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Role of scavenger receptors in silica nanoparticle-induced cytokine responses in bronchial epithelial cells

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HIGHLIGHTS

- Silica nanoparticles (SiNP) induce IL-6, CXCL8, IL-1α and IL-1β and TGF-α responses in epithelial lung cells.
- The scavenger-receptors SR-B1, LOX-1 and CXCL16 are involved in the SiNP-induced cytokine and TGF-α responses.
- The SR-B1-induced responses are mediated via MAP-kinase- and NF-κB-independent mechanisms.

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ABSTRACT

A major challenge in nanoparticle (NP) research is to elucidate how NPs activate initial targets in cells, leading to cytotoxicity and inflammation. We have previously shown that silica (Si)NPs induce pro-inflammatory responses in bronchial epithelial cells (BEAS-2B) via mechanisms involving transforming growth factor (TGF)-α release, and activation of MAP-kinase p38 and JNK besides NF-κB (p65). In the present study, the roles of scavenger receptors (SRs) in SiNP-induced cytokine responses in BEAS-2B cells were examined by siRNA silencing. Cells exposed to Si10 and Si50 (nominal sizes 10 and 50 nm) showed marked interleukin (IL)-6, CXCL8, IL-1α, IL-1β responses. Transient knockdown of SR-B1, LOX-1 and CXCL16 reduced the Si10- and Si50-induced cytokine responses, to a different magnitude dependent on the particle size, SR and cytokine. Si10-induced TGF-α responses were also markedly reduced by knockdown of SR-B1 and CXCL16. Furthermore, the role of SR-B1 in Si10-induced phosphorylations of p65 and MAP-kinases p38 and JNK were examined, and no significant reductions were observed upon knockdown of SR-B1. In conclusion, LOX-1 and CXCL16 and especially SR-B1 seem to have important roles in mediating cytokine responses and TGF-α release due to SiNP exposure in BEAS-2B cells, without a down-stream role of MAP-kinase and NF-κB.

1. Introduction

The use of engineered nanoparticles (NPs) in the development of new materials, giving advantageous properties for consumer products and therapeutics, is rapidly increasing word-wide. With the emerging use, it is of uttermost importance to avoid that these new NP/nanomaterials (NM) possess properties that induce adverse acute or chronic health effects in humans or affect the plethora of organisms in the environment. A lot of research has focused on hazard characterization of NPs (Nel et al., 2013) and also on exposure assessment, to identify which NPs may represent a potential health problem for humans, either in occupational settings, upon drug therapy or for the general population/consumers. To better predict NP toxicity and develop adverse outcome pathways (AOPs) for different classes of NPs, it is essential to elucidate the molecular initiating events ultimately causing effects observed from NP exposure.

Non-crystalline (amorphous) silica nanoparticles (SiNPs) are known to induce cytotoxic, genotoxic and immunotoxic responses (Napierska et al., 2010; Murugadoss et al., 2017; Pavan et al., 2019). A large range of *in vitro* studies has shown that SiNPs induce inflammation-related responses, including release of the cytokines interleukin (IL)-6 and CXCL8. Furthermore, small SiNPs appear to be far more potent on a mass basis than similar submicro-sized

Abbreviations: NPs, Nanoparticles; SiNPs, Silica nanoparticles; Si10, SiNPs with nominal size 10 nm; BEAS-2B, bronchial epithelial cells 2B; SR-B1, scavenger receptor B1; LOX-1, lectin-like oxidized LDL receptor-1; CXCL16, C-X-C motif chemokine ligand 16; IL, interleukin; TGF-α, transforming growth factor-α; TACE, tumour necrosis factor-α converting enzyme.

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particles (Lin et al., 2006; Napierska et al., 2009; Napierska et al., 2012; Låg et al., 2018; Sandberg et al., 2012; Skuland et al., 2014a). We have previously shown that the SiNP-induced IL-6 and CXCL8 responses in BEAS-2B cells involve p38 mitogen-activated protein kinase (MAP-kinase) and c-Jun N-terminal kinases (JNK), and nuclear factor-κB (NF-κB) signalling, as well as cleavage and release of membrane-bound transforming growth factor-α (TGF-α) through tumour necrosis factor-α converting enzyme (TACE) (Låg et al., 2018; Skuland et al., 2014b). Furthermore, SiNP -induced cytokine responses partially seem to involve reactive oxygen species (ROS)-dependent mechanisms in the BEAS-2B cells, at high, but not low concentrations of SiNPs with nominal size of 50 nm (Si50) (Refsnes et al., 2019).

