCL8 expression in BEAS-2B cells, providing a possible mechanistic link between EGFR activation and increased CXCL8 levels in the airways of DEP-exposed human volunteers [48, 49].

In the case of both neutrophil elastase and TLR ligand exposure, TACE activation in airway epithelial cells appears to involve ROS production by dual oxidase (Duox)1 [20, 25]. Oxidative stress is also considered a central mechanism in cellular responses to PM [50]. Thus, ROS formation either directly by the compounds or indirectly *via* Duox1 activation may explain why the effects of such a diverse group of PM compounds may converge onto the same pathway. Notably, PKCδ/θ has been implicated in activation of the Duox1/ROS/TACE/TGF-α/EGFR cascade [20, 51]. In accordance with this, we observed that rottlerin significantly reduced CXCL8 release induced by Zn^{2+}, Fe^{2+} and 1-NP, and partially (but not significantly) that induced by silica. However, our data do not support a role of PKCδ/θ in LPS-induced CXCL8, in contrast to what has been proposed by others [3]. Several studies imply that IL-1α/β is also important in regulation of CXCL8 through an autocrine signalling loop [52–54], and preliminary results from our laboratory suggest that the IL-1-antagonising drug anakinra/Kineret (soluble IL-1 receptor (IL-1R) antagonist) represses CXCL8 induction by all test compounds except LPS (unpublished results). IL-1Rs and TLRs belong to the same superfamily and use many of the same adaptor/signalling molecules, including MyD88 and IL-1R-associated kinase [43]. It is therefore intriguing to speculate that IL-1 may activate TACE and that signalling pathways induced by several test compounds converge on IL-1 regulation. Indeed, IL-1β may induce shedding of EGFR ligands in other cell types and CXCL8 may even induce such effects, leading to a self-perpetuating loop [55].

Extrapolation from data obtained in immortalised cell lines has obvious pitfalls. However, studies with normal human bronchial epithelial cells have shown that CXCL8 regulation by TACE/TGF-α/EGFR signalling is not restricted to cell lines [25]. Although one cannot exclude that other pathways may be predominant in epithelial cells from at-risk subjects, EGFR expression is increased in the airway epithelium of asthmatics, smokers, and patients with COPD and CF, and the pulmonary expression of EGFR and CXCL8 correlates in patients with severe asthma [3]. Accordingly, animal models have revealed elevated tissue levels of TACE in COPD [56] and that TACE inhibitors decrease neutrophil influx in both allergic and nonallergic airway inflammation [57]. Notably, TACE not only cleaves growth factors involved in CXCL8 regulation, but also proteins involved in adhesion and transmigration of leukocytes across the endothelium, including vascular cell adhesion molecule/intercellular adhesion molecule-1, L-selectin and tight-junction molecules [58]. Thus, activation of TACE may trigger a number of reactions participating in the promotion of neutrophil inflammation in the airways. In addition, TACE/TGF-α/EGFR signalling also regulates mucin production, which contributes to exacerbation of asthma, COPD and CF along with neutrophilia [3, 24, 59–61].

Our present data confirm that CXCL8 regulation is complex and involves a number of signalling pathways that conceivably act in concert to elicit the final response. Thus, interference with just one pathway may not be sufficient to abrogate CXCL8 responses induced by air pollutants, such as PM. Nevertheless, our present findings strengthen the impression that TACE/TGF-α/EGFR is an important convergent pathway regulating innate immune responses induced by a variety of inhaled compounds in airway epithelial cells. It will be of interest to clarify any synergy between different PM components or other

inhalable irritants on TACE/EGFR signalling and whether existing inflammation or airway disease may lower the threshold level for TACE/EGFR activation.

SUPPORT STATEMENT

The work was supported by the Research Council of Norway, through the Environment, Genetics and Health programme (grants 165386 and 160863).

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We thank E. Lilleaas and T. Skuland (Dept of Air Pollution and Noise, Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway) for expert assistance throughout the study.

