Crystalline Silica Mediated Inflammation: Role of Mast Cells

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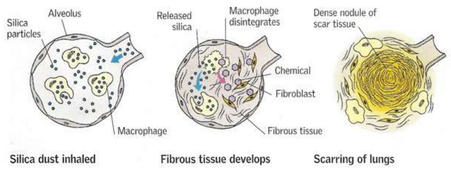
Abstract

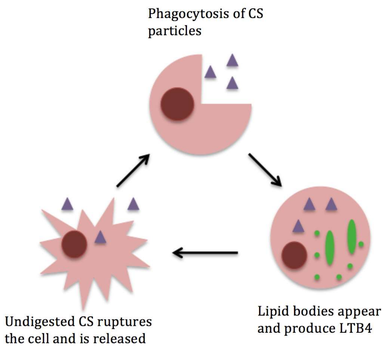
Prolonged exposure to crystalline silica (CS) leads to silicosis due to chronic inflammation and fibrosis of the lungs. Individuals with silicosis are twice as likely to develop lung cancer. A key aspect of this CS-induced inflammation is the migration of neutrophils to the lungs. The process of neutrophil recruitment to the lungs begins with the chemoattractant Leukotriene B_4 (LTB_4) binding to BLT1 and BLT2 receptors. The main producers of LTB_4 are macrophages and mast cells. Once this process has begun other mediators such as IL1-β and neutrophil active chemokines also play a role promoting the inflammation. When inhaled CS travels to the alveoli of the lungs where it enters mast cells, macrophages, and epithelial cells through phagocytosis. As the phagosome progresses, lipid bodies begin to appear in the cytosol. After fusion of the lysosome and phagosome the inflammasome protein complex appears and produces IL1-β. By inhibiting the formation of the phagolysosome, IL1-β production is stunted while LTB_4 production is heightened. This shows that the production of LTB_4 and IL1-β are triggered independently of one another. The pathway through which inflammasomes are constructed and produce IL1-β is understood, while the pathway through which LTB_4 is produced is not yet clear. The first objective of this study is to stain different cellular compartments using microscopy to determine the connection between phagocytosis and LTB_4 production in macrophages. While mast cells are known to play an important role in inflammation and produce even more LTB_4 than macrophages, little is known about the process through which this occurs. Rat basophilic leukemia (RBL) cells share many properties with mast cells. The second objective of this study is to determine if RBL cells make LTB_4 in response to CS.

Background

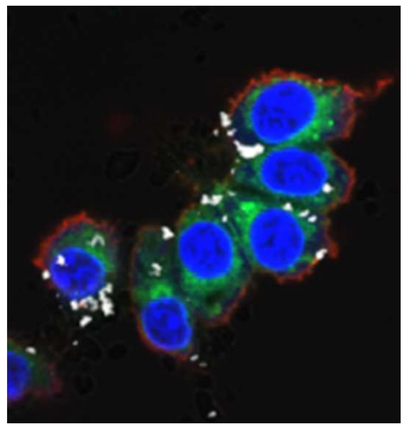
In 2015, lung cancer was responsible for approximately 27% of all cancer deaths, making it the number one cancer killer of both men and women (1). Factors such as smoking, genetic susceptibility and various environmental hazards are known to increase the risk of lung cancer. One of these environmental hazards is crystalline silica, the second most abundant element on the earth. Millions of workers in the US alone are exposed to CS every year. When in crystalline form silica is inhaled into the lungs where the particles become trapped and damage lung tissue. The damaged tissue eventually becomes scar tissue that forms granulomas. The damage done by CS causes the incurable, but preventable, lung disease silicosis. Individuals with silicosis are at an increased risk of developing lung cancer, making CS a human carcinogen.