Scavenger receptors (SRs) are a superfamily of receptors that might mediate the effect of NPs. SRs were initially shown to bind and internalize oxidized lipoproteins, but are currently known to bind a variety of ligands including exogenous pathogens, and negatively charged particles. SRs and their inherent properties have been identified and classified into 10 eukaryote families (A-J). These receptors are expressed in a variety of cell types, including macrophages, dendritic -, endothelial - and epithelial cells. Binding of ligands to SRs facilitates cellular uptake and ligand removal/clearance. SR members are known to regulate pathophysiological states including airway diseases as silicosis, atherosclerosis, pathogen infections, immune surveillance, and cancer. In general, studies have focused on Class A scavenger receptors (SR-A), however, research has also been directed against the role of Class B scavenger receptors (SR-B) (Gowdy et al., 2015; Zani et al., 2015; Shannahan et al., 2015a; Hoekstra, 2017). Different SRs have been shown to be involved in particle- and nanoparticle recognition, uptake, inflammatory (cytokine) and /or cytotoxic responses, as revealed in different cell types (Shannahan et al., 2015a; Nakayama, 2018). SR-A1 and also another member of the SR-A class (MARCO), were early reported to mediate the uptake, cytotoxicity and/or inflammatory responses (cytokines) of larger crystalline silica particles in alveolar macrophages and mast cells upon inhalation or instillation in mice (Hamilton et al., 2006; Brown et al., 2007). Subsequently, it has been reported that SR-A1 and MARCO also might mediate such responses in macrophages when exposed to various NPs, including SiNPs (Orr et al., 2011), carbon nanotubes (Wang et al., 2020), gold-NPs (França et al., 2011) and polystyrene-NPs (Kanno et al., 2007).

The scavenger receptor SR-B1 is expressed in multiple tissues and cell types across the body, although expressed at varying levels. The SR-B1 is able to bind to a wide variety of ligands, including anionic phospholipids and native and modified lipoproteins. Interestingly, SR-B1 and also SR-B2 (CD36), have been reported to mediate the effect of silica and other nano-sized and micro-sized particles (Nakayama, 2018; Shannahan et al., 2015b). Evidence also suggests that SR-B1 is involved in canonical inflammasome activation and subsequent pro-inflammatory responses in mouse macrophages and human peripheral blood monocytes, after exposure to crystalline silica and amorphous SiNPs (Tsugita et al., 2017). In contrast to SR-A, SR-B1 also seems to play a more prominent role in epithelial cells (Hoekstra et al., 2010; Sharma et al., 2015), but its role with respect to NPs has been less elucidated. It has been reported that SR-B1 mediates the uptake of silver-NPs in rat lung epithelial cells, leading to cell toxicity and an IL-6 response (Shannahan et al., 2015b). Both SR-B1 and the class-E SR, LOX-1 (lectin-like oxidized LDL receptor-1), has been reported to be highly expressed in human bronchial epithelial cells. Others, such as CXCL16 (chemokine (C-X-C motif) ligand 16) a chemokine with a dual role as class G SR, and SREC-1 (scavenger receptors expressed on the endothelial cells), appear to be more moderately expressed in bronchial epithelial cells (Dieudonné et al., 2012).

In the present study, we examine the role of three SRs, SR-B1, LOX-1 and CXCL16, in regulation of 10 nm SiNP (Si10)-induced cytokine and growth factor responses in BEAS-2B cells. The involvement of these SRs was also examined after exposure with larger Si50 to verify the role of SRs in effects from NPs of different sizes. Finally, the roles of MAPK- and NF-κB-pathways in Si10-induced SR-B1-mediated responses were investigated.

2. Methods

2.1. Materials

LHC-9 and Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) medium were purchased from Gibco, Life technologies, NY, USA. PureColTM collagen from Advanced BioMatrix, Inc, CA, USA. The cell culture flasks were obtained from Nunc A/S, Roskilde, Denmark and the 6-well plates and 10 cm culture discs from Corning, Lowell, MA 01851 USA. Silica nanoparticle with nominal size of 10 nm (Si10) and 50 nm (Si50) were from Kisker Biotech, Steinfurt, Germany. ELISA cytosets for IL-6 and CXCL8 were purchased from Invitrogen Life Technologies Ltd, UK, while IL-1α, IL-1β and TGF-α DuoSet were from R&D Systems, Inc, UK. The specific antibodies against p-p38, p-JNK and p-p65 were bought from Cell Signaling Technology, Inc., Danvers, MA, USA, β-actin was delivered from Sigma-Aldrich, St. Louis, MO, USA. Supersignal West Dura was obtained from Thermo Fisher Scientific Inc, Rockford, IL, USA, while the Re-blot stripping solution was from Chemicon, Millipore, Billerica, MA, USA. DC-Protein assay, 30 % acrylamid-BIS, TEMED, APS used for Western analysis, were purchased from BioRad Laboratories Ltd, UK and the nitrocellulose membrane from Whatman GmbH, Dassel, Germany. siRNA against SR-B1, LOX-1and CXCL16, and control siRNA were bought from Cell Signaling Technology, Inc., Danvers, MA 01923, USA and HiPerfect transfection reagent from Qiagen GmbH, Hilden, Germany. PerfectPure RNA isolation kit was bought from 5 PRIME, Gaithersburg, MD 20878, USA. High Capacity cDNA Archive Kit, SYBR™ Green PCR Master Mix, TaqMan Universal PCR Mastermix and TaqMan Gene Expression Assays were obtained from Life Technologies (Thermo Fisher Scientific Inc, Waltham, MA 02454, USA). The primers for SR-B1, LOX-1, SREC-1 and CXCL16 were made for us by Integrated DNA Technologies, Inc., IA, USA. The LDH kit was obtained from Sigma-Aldrich (Merck, Darmstadt, Germany). Anti-EGFR, neutralizing, was from Millipore, Billerica, MA, USA.

2.2. Cell culture and exposure conditions

BEAS-2B cells, a SV-40 hybrid (Ad12SV40) transformed human bronchial epithelial cell line (European Collection of Cell Cultures, ECACC, Salisbury, UK) were maintained in LHC-9 medium in collagen-coated flasks (PureColTM) in a humified atmosphere at 37 ° C with 5% CO_2, with refreshment of medium every second day. The cells (passages 8–50) were subsequently seeded in 35 mm 6-well culture plates (300 000 cells per well) or on 100 mm culture dishes in LHC-9 medium and cultured for 24 h. Thereafter, the cells were starved for 24 h in serum-free DMEM/F12 before exposure to Si10 and Si50. Cytokine release (CXCL8, IL-6, IL-1α and IL-1β) was assessed after 20 h exposure, while TGF-α release was assessed after 4 h exposure. Analysis of p38-, JNK- and NF-κB-phosphorylation by Western blotting was performed after 2 h exposure. Total exposure volume was 1.0 mL in 35 mm (six-well) and 5 mL in the culture dishes.

2.3. Preparation and characterisation of the nanoparticle solution

Stock solutions (25 mg/mL) of the Si10 and Si50 nanoparticles were dispersed in sterile water (2.3 mg/mL) and sonicated for

approximately 2 min on ice (until specific ultrasound energy of 420 J was given to the nanoparticles). Bovine serum albumin (BSA, final concentration 0.15 %) and phosphate buffed saline (PBS, final dilution 1x) were added to the particle solution, according to the method by Bihari and co-workers (Bihari et al., 2008). This gave a "particle working solution" with a final particle concentration of 2 mg/mL. Dynamic light scatter (DLS), transmission electron microscopy (TEM), surface area and chemical element composition of Si10 and Si50 were previously reported (Låg et al., 2018). The Si10 and Si50 sizes as measured by TEM analysis were 10.8 ± 1.6 and 34.7 ± 1.7 nm, respectively, whereas the surface areas as measured by Brunauer -Emmett-Teller (BET) analysis were 244 and 86.2 m^2/g, respectively.

2.4. Quantification of scavenger receptor expressions

In the present study, the mRNA expression levels of SR-B1, LOX-1, SREC-1 and CXCL16 were investigated in BEAS-2B cells by use of qPCR. RNA was isolated and cDNA synthesised as previously described (Låg et al., 2018). qPCR was performed with forward and reverse primers for SR-B1 (*forward:* 5′*-TTT GAA GGC ATC CCC ACC TA, reverse:* 5′*- TGA ATT CCA GAC TCC AGG CAC*), LOX-1 (f*orward:* 5′*-TTG TCC GCA AGA CTG GAT CTG,reverse:* 5′*-TGG CAT CCA AAG ACA AGC ACT*), SREC-1 (f*orward:* 5′*-GAC TCC TTC TCA TCC GAT CCreverse:* 5′*-GGC GCG GAG GCT TAG GGA TGG*) and CXCL16 (*forward:* 5′*-CAT CAA TTC CTG AAC CCA TGG, reverse:* 5′*-GAA TCG TCT CCG GAA ACA CCT*) in SYBR™ Green PCR Master Mix and for β-actin with a pre-designed TaqMan Gene Expression Assays (Hs01060665_g1) in TaqMan® Real-Time Universal PCR Master Mix. The expression of the different genes was normalized against β-actin, a house-keeping gene, and expressed as fold change compared to the untreated control as calculated by the ΔΔCt-method (Låg et al., 2018).

2.5. Suppression of scavenger receptors

The BEAS-2B cells were transfected with HiPerfect (6 μL/well) and siRNA against SR-B1, LOX-1 and CXCL16 (10 nM) immediately after seeding the cells (200 000 cells in 2.3 mL medium/well in a 6-well culture plate). After 2 days in culture the cells were exposed to Si10 and Si50.The transfection efficiency was verified by qPCR of the gene-expression at 48 and 72 h after the transfection process. To ensure that the cytokine-inhibition was due to knock-down of the mRNA of the respective scavenger receptors, and not an unspecific reaction, the cells were also transfected with non-sense siRNA (10 nM).

2.6. Cytokine analysis of IL-6, CXCL8, IL-1α and IL-1β

Cell culture media were collected 20 h after exposure and centrifuged at 300 x g to remove cell debris and at 10,000 x g to remove floating SiNPs. IL-6, CXCL8, IL-1α and IL-1β protein levels were determined by sandwich ELISA according to the manufacturer's guidelines. Absorbance was measured and quantified by a plate reader (TECAN Sunrise) equipped with a dedicated software (Magellan V 1.10).

2.7. Analysis of TGF-α release

The release of TGF-α was determined 4 h after exposure with a 30 min pre-treatment of the cell cultures to a neutralization EGFR antibody (anti-EGFR 5 μg/mL). After exposure to Si10 particles the media were collected and centrifuged at 300 x g and 10,000 x g to remove cell debris and floating silica particles. The protein levels of TGF-α were determined by sandwich ELISA, according to the manufacturer's guidelines.

2.8. Cytotoxicity

The cytotoxicity was evaluated by measuring lactate dehydrogenase (LDH) release in the cell culture media after 20 h of exposure and calculated as described in previous studies (Låg et al., 2018). The measurements were done according to the manufacture's procedures.

2.9. Western analysis

Phosphorylations of the MAPKs p38 and JNK, and the transcription factor p65 in BEAS-2B cells after exposure to Si10 have previously been investigated by Western analysis, with characterization of the time-course relationships (Låg et al., 2018). Based on these time-courses, the BEAS-2B cells were exposed to Si10 particles for 2 h, and the cell lysate analysed for p-p38, p-JNK and p-p65. The cell lysates were prepared and analysed as described in the previous study with β-actin as an endogenous control (Låg et al., 2018).

2.10. Statistical analysis

Statistical analyses of 4–6 independent biological replicates were performed by using one-way ANOVA with Dunnets Multiple comparison test (using GraphPad Prism software (version 8.0 Inc., San Diego, CA).

3. Results

3.1. SiNP-induced cytokine responses

In line with a previous study (Låg et al., 2018) Si10 and Si50 exposure (0−50/0−200 μg/mL) induced a concentration-dependent and progressive increase in IL-6, CXCL8, IL-1α and IL-1β releases in BEAS-2B cells (Fig. 1). Some reduction in cell viability was observed at 50 μg/mL Si10 and 200 μg/mL Si50 in accordance with our previous study (data not shown). Thus, in further experiments 25 μg/mL Si10 was chosen to minimize that NP-induced cytotoxicity could influence the cytokine responses.

3.2. Involvement of scavenger-receptors in SiNP-induced cytokine responses

Initially analysis of SR expressions by qPCR showed relatively high levels of SR-B1, LOX-1 and CXCL16 (Ct 20–25) in unexposed BEAS-2B cells, but a minimal level (Ct > 35) of SREC-1. Thus, to address the potential role of SRs in SiNP-induced cytokine responses in BEAS-2B cells, the cells were transfected with siRNA against SR-B1, LOX-1 and CXCL16, 48 h before exposure. The transient knockdown of SR-B1 significantly reduced the Si10-induced CXCL8, IL-6, IL-1α and IL-1β responses (Fig. 2A). The siRNA against LOX-1 and CXCL16 also significantly reduced the CXCL8, IL-6 and IL-1β responses. However, Si10-induced IL-1α response was only reduced by the siRNA against CXCL16, not LOX-1. The Si10-induced IL-1β responses were reduced to approximately the same magnitude by siRNAs against SR-B1, LOX-1 and CXCL16, whereas the other cytokines seemed to be less reduced by siRNAs against CXCL16 and LOX-1 than SR-B1, although not significantly. Next, we explored whether SRs could be involved in effects from SiNPs of other sizes. Thus, the effects of SR-B1, LOX-1 and CXCL16 silencing by siRNA were examined for Si50-induced cytokine responses. Similar patterns as for Si10 were observed, but with less significant reductions in the cytokine responses (Fig. 2B). Thus, for CXCL8 no significant reduction was observed after silencing of LOX-1. For IL-1α no reductions were observed neither for LOX-1 nor CXCL16, and for IL-1β not for any of the SR-receptors. The efficiency of siRNA-

