Fibronectin Is Regulated During Microbial Lung Injury and Mediates Chronic Inflammation

Saralili Robertson, Monika Hermansson, James Moffatt, Debbie Baines, Emma Baker, Nidhi Sofat

St Georges, University of London, Dept of Biomedical Sciences, SW17 ORE, United Kingdom

Background

Recurrent respiratory infections are a common cause of morbidity in subjects with chronic lung conditions. The lung microenvironment, including the extracellular matrix (ECM), is remodelled in chronic lung injury and may potentiate the propensity to develop further pulmonary infections. Fibronectin (FN) is one such ECM molecule that is also known as a MSCRAMM (microbial surface component recognizing adhesive matrix molecules) \(^1\). Although the host MSCRAMM response to gram positive organisms is well characterised \(^1\), the lung response to Gram negative organisms containing lipopolysaccharide (LPS) is less well understood.

Aims

We aimed to: 1) investigate the regulation of FN, an ECM molecule implicated in the host response to injury using a murine lung model of infection; and 2) to validate our results in samples from human subjects.

Methods

For murine experiments, 6 – 8 week old female BALB/c mice were administered 0.125 mg/kg of LPS from \(E.\ coli\) serotype 0127:B8. LPS or saline control were administered intranasally in 50 µL volumes whilst the mice were anaesthetised with isoflurane (under full institutional Ethics Approval). Mice were sacrificed after 24 hours: the lungs were dissected out and fixed for 4 hours in 4% paraformaldehyde and washed before embedding in paraffin wax. For immunohistochemistry, lung tissue was sectioned into 4 µm slices which were stained with H & E or primary rabbit anti-fibronectin antibody. FN antigen was detected using secondary antibodies conjugated to horseradish peroxidase and analysed by light microscopy. Bronchoalveolar lavage fluid (BALF) from human subjects with asthma and/or COPD was immunostained for fibronectin using SDS-PAGE and Western blotting. The same samples were also analysed by mass spectrometry on 1-D gels by in gel digestion. LC-MS was used with an LCQ Deca Plus apparatus.

Results

Our murine model showed an inflammatory response to LPS, with oedema, destruction of alveolar architecture and a cellular infiltrate. Expression of FN was highly upregulated in LPS treated mice vs. saline controls (n=5 in each group). FN protein was detected in the surrounding ECM of alveolar tissue, type II pneumocytes and the cellular infiltrate. Studies with human BALF found increased FN protein expression in normal and disease samples. Higher levels of FN expression were found in some normal subjects compared with subjects with asthma/COPD. Controls and asthma BALF samples subjected to LC-MS identified specific regions of FN from human samples mapping to the cell-binding region and the C-terminal heparin-binding region of FN.

Discussion

Our results show that fibronectin is upregulated in a murine model of lung infection using LPS with localisation to the alveolar cells and pro-inflammatory infiltrate. FN is also expressed in human BALF from subjects with COPD/asthma mapping to FN regions previously implicated in mediating chronic inflammation in arthritis. Since full-length FN was reduced in diseased BALF, this may represent fragmentation by proteases. It is possible that FN and/or its fragments mediate chronic inflammation during lung injury such as COPD/asthma. ECM molecules such as FN may provide new therapeutic targets aimed at inhibiting the development of chronic inflammation.

(491 words)
Reference: