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Differential activation of the inflammasome in THP-1 cells exposed to chrysotile asbestos and Libby "six-mix" amphiboles and subsequent activation of BEAS-2B cells

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ABSTRACT

Inflammatory responses of THP-1 cells (macrophage cell line) exposed to chrysotile asbestos (Chry) and Libby six-mix (LIB) and the subsequent impact on bronchial epithelial cells were determined. Direct treatment of THP-1 cells with Chry caused cell death, activation of caspase-1 and release of IL-1β, while the addition of caspase-1 inhibitor, Z-YVAD-FMK, reduced IL-1β, suggesting that Chry activated the caspase-1 mediated Nod-like receptor protein 3 (NLRP3) inflammasome; by comparison, LIB had less effects on all of these parameters. Expression of antioxidant enzymes, protein oxidation and nitration, and lipid peroxides in THP-1 cells treated with the two particles suggest that LIB generated more reactive oxygen species (ROS) than the same dose of Chry. Differences in fiber length and surface area suggest a possible role for particulate size in the differential activation of the inflammasome. BEAS-2B cells, representing the bronchial epithelium, treated with supernatants of medium from Chry- or LIB-treated THP-1 cells (conditioned medium) activated the MAPK cascade, increased phosphorylation of ERK and Cot (MAP3K8), increased AP-1 binding activity and induced IL-6 release. To verify that IL-1β from THP-1 cells was responsible for activation of BEAS-2B, conditioned medium with added IL-1Ra, an IL-1β antagonist, was applied to BEAS-2B. Results show that IL-1Ra attenuated effects of conditioned medium, supporting a role of IL-1β, as a secondary mediator, in the transduction of inflammatory signaling from the macrophage to epithelial cells. The effects of LIB-conditioned medium appeared to be less dependent on IL-1β. In conclusion, Chry and LIB induce differential inflammatory responses in THP-1 cells that subsequently lead to differential effects in epithelial cells.

1. Introduction

Human exposure to asbestos is associated with an increased incidence of pleural disease, asbestosis, lung cancer, and mesothelioma [1]. Inflammation plays a key role in lung injury, involving different cell types, such as alveolar macrophages and bronchial epithelial cells that initiate and/or sustain inflammatory processes. Alveolar macrophages provide surveillance of the major boundaries between the body and the outside world. Macrophages are innate immune cells with well-established roles in the primary response to pathogens. These cells recognize danger signals through receptors and activate specific signaling pathways [2]. The macrophage-like cell line, THP-1, has been shown to secrete the proinflammatory cytokine, IL-1β, upon contact with crocidolite asbestos [3–5]. Mature IL-1β is processed through cleavage of the inactive pro-IL-1β precursor by caspase-1/ICE (IL-1β converting enzyme). Caspase-1 was the first caspase to be discovered in mammals, but only recently has the pathway leading to its activation been elucidated and shown to involve a series of large complexes, called inflammasomes [6,7]. Stimulation of macrophages with asbestos resulted in cell death and robust secretion of IL-1β in a manner dependent on the Nod-like receptor protein 3 (NLRP3),

Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; Chry, chrysotile; Cot, cancer osaka thyroid oncogene; DNPH, 2,4-dinitrophenylhydrazine; EMSA, electrophoretic mobility shift assay; GB, glass beads; GCLC, γ-glutamate cysteine ligase catalytic subunit; IKKs, IκB kinases; IL-1Ra, IL-1 receptor antagonist; IL-1RAcP, IL-1 receptor accessory protein; IRAK, interleukin-1 receptor-activated protein kinase; LIB, Libby "six-mix amphiboles"; MAP3K, MAPKKK, MAPK kinase kinase; MnSOD, manganese SOD; MYD88, myeloid differentiation primary response gene 88; NLRP3, NOD-like receptor family, pryin domain containing 3; PMA, phorbol-12-myristate-13-acetate; SA, surface area; SOD, superoxide dismutase; TRAF6, TNF receptor-associated factor 6; TIR, Toll- and IL-1R-like; Tpl2, tumor progression locus 2; ZYF, Z-YVAD-FMK.

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also called Nalp3 (NACHT, LRR and PYD domains-containing protein 3) inflammasome [3,4,8,9]. Previous reports have indicated that macrophages from wild-type mice could undergo caspase-1-mediated IL-1β maturation but macrophages deficient in the NLRP3 inflammasome were incapable of secreting IL-1β [3,4,8].

Following the initiation of proinflammatory responses upon exposure to asbestos with the release of cytokines, such as IL-1β, alveolar macrophages often die and are cleared from the airway. In contrast, neighboring bronchial epithelial cells can sustain pathogenic responses and lead to asbestos-related lung disease, even if they may not be in direct contact with the asbestos fibers. IL-6 is considered to be an important mediator of acute inflammation [10] and implicated in the pathogenesis of particle-related lung disease. The MAPK pathways contribute to epithelial cell proliferation, which is a major risk factor for the development of lung cancer or fibro-proliferative diseases [11,12]. Whether IL-1β secreted by THP-1 cells affects the alveolar epithelium leading to cell proliferation, inflammation and eventual fibrosis via MAPK-IL-6 pathways is uncertain although it is well established that direct exposure of pulmonary epithelial cells [13], alveolar [14], and bronchial epithelial cells [15,16] to asbestos leads to activation of MAPK cascades and induction of ERK1/2 phosphorylation.