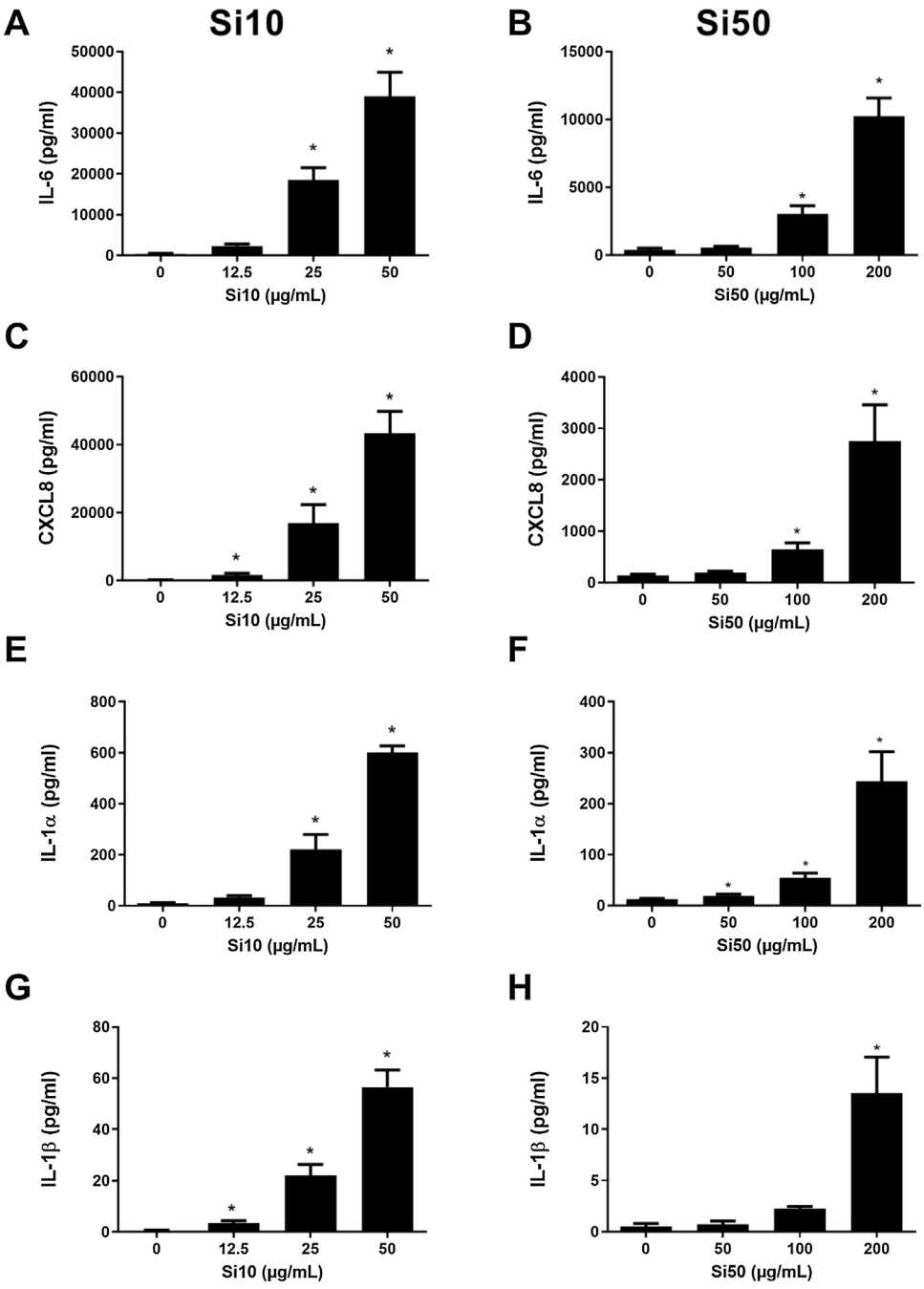

Fig. 1. Concentration-dependent effects of Si10 and Si50 exposure on cytokine release. The BEAS-2B cells were exposed to Si10 (0-50 µg/mL) and Si50 (0-200 µg/mL) for 20 h before analysis for IL-6 (A, B), CXCL8 (C, D), IL-1α (E, F) and IL-1β (G, H) by ELISA. The results are presented by the mean ± SEM of 4 independent experiments. *Significantly different from control, $p \leq 0.05$.

mediated silencing of SRs were validated by qPCR. SiRNA transfections against SR-B1, LOX-1 and CXCL16, almost completely knocked down (approximately 80–90 %) their respective receptor mRNA levels (Fig. 2C). The LOX-1 and CXCL16 siRNAs did not affect the SR-B1 levels. However, siRNA against SR-B1 appeared to cause a slight, but not statistically significant reductions in LOX-1 and CXCL16 expressions (Fig. 2C).

3.3. The involvement of scavenger receptors in Si10-induced TGF-α release

Previously we have shown that SiNPs induce TGF-α via a TACE-mediated mechanism, and that TGF-α contributed to SiNP-induced cytokines (Låg et al., 2018; Skuland et al., 2014b). We therefore investigated the involvement of the different SRs in Si10-induced TGF-α release by transfecting with the siRNA against SR-B1, LOX-1 and CXCL16. In line with previous observations, Si10 exposure induced a marked TGF-α release in BEAS-2B cells after 4 h exposure, and significant reductions by siRNAs against SR-B1 and CXCL16 were observed, but not by siRNA against LOX-1 (Fig. 3).