REFERENCES

1 Salvi S, Holgate ST. Mechanisms of particulate matter toxicity. *Clin Exp Allergy* 1999; 29: 1187–1194.
2 Holtzman MJ, Morton JD, Shornick LP, *et al.* Immunity, inflammation, and remodeling in the airway epithelial barrier: epithelial–viral–allergic paradigm. *Physiol Rev* 2002; 82: 19–46.
3 Burgel PR, Nadel JA. Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases. *Eur Respir J* 2008; 32: 1068–1081.
4 Mayer AK, Dalpke AH. Regulation of local immunity by airway epithelial cells. *Arch Immunol Ther Exp (Warsz)* 2007; 55: 353–362.
5 Øvrevik J, Lag M, Holme JA, *et al.* Cytokine and chemokine expression patterns in lung epithelial cells exposed to components characteristic of particulate air pollution. *Toxicology* 2009; 259: 46–53.
6 Øvrevik J, Refsnes M, Schwarze P, *et al.* The ability of oxidative stress to mimic quartz-induced chemokine responses is lung cell line-dependent. *Toxicol Lett* 2008; 181: 75–80.
7 Øvrevik J, Arlt VM, Oya E, *et al.* Differential effects of nitro-PAHs and amino-PAHs on cytokine and chemokine responses in human bronchial epithelial BEAS-2B cells. *Toxicol Appl Pharmacol* 2010; 242: 270–280.
8 Lezcano-Meza D, Teran LM. Occupational asthma and interleukin-8. *Clin Exp Allergy* 1999; 29: 1301–1303.
9 Barnes PJ. New molecular targets for the treatment of neutrophilic diseases. *J Allergy Clin Immunol* 2007; 119: 1055–1062.
10 Harada A, Mukaida N, Matsushima K. Interleukin 8 as a novel target for intervention therapy in acute inflammatory diseases. *Mol Med Today* 1996; 2: 482–489.
11 Xie K. Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev* 2001; 12: 375–391.
12 Hoffmann E, Dittrich-Breiholz O, Holtmann H, *et al.* Multiple control of interleukin-8 gene expression. *J Leukoc Biol* 2002; 72: 847–855.
13 Zhu W, Downey JS, Gu J, *et al.* Regulation of TNF expression by multiple mitogen-activated protein kinase pathways. *J Immunol* 2000; 164: 6349–6358.
14 Li J, Kartha S, Iasvovskaia S, *et al.* Regulation of human airway epithelial cell IL-8 expression by MAP kinases. *Am J Physiol Lung Cell Mol Physiol* 2002; 283: L690–L699.
15 Wu W, Samet JM, Ghio AJ, *et al.* Activation of the EGF receptor signaling pathway in airway epithelial cells exposed to Utah Valley PM. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L483–L489.
16 Keates S, Sougioultzis S, Keates AC, *et al. cag+ Helicobacter pylori* induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. *J Biol Chem* 2001; 276: 48127–48134.
17 Tjabringa GS, Aarbiou J, Ninaber DK, *et al.* The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor. *J Immunol* 2003; 171: 6690–6696.
18 Øvrevik J, Refsnes M, Namork E, *et al.* Mechanisms of silica-induced IL-8 release from A549 cells: initial kinase-activation does not require EGFR activation or particle uptake. *Toxicology* 2006; 227: 105–116.
19 Refsnes M, Skuland T, Schwarze PE, *et al.* Fluoride-induced IL-8 release in human epithelial lung cells: relationship to EGF-receptor-, SRC- and MAP-kinase activation. *Toxicol Appl Pharmacol* 2008; 227: 56–67.