Several investigators have examined the interaction between macrophages and bronchial epithelial cells, employing a co-culture system (Transwell) in which two different cell types were separated by a membrane with only macrophages exposed to the particles of asbestos or silica [17–19]. In the other compartment, the epithelial cells were bathed by the same culture medium. Although interesting connections were suggested, the underlying mechanisms whereby primary responses of the macrophage initiated and interacted with lung epithelial cells remain unclear. We have investigated signal transduction from THP-1 macrophage-like cells to BEAS-2B bronchial epithelial cells through a relatively simple method: that is, to initially determine the inflammatory responses of THP-1 exposed to different particulate fibers followed by collection of the supernatants of culture medium without any fibers (termed as conditioned medium) in which BEAS-2B cells were then grown and their responses assessed.

Asbestos occurs naturally and is composed of long thin fibers broadly classified into two groups: serpentine and amphibole [20]. Chrysotile asbestos (the asbestiform variety of the serpentine group) is the most commonly used asbestos type in the US [21]. The amphibole asbestos group consists of five mineral species: amosite, crocidolite, tremolite, actinolite, and anthophyllite. The amphibole asbestos occurring in the vermiculite mine near Libby, Montana, is a mixture of several different species of amphiboles [22]. Several research groups have studied this material, but the samples used in the present work are a subset of those discussed in the publication by Meeker et al. [23] and morphologically characterized by Bellamy and Gunter [24]. Bellamy and Gunter referred to the Libby material as "six-mix". Because many of the studies examining the pathogenic mechanisms related to aberrant cellular responses to asbestos exposure utilized different forms of asbestos, the present work contrasts the signal transduction pathways induced by chrysotile asbestos (Chry) and Libby six-mix (LIB). The role of IL-1β in the THP-1-conditioned medium secreted in response to Chry or LIB was compared and its subsequent effects on BEAS-2B immune responses, specifically IL-6 secretion, were examined.

2. Materials and methods

2.1. Cell culture

THP-1 cells: THP-1 cells from a human monocyte cell line were grown in RPMI 1640 medium (Invitrogen) containing 10% FBS; 100 U/ml Penicillin/Streptomycin; 10 mM HEPES and 1 mM Sodium pyruvate at 37.0 °C in 5% CO_2. Cells from this monocytic cell line require differentiation into cells with the functional characteristics of mature macrophages [25,26], accomplished by treatment with 100 nM PMA overnight and then refreshing the cells with medium without PMA. *BEAS-2B cells*: BEAS-2B cells from the human bronchial epithelial cell line (ATCC) were seeded into flasks or plates pre-coated with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin in medium. The cells were grown in DMEM/F12 medium (Invitrogen) containing 10% FBS; 100 U/ml of Penicillin/Streptomycin, 1 μg/ml Insulin-Transferrin-Na selenite (Sigma), 1 μg/ml Hydrocortisone (Sigma), and 10 mM HEPES at 37.0 °C in 5% CO_2.

2.2. Chrysotile asbestos and Libby six-mix treatments

Chrysotile asbestos fibers (National Institute of Environmental Health Sciences reference sample, NIEHS), and Libby six-mix (United States Geological Service, USGS) have been physically and chemically characterized previously [21,24,27–30]. Glass beads (GB) from Polysciences Inc. were used as a noncytotoxic particle control [29]. The surface area of chrysotile measured using Micromeritics Tristar equipment for nitrogen gas sorption analysis by Happond Expander™ is reported to be 28.83 m^2/g (Table 1). The Chry and LIB fibers were sterilized under ultraviolet light overnight prior to suspension in HBSS at 1 mg/ml, sonication for 15 min in a water bath sonicator, and trituration eight times through a 22-gauge needle before treatment of the cells [31]. Two mass-based concentration systems have been commonly used in studies of asbestos exposure: (1) mass concentration, which means the mass of a constituent divided by the volume of a carrier and (2) mass-per-area, which is the mass divided by the exposed surface area. The asbestos suspensions were directly added to differentiated macrophages or BEAS-2B cells in medium at a mass concentration of 20 μg/ml and 40 μg/ml for 24 h, which was equivalent to mass per area of 5 $\mu g/cm^2$ and 10 $\mu g/cm^2$ achieved by adjusting the volume of medium based on the surface area of the culture dishes. The culture medium collected after exposure of THP-1 cells for 24 h was centrifuged at the highest speed of an Eppendorf 5430R centrifuge with 50 ml-tube rotor. Then the supernatant of medium was used to treat BEAS-2B cells and referred to as conditioned medium with indicated asbestos concentration from the original THP-1 treatment. Since the conditioned medium contained no particles and the cellular responses to GB or medium alone were similar, a GB control was not included in the experiments using conditioned medium.

2.3. Caspase-1 inhibitor treatments

A caspase-1 inhibitor VI, Z-YVAD-FMK (ZYF), was purchased from BioVison and a caspase-1 inhibitor V, Z-D-CH2-DCB (ZD), from Calbiochem. In preliminary experiments, 10, 20, 40 and 50 μM of inhibitors were applied to THP-1 cell 30 min prior to asbestos treatment. After 24 h, the inhibitory effects on production of IL-1β were compared, suggesting that ZYF was more efficient than ZD. Based on these preliminary results, only 40 μM of ZYF was used in the subsequent experiments.

2.4. IL-1β and IL-1Ra treatments

Recombinant human IL-1β protein and the antibody for cleaved IL-1β were purchased from Cell Signaling. The IL-1 receptor antagonist (IL-1Ra) was purchased from Abcam. BEAS-2B cells were challenged in preliminary experiments by 10, 20, 40, 80, and 100 ng/ml recombinant IL-1β to determine its effect on the secretion of IL-6. The inhibitory effects of both IL-1Ra and anti-IL-1β