3.4. Scavenger receptor-B1 and intracellular signalling

Previous studies suggest that activation of MAP-kinases p38 and JNK and the transcription factor NF-κB (p65) partly mediate SiNP-induced IL-6 and CXCL8 formation (Låg et al., 2018; Skuland

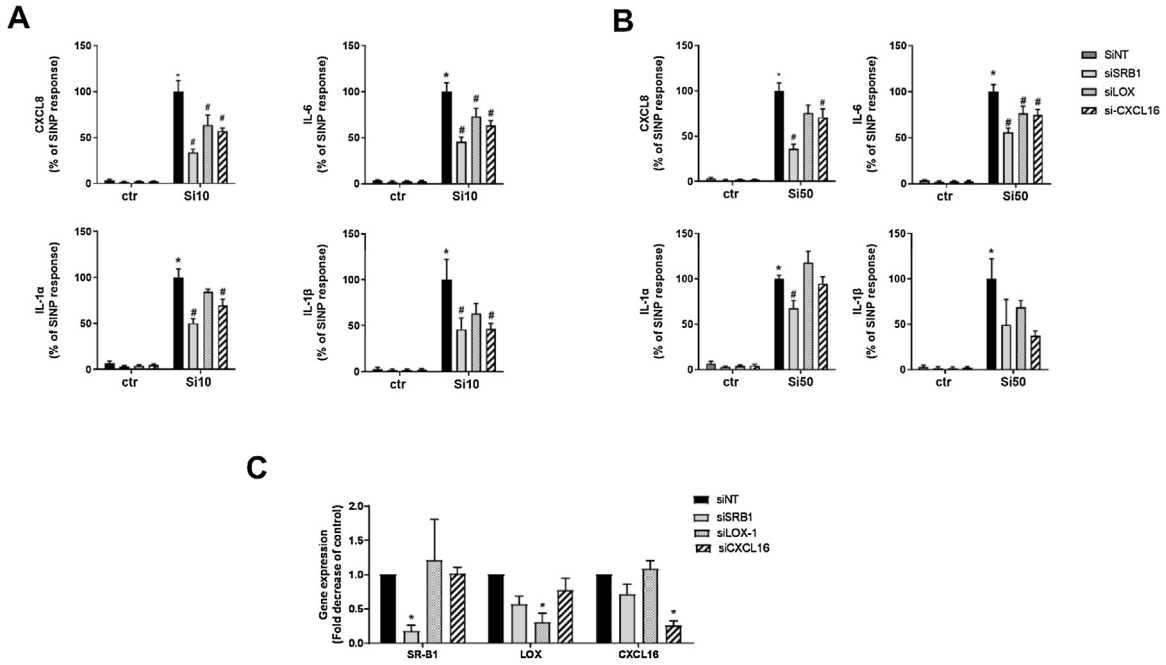

Fig. 2. The role of different scavenger receptors in mediating Si10–and Si50-induced cytokine responses. The BEAS-2B cells were transfected with siRNAs against SR-B1, LOX-1 and CXCL16 or with si-non targeting RNA (siNT) two days prior to the exposure of Si10 (25 μg/mL) and Si50 (200 μg/mL) for 20 h. A) Si10-induced cytokine responses B) Si50-induced cytokine responses. C) Gene expression levels of SR-B1, LOX-1 and CXCL16 before and after treatment with siRNAs against the different SRs as analysed by qPCR. In A) and B) cell supernatants were analysed for CXCL8, IL-6, IL-1α and IL-1β by ELISA. The results are presented as % of SiNP-induced responses and represent the mean ± SEM of 4-6 independent experiments, *significantly different from control, $p \leq 0.05$; #significantly different from Si10-and Si50-exposed cells, $p \leq 0.05$. The mean ± SEM control values were 580 ± 146, 590 ± 130, 16 ± 2.8 and 3.0 ± 2.5 pg /mL for CXCL8, IL-6, IL-1α and IL-1β, respectively. The mean of Si10-responses was 25800 pg/mL for CXCL8, 28 000 pg/mL for IL-6, 390 pg/mL for IL-1α and 96 pg/mL for IL-1β. The mean of the Si50-induced responses was 5900 pg/mL for CXCL8, 10 000 pg/mL for IL-6, 170 pg/mL for IL-1α and 17 pg/mL for IL-1β. The gene expression (C) was performed by qPCR.

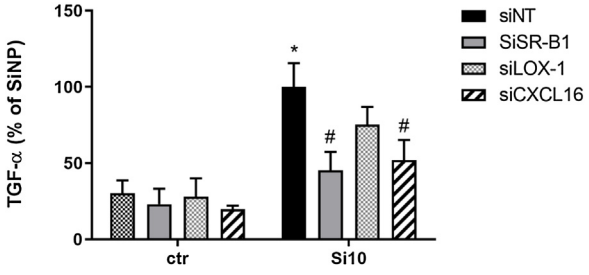

Fig. 3. The role of different scavenger receptors in mediating Si10-induced TGF-α release. The BEAS-2B cells were transfected with siRNAs against SR-B1, LOX-1 and CXCL16 or with non-targeting siRNA (siNT) two days prior to the exposure of Si10. One h before exposing the cells to Si10 (25 μg/mL) for 4 h, the cells were pre-treated with a neutralization EGFR antibody (anti-EGFR, 5 μg/mL). The cells were analysed for TGF-α by ELISA. The results are presented as % of Si10-induced responses by the mean ± SEM of 4 experiments, *significantly different from control (siNT), #significantly different from Si10 (siNT), $p \leq 0.05$. The mean ± SEM control value was 6.5 ± 2 pg/mL and the mean ± SEM Si10 (NT) response was 20.6 ± 8.5 pg/mL.

et al., 2014b). We therefore explored whether SRs could be involved in SiNP-induced p38, JNK or NF-κB activation in BEAS-2B cells, by transient knockdown of SR-B1. However, silencing of SR-B1 did not affect the Si10-induced phosphorylation of p38 and JNK (2 h), indicating that these signalling pathways were activated through a SR-B1-independent mechanism, at least at early timepoints (Fig. 4). Although Si10-induced p65-phosphorylation seemed partially reduced by siRNA against SR-B1, the reduction was not significant, indicating no or little down-stream role for NF-κB in mediating regulation of cytokine responses in BEAS-2B cells (Fig. 4).