20 Kuwahara I, Lillehoj EP, Lu W, *et al.* Neutrophil elastase induces IL-8 gene transcription and protein release through p38/NF-κB activation *via* EGFR transactivation in a lung epithelial cell line. *Am J Physiol Lung Cell Mol Physiol* 2006; 291: L407–L416.
21 Nakanaga T, Nadel JA, Ueki IF, *et al.* Regulation of interleukin-8 *via* an airway epithelial signaling cascade. *Am J Physiol Lung Cell Mol Physiol* 2007; 292: L1289–L1296.
22 Hamilton LM, Torres-Lozano C, Puddicombe SM, *et al.* The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. *Clin Exp Allergy* 2003; 33: 233–240.
23 Burgel PR, Montani D, Danel C, *et al.* A morphometric study of mucins and small airway plugging in cystic fibrosis. *Thorax* 2007; 62: 153–161.
24 Shao MX, Nadel JA. Neutrophil elastase induces MUC5AC mucin production in human airway epithelial cells *via* a cascade involving protein kinase C, reactive oxygen species, and TNF-α-converting enzyme. *J Immunol* 2005; 175: 4009–4016.
25 Koff JL, Shao MX, Ueki IF, *et al.* Multiple TLRs activate EGFR *via* a signaling cascade to produce innate immune responses in airway epithelium. *Am J Physiol Lung Cell Mol Physiol* 2008; 294: L1068–L1075.
26 Schwarze PE, Ovrevik J, Lag M, *et al.* Particulate matter properties and health effects: consistency of epidemiological and toxicological studies. *Hum Exp Toxicol* 2006; 25: 559–579.
27 Totlandsdal AI, Cassee FR, Schwarze P, *et al.* Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses *via* differential pathways in human bronchial epithelial cells. *Part Fibre Toxicol* 2010; 7: 41.
28 Øvrevik J, Lag M, Schwarze P, *et al.* p38 and Src-ERK1/2 pathways regulate crystalline silica-induced chemokine release in pulmonary epithelial cells. *Toxicol Sci* 2004; 80: 480–490.
29 Lag M, Refsnes M, Lilleaas EM, *et al.* Role of mitogen activated protein kinases and protein kinase C in cadmium-induced apoptosis of primary epithelial lung cells. *Toxicology* 2005; 211: 253–264.
30 Samuelsen JT, Schwarze PE, Huitfeldt HS, *et al.* Regulation of rat alveolar type 2 cell proliferation *in vitro* involves type II cAMP-dependent protein kinase. *Am J Physiol Lung Cell Mol Physiol* 2007; 292: L232–L239.
31 Refsnes M, Thrane EV, Lag M, *et al.* Mechanisms in fluoride-induced interleukin-8 synthesis in human lung epithelial cells. *Toxicology* 2001; 167: 145–158.
32 Chen YR, Tan TH. Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. *Oncogene* 1998; 17: 173–178.
33 Lin JK. Molecular targets of curcumin. *Adv Exp Med Biol* 2007; 595: 227–243.
34 Kocbach A, Totlandsdal AI, Lag M, *et al.* Differential binding of cytokines to environmentally relevant particles: A possible source for misinterpretation of *in vitro* results? *Toxicol Lett* 2008; 176: 131–137.
35 Davies DE, Holgate ST. Asthma: the importance of epithelial mesenchymal communication in pathogenesis: inflammation and the airway epithelium in asthma. *Int J Biochem Cell Biol* 2002; 34: 1520–1526.
36 Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L566–L577.

