Tissues such as the skin and intestinal epithelium experience physiological hypoxia whereas pathological hypoxia occurs at inflammatory sites. Neutrophils are recruited to infective/inflamed areas and are thus required to operate under low oxygen tensions. We have shown previously that hypoxia delays neutrophil apoptosis (JEM 2005; 201:105) and impairs bacterial killing (J Immunol 2011; 186:453) and have now studied the effect of hypoxia on the release of histotoxic neutrophil proteases.

Neutrophils isolated from healthy volunteers were subjected to normoxia or hypoxia (5 kPa). Superoxide anion release was measured by the reduction of cytochrome. C Elastase release was quantified by the cleavage of labelled elastin.

Hypoxic incubation for 4 hours resulted in a 3-fold reduction in superoxide anion release from cells stimulated with GM-CSF and fMLP. In contrast, elastase release from the azurophilic granules was augmented almost 3-fold under hypoxia. The release of MMP-9 and lactoferrin was similarly up-regulated, suggesting a more generalised increase in degranulation under hypoxia. In addition to this electron microscopy showed that hypoxia induced a more activated phenotype (e.g. increased membrane ruffling and cell spreading).

We show that hypoxia can induce a more destructive neutrophil phenotype, with enhanced degranulation and release of potentially histotoxic proteases, impaired bacterial killing, and delayed apoptosis. These data suggest that hypoxia adversely affects neutrophil function and may augment neutrophil mediated tissue destruction.

**Methods**

In vitro cleavage assays: After literature review the pro-inflammatory mediators osteopontin and tumour necrosis factor (TNF)-α were selected as potential MMP-12 substrates in COPD. Both were incubated with MMP-12 and reaction products analysed by silver stain and western blot. EDTA was used as a metalloprotease inhibitor and thrombin as positive control. COPD cohort: Patients with COPD were recruited during exacerbations at the Nottingham University Hospitals NHS Trust. Sputum, lung function and other parameters were recorded. Aims To identify MMP-12 substrates of relevance to COPD and determine how their activity affects disease progression in vitro and in vivo.

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Results

MMP-12 cleaved osteopontin and pro-TNF-α in a dose and time-dependent manner when visualised by silver staining. Cleavage was dependent on MMP-12 activity as it was inhibited by EDTA. Western blot of cleaved protein fragments gave a characteristic band signature. MMP-12 was present in sputum of patients with COPD as demonstrated by western blotting, ELISA and casein zymography. Western blot analysis of sputum with anti-osteopontin antibodies showed a similar band signature to the in vitro cleavage suggesting osteopontin is cleaved in the airways of patients with COPD.

Discussion

MMP-12 possesses proteolytic activity against osteopontin and pro-TNF-α in vitro. MMP-12, osteopontin and TNFα are present in COPD sputum and our data suggest that MMP-12 may target osteopontin in COPD. Further work is needed to determine the precise mechanisms of such MMP-12 substrate activity in COPD.