4. Discussion

The present results suggest that the scavenger-receptors SR-B1, LOX-1 and CXCL16 have important roles in mediating SiNPs (Si10 and Si50)-induced activation of IL-6, CXCL8, IL-1α and IL-1β responses, in bronchial epithelial cells (BEAS-2B). SR-B1 and CXCL16 were also involved in SiNP-induced TGF-α release, as demonstrated for Si10. Furthermore, SR-B1 seems to mediate SiNP-induced cytokine responses through MAPK- and NF-κB-independent mechanisms, as presently shown for Si10.

An important finding in the present study with BEAS-2B was that silencing of SR-B1 by transient knockdown reduced the SiNP-induced increase of IL-6 and CXCL8, supporting an up-stream involvement of SR-B1 for the induction of these cytokines. Furthermore, SiNP-induced IL-1α and IL-1β responses, were similarly affected as IL-6 and CXCL8 upon transfection with siRNA against SR-B1. This may suggest that SiNP-induced IL-1α and IL-1β formation via SR-B1 is an initial critical event involved in SiNP-induced IL-6 and CXCL8 responses. However, it should not be excluded that IL-6 and CXCL8 formation also involves IL-1-independent pathways. Previously, many studies have shown that IL-1, in addition to TNF-α, is important in triggering of pro-inflammatory responses, including cytokines as CXCL8 and IL-6, and ultimately in exacerbation and development of inflammatory diseases (Dinarello, 2000). Interestingly, we have shown that larger-sized crystalline silica (Min-U-Sil) and Si10 are promoting the induction of IL-6 and CXCL8 release at least partly via IL-1, but not TNF-α, in tri-culture models with human typeIIlike alveolar epithelial A549 cells cultured in combination with THP-1 monocytes/macrophages and endothelial cells (Herseth et al., 2009; Skuland et al., 2020a).

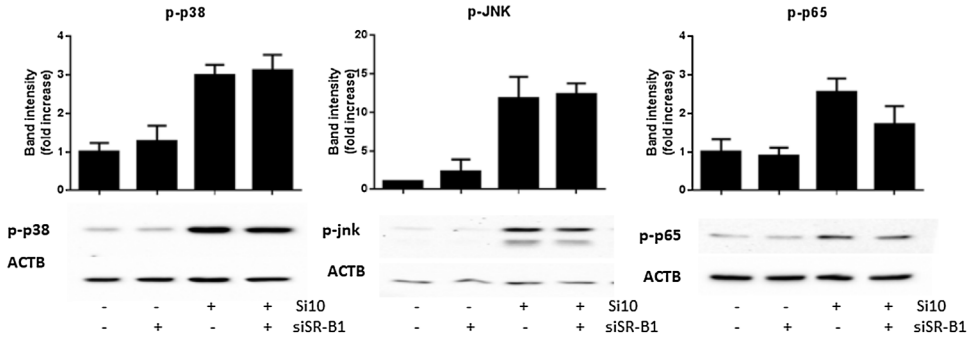

Fig. 4. The role of SR-B1 in Si10–induced phosphorylation of MAPK p38 and JNK, and NF-κB (p65). The BEAS-2B cells were pre-treated with siRNAs against SR-B1, two days prior to the exposure to Si10 (25 μg/mL) for 2 h. The controls were treated with non-sense siNT. The cells were analysed for p38-, JNK- and p65-phosphorylation, and β-actin (ACTB, loading control) by Western analysis. The results are presented by representive blots as well as the mean ± SEM of 4-5 independent experiments after quantification.

It should be noted that the Si10-induced cytokine responses in the bronchial epithelial cells does not solely seem to be mediated by SR-B1. Our data suggest that LOX-1 and CXCL16, also reported to be localized on epithelial lung cells (Dieudonné et al., 2012), may be important in mediating SiNP-induced cytokine regulation. Thus, transfections with siRNAs against LOX-1 and CXCL16 also showed significant, reductions of Si10-induced IL-6, CXCL8, IL-1α and IL-1β responses in the BEAS-2B cells. Although not significantly different from the effect of SR-B1 siRNA, the impact of LOX-1 and CXCL16 silencing on SiNP-induced cytokine responses, seemed somewhat lower. Notably, the involvement of SR-B1, LOX-1 and CXCL16 was observed for different sizes of SiNP, supporting a more general role of SRs in SiNP-induced cytokine responses.