37 Brach MA, deVos S, Gruss HJ, *et al.* Prolongation of survival of human polymorphonuclear neutrophils by granulocyte-macrophage colony-stimulating factor is caused by inhibition of programmed cell death. *Blood* 1992; 80: 2920–2924.

38 Saba S, Soong G, Greenberg S, *et al.* Bacterial stimulation of epithelial G-CSF and GM-CSF expression promotes PMN survival in CF airways. *Am J Respir Cell Mol Biol* 2002; 27: 561–567.

39 Nebert DW, Dalton TP, Okey AB, *et al.* Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem* 2004; 279: 23847–23850.

40 Pelkonen O, Nebert DW. Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. *Pharmacol Rev* 1982; 34: 189–222.

41 Palsson-McDermott EM, O'Neill LA. Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. *Immunology* 2004; 113: 153–162.

42 Scapoli L, Ramos-Nino ME, Martinelli M, *et al.* Src-dependent ERK5 and Src/EGFR-dependent ERK1/2 activation is required for cell proliferation by asbestos. *Oncogene* 2004; 23: 805–813.

43 Subauste MC, Proud D. Effects of tumour necrosis factor-α, epidermal growth factor and transforming growth factor-α on interleukin-8 production by, and human rhinovirus replication in, bronchial epithelial cells. *Int Immunopharmacol* 2001; 1: 1229–1234.

44 Chokki M, Mitsuhashi H, Kamimura T. Metalloprotease-dependent amphiregulin release mediates tumour necrosis factor-α-induced IL-8 secretion in the human airway epithelial cell line NCI-H292. *Life Sci* 2006; 78: 3051–3057.

45 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.

46 Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. *Mol Aspects Med* 2008; 29: 258–289.

47 Liu K, Gualano RC, Hibbs ML, *et al.* Epidermal growth factor receptor signaling to Erk1/2 and STATs control the intensity of the epithelial inflammatory responses to rhinovirus infection. *J Biol Chem* 2008; 283: 9977–9985.

48 Pourazar J, Blomberg A, Kelly FJ, *et al.* Diesel exhaust increases EGFR and phosphorylated C-terminal Tyr 1173 in the bronchial epithelium. *Part Fibre Toxicol* 2008; 5: 8.

49 Stenfors N, Nordenhall C, Salvi SS, *et al.* Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. *Eur Respir J* 2004; 23: 82–86.

50 Borm PJ, Kelly F, Kunzli N, *et al.* Oxidant generation by particulate matter: from biologically effective dose to a promising, novel metric. *Occup Environ Med* 2007; 64: 73–74.

51 Shao MX, Nadel JA. Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. *Proc Natl Acad Sci USA* 2005; 102: 767–772.

52 Griffith DE, Miller EJ, Gay LD, *et al.* Interleukin-1-mediated release of interleukin-8 by asbestos-stimulated pleural mesothelial cells. *Am J Respir Cell Mol Biol* 1994; 10: 245–252.

53 Patel JA, Jiang Z, Nakajima N, *et al.* Autocrine regulation of interleukin-8 by interleukin-1α in respiratory syncytial virus-infected pulmonary epithelial cells *in vitro*. *Immunology* 1998; 95: 501–506.

54 Schwarz YA, Amin RS, Stark JM, *et al.* Interleukin-1 receptor antagonist inhibits interleukin-8 expression in A549 respiratory epithelial cells infected *in vitro* with a replication-deficient recombinant adenovirus vector. *Am J Respir Cell Mol Biol* 1999; 21: 388–394.

55 Berasain C, Perugorria MJ, Latasa MU, *et al.* The epidermal growth factor receptor: a link between inflammation and liver cancer. *Exp Biol Med (Maywood)* 2009; 234: 713–725.

56 Ju CR, Xia XZ, Chen RC. Expressions of tumour necrosis factor-converting enzyme and ErbB3 in rats with chronic obstructive pulmonary disease. *Chin Med J (Engl)* 2007; 120: 1505–1510.

57 Trifilieff A, Walker C, Keller T, *et al.* Pharmacological profile of PKF242-484 and PKF241-466, novel dual inhibitors of TNF-α converting enzyme and matrix metalloproteinases, in models of airway inflammation. *Br J Pharmacol* 2002; 135: 1655–1664.

58 Gooz M. ADAM-17: the enzyme that does it all. *Crit Rev Biochem Mol Biol* 2010; 45: 146–169.

59 Shao MX, Ueki IF, Nadel JA. Tumour necrosis factor α-converting enzyme mediates MUC5AC mucin expression in cultured human airway epithelial cells. *Proc Natl Acad Sci USA* 2003; 100: 11618–11623.

60 Shao MX, Nakanaga T, Nadel JA. Cigarette smoke induces MUC5AC mucin overproduction *via* tumour necrosis factor-α-converting enzyme in human airway epithelial (NCI-H292) cells. *Am J Physiol Lung Cell Mol Physiol* 2004; 287: L420–L427.

61 Gomez MI, Seaghdha MO, Prince AS. *Staphylococcus aureus* protein A activates TACE through EGFR-dependent signaling. *EMBO J* 2007; 26: 701–709.