Results

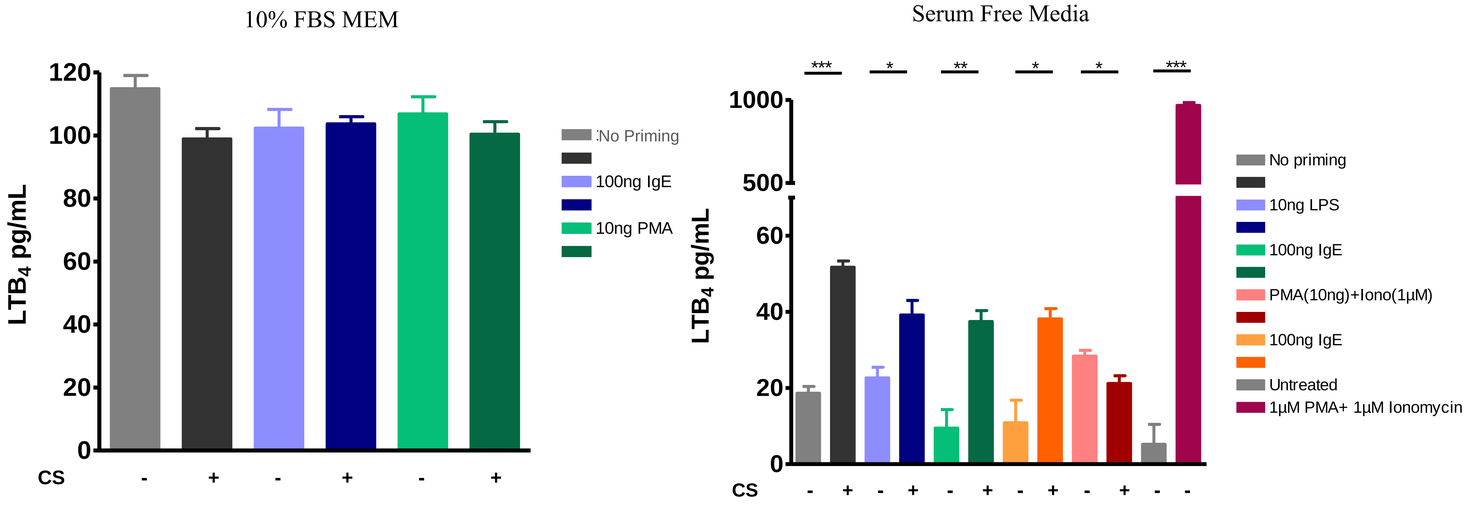


This cycle attracts neutrophils and causes chronic inflammation



Confocal microscopy of nucleus (blue), CS (white), lipid bodies (green), and membrane (red) in a macrophage

LTB_4 Production by RBLs in Response to CS



Methods

RBL-2H3 cells were plated in a 24-well cell culture plate with a cell density of $1x10^5$ cells/well. Cells adhered to the cell plate overnight in 500 μl of 10% FBS MEM. A variety of priming agents were used. Cells were primed overnight with 10 μg PMA, 100 μg IgE, 10 μg LPS, 10ng PMA+ + 1 μM Ionomycin. Before CS stimulation the cells were switched to either 200 μl of 1% FBS media or serum free media. 1 μM PMA + 1 μM Ionomycin was used as a positive control. After the cells were stimulated with 100-$\mu g/$ cm^{-2} of CS for 5 hours, the supernatant was removed and a LTB_4 Elisa was run following the manufacturers protocol to determine the levels of LTB_4 produced. Experiments were done in triplicate cultures.

Conclusions

- In 10% FBS MEM, with and without priming, CS does not induce LTB_4 production in RBL cells.
- In serum free media, with and without priming, CS induces LTB_4 production in RBL cells for all conditions except PMA (10ng)+Iono (1μM).
- CS induces the most LTB_4 production in RBL cells without priming in serum free media. This was expected because CS also induces the most LTB_4 production in bone marrow derived mast cells without priming in serum free media.
- Using RBL cells in place of mast cells will allow for studies to be completed using a more durable and accessible cell line.

Future Directions

- Refine the conditions in which CS induces LTB_4 production in RBL cells.
- Use RBL cells without priming in serum free media to study LTB_4 production in mast cells.
- Continue with confocal microscopy to determine the connection between phagocytosis and LTB_4 production.

Acknowledgements

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References

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