The pathogenesis of silica-induced airway disease has been extensively examined. The silanol groups are regarded as critical for the silica-induced responses (Pavan and Fubini, 2017). Furthermore, the major focus has been on inflammasome (NLRP3) activation and IL-1β formation in macrophages. These events are regarded as essential in the adverse outcome pathways for inflammation in silicosis. A number of studies has shown that phagocytosis/internalization of crystalline and amorphous silica in macrophages is crucial for the inflammatory responses via induction of lysosomal rupture and activation of the NLRP3 inflammasome. These processes subsequently induce caspase-1 activation and cleavage of pro-IL-1β and enhance IL-1β secretion (Dostert et al., 2008; Hornung et al., 2008; Kusaka et al., 2014) that is crucial for lung disease development (Schroder and Tschopp, 2010). Notably, it has also been demonstrated a critical role for scavenger receptors as an initial event in triggering internalization of silica particles and subsequent inflammasome activation. This has especially been shown for SR-A1 and Marco (Hamilton et al., 2006; Brown et al., 2007). However, the SR-B1 has also been reported to be important for internalization of silica particles in macrophages. Thus, silica particles interact with SR-B1 via a binding site in the extracellular helix, thus leading to internalization of silica particles and subsequent inflammasome activation (and IL-1β formation) and pro-inflammatory responses as extensively characterized by Tsugita and coworkers (Tsugita et al., 2017). In the present study, SR-B1 was shown to be involved in Si10-induced cytokine responses in human bronchial epithelial cells (BEAS-2B). In general, the role of scavenger receptors in lung epithelial cells has been much less examined than in macrophages. However, SR-B1 has been reported to be localized in human bronchial epithelial lung cells, and to internalize exogenous agents such as double-stranded RNA (Dieudonné et al., 2012). In other cell types as HEK293 cells, SR-B1 has been reported to mediate fatty acid uptake (Wang et al., 2020). The partial reduction of the SiNP-induced cytokine responses obtained by the siRNAs against SR-B1, LOX-1 and CXCL16, respectively, may possibly be explained by the reserve capacity for internalization of SiNP via these SRs. Although it may appear that SR-B1 and other SRs primarily are involved in SiNP binding, internalization and subsequent inflammasome activation, more indirect effects involving uptake of SiNP-induced mediators should not be excluded.

Overall, the present study extends our previous characterization of cellular signaling pathways for SiNP-induced IL-6 and CXCL8 responses (Låg et al., 2018; Skuland et al., 2014b) and also on IL-1α and IL-1β responses (Skuland et al., 2020b), and suggests that several independent pathways may be involved. Our current data suggest that SiNPs induce cytokine responses via SR-B1, independent of the short-term activation of the MAP-kinases p38 and JNK, and probably the NF-κB pathway. The role of MAP-kinases and NF-κB in SR-mediated responses remains unclear. With few exceptions SRs have only short cytosolic tails that lack discernible signalling motifs (Canton et al., 2013). However, it has been reported that SRs, as especially examined for CD36, interact with various co-receptors/signalling proteins in hetero-multimeric signalling complexes known as signalosomes. These have also been reported to include MAPK. However, these complexes have shown a large context-dependent variability, with differences depending on the exogenous agents, cell types and other factors (Canton et al., 2013). Notably, we have previously shown that larger micron-sized crystalline silica particles trigger a rapid MAPK-phosphorylation within minutes of contact with the cell membrane of A549 cells. These early signalling responses were triggered prior to particle uptake, which occurred from 1–4 h after exposure (Ovrevik et al., 2006). Thus, it is tempting to speculate that MAPK signalling could derive from early signalling induced through interactions between SiNPs and cell membrane components (Kettiger et al., 2016; Alkhammash et al., 2015), while SRs, may be involved in the later binding and internalisation of SiNPs, possibly leading to lysosome damage and inflammasome activation (Gómez et al., 2017; Peeters et al., 2013). Interestingly, it has been reported that JNK- and MEK inhibition reduce SR-mediated internalization (Sulahian et al., 2008). MAPK-activation could therefore be an upstream event. Such an upstream or independent role would explain why SR-B1 silencing did not affect MAPK responses in SiNP-exposed BEAS-2B cells, despite its effect on cytokine regulation. An upstream role of MAPK would comply with a more indirect activation of SRs through SiNP-induced mediators. However, the potential mechanisms behind this need to be clarified. In addition, the roles of MAPKs and NF-κB in LOX-1- and CXCL16-mediated cytokine responses in SiNP-exposed cells remain to be addressed, as well as the role of these SRs and mechanisms in other cell types.

5. Conclusions

The scavenger-receptor SR-B1, as well as LOX-1 and CXCL16, are shown to be involved in induction of IL-6, CXCL8, IL-1α and IL-1β responses, as well as TGF-α formation, due to SiNP exposure in BEAS-2B cells. Lack of effect from SR-B1 silencing on SiNP-induced phosphorylation of MAPKs and NF-κB is compatible with an independent or upstream role of these pathways.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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