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**Title:** The effect of in vitro culture methods on corticosteroid sensitivity in COPD alveolar macrophages

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**Body:** Introduction The culture of COPD alveolar macrophages is used to investigate the effects of corticosteroids. The cell culture methods may influence the results obtained. The aim was to investigate the effect of culture time on the corticosteroid sensitivity of COPD alveolar macrophages. Methods Alveolar macrophages were isolated from resected lung tissue; 10 COPD patients and 10 smokers were recruited. Two different culture methods were compared; (1) cells were rested for 1 hr prior to experiment, and (2) cells were rested overnight prior to experiment. The effect of dexamethasone on LPS-induced TNFα and CXCL8 release after a further 24 hrs culture was investigated. p38 MAPK activation and the effect of dexamethasone and birb-796 on TNFα and CXCL8 release from unstimulated cells was also measured. Results The effect of dexamethasone on LPS-induced TNFα and CXCL8 was significantly reduced in method 1 in both COPD patients and controls ($p<0.05$). The basal levels of activated p38 were higher in macrophages cultured using method 1. In addition, dexamethasone was less effective than birb-796 in reducing the basal release of TNFα in condition 1. The level of dexamethasone mediated inhibition reached 37% and 60% for COPD patients and controls respectively, whereas the level of birb-796 mediated inhibition reached 74% and 80% for COPD patients and controls respectively. Discussion We have shown that variations in culture methods can alter the corticosteroid sensitivity of COPD alveolar macrophages in vitro. Activation of p38 MAPK occurs to a greater extent in cells used for culture soon after being extracted from the lungs and appears to contribute to corticosteroid insensitivity.