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Original article

Biphasic lung injury during *Streptococcus pneumoniae* infection in a murine model

Réponse pulmonaire biphasique lors de l'infection à Streptococcus pneumoniae dans un modèle murin

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Abstract

Objectives. – *Streptococcus pneumoniae* is the leading cause of community-acquired pneumonia. We aimed to analyze the epithelial response to *S. pneumoniae*-induced lung injury.

Methods. – Using an in vitro model with 16HBE cells and experimental in vivo murine model of acute lung injury, we analyzed the epithelial response to *S. pneumoniae*. Lung epithelial cell monolayers were exposed to *S. pneumoniae* and permeability was assessed by transepithelial resistance (TER) measurement and organization and expression of junction proteins. Functional consequences were studied with an in vivo murine model measuring alveolar permeability, distal alveolar fluid clearance (DAFC), and the alveolar inflammatory response.

Results. – In vitro, *S. pneumoniae* induced a dose-dependent decrease in transepithelial resistance, which was associated with significant modifications in the organization of junction proteins assessed by immunofluorescence staining and expression after 6 hours of exposure. In vivo, *S. pneumoniae* induced a transient increase in alveolar permeability with an adequate increase in DAFC 6 hours post infection. In a second phase, a permanent increased permeability was associated with a major decrease in DAFC.

Conclusion. – Overall, the epithelial response to *S. pneumoniae* followed a biphasic pattern with an initial reversible increase in permeability related to the alteration of tight and adherens junctions and a second phase associated with an epithelial injury with a major increase in permeability with a decreased DAFC reflecting an injured alveolar capillary barrier.

Keywords: Acute lung injury; Pneumonia; *Streptococcus pneumoniae*

Résumé

Objectifs. – *Streptococcus pneumoniae* est le principal pathogène impliqué dans les pneumonies communautaires. L'objectif de ce travail était de caractériser le mécanisme lésionnel lié à *S. pneumoniae*.

Méthodes. – Nous avons analysé, en utilisant un modèle cellulaire sur cellules 16HBE et un modèle animal murin, la réponse à l'agression épithéliale par *S. pneumoniae*. Des cellules 16HBE en monocouches ont été exposées à *S. pneumoniae*, la perméabilité a été mesurée par la résistance transépithéliale (RTE), ainsi que l'organisation et l'expression des protéines de jonction. Les conséquences fonctionnelles ont été évaluées in vivo dans un modèle murin par le trouble de perméabilité de la barrière alvéolocapillaire, la clairance liquidienne alvéolaire et la réponse inflammatoire.

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Résultats. – In vitro, *S. pneumoniae* induit une diminution dose dépendante de RTE associée à une modification de l'organisation des protéines de jonction évaluée par immunofluorescence à 6 heures. In vivo, *S. pneumoniae* induit une augmentation transitoire de la perméabilité avec une réponse adaptée de la clairance alvéolaire à 6 heures post-infection. Dans une seconde phase, une augmentation pérenne de la perméabilité est associée à une baisse majeure de clairance.

Conclusion. – Globalement, la réponse épithéliale est biphasique, avec une augmentation initiale de perméabilité compensée, par une adaptation de clairance alvéolaire et une seconde phase associée à une altération épithéliale se traduisant par un trouble de perméabilité sans adaptation de clairance alvéolaire.

Mots clés : Agression pulmonaire aiguë ; Pneumonie ; Pneumocoque

1. Introduction

Streptococcus pneumoniae is the leading cause of community-acquired pneumonia [1]. The case fatality rate can reach more than 20% in patients presenting with bacteremia [2]. It is also a predominant cause of severe infections such as bacterial meningitis, septic shock, or frequent but less severe infections such as otitis and sinusitis. Pneumonia develops after inhalation of the pathogen which colonizes the mucosal surface of the host's nasopharynx and upper airways [3]. Through a combination of virulence factors, this pathogen can spread from the upper to the lower respiratory tract.

The pathogenicity of *S. pneumoniae* in the lung is complex and is only starting to be understood. *S. pneumoniae* has the capacity to invade lung tissue to ultimately reach the blood and therefore has to cross both epithelial and endothelial structures. Talbot et al. demonstrated that *S. pneumoniae* could penetrate A549 cells, by both passive and actin microfilament-dependent mechanisms [4]. Many virulence factors of *S. pneumoniae* have been characterized and associated with lung injury such as pneumolysin, pneumococcal cell surface proteins (choline-binding proteins, Psp A, Lyt A), divalent metal-ion-binding lipoproteins, or LPXTG-anchored proteins [5]. Several authors have underlined the key role of pneumolysin in the development of pneumococcus-induced lung injury. Using a mouse model Witzenrath et al. showed in vitro and in vivo that pneumolysin played a key role in early onset acute lung injury [6]. This study was performed with recombinant pneumolysin instilled in the airway. However, the role of the host and of other virulence factors in the development of lung injury was not investigated.

Lung injury is associated with an increased lung permeability. The regulation of paracellular permeability is based on the function of cellular junctions in lung epithelial cells [7]. In these complex structures, tight junctions (TJ) and adherens junctions (AJ) are essential for the control of lung permeability. TJ complex separates apical and basal cell compartments defining cell polarity, and constitutes a continuous epithelial barrier regulating fluid transport. AJ complex maintains intercellular adhesion and regulates macromolecule transport.

To further study the pathogenicity of *S. pneumoniae*, we decided to analyze the lung permeability in vitro and in an in vivo mouse model. We evaluated the consequences of *S. pneumoniae* infection on epithelial permeability and intercellular junctions in a model of human polarized airway epithelium. We then studied in vivo the functional consequences of this infection by measuring alveolar permeability, alveolar fluid clearance, and the alveolar inflammatory response. We demonstrated that *S. pneumoniae* initially induces a transient increase in permeability with an adequate alveolar response, which can be explained by intercellular junction disorganization. After this, a genuine acute lung injury with an impairment of the alveolar response is observed, associated with an important local inflammation.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Streptococcus pneumoniae strain is a clinical serotype 1 isolate grown from a blood culture of a pneumonia patient from the Hospital of Valenciennes, France. The strain was stored in a -80 °C frozen stock. From this stock, bacteria were inoculated on blood agar medium plate and incubated for 15–18 hours under 5% CO_2 atmosphere (C02Gen, Oxoid Ltd, UK). Then, 15 to 20 colonies of this plate were inoculated in 10 ml of a Todd Hewitt growth medium with 0.5% yeast extract (Sigma®) for 4 to 6 hours. Bacterial concentration was determined by measurement of the optical density (CO 8000 Cell Density Meter, WPA Biowave®). The dilutions of the final suspension were cultured on blood agar medium to determine the size of the final inoculum.

2.2. Cell culture

16HBE cells were obtained from Dr. D. Guenert, Colchester, VE. These cells grow on collagen G coated flasks (Biochrom KG®) with DMEM Glutamax medium (Life technologies®) supplemented by 10% Fetal Calf Serum (FCS), 1% antibiotic (Penicillin G sodium 10,000 UI/ml-streptomycin sulfate 10,000 pg/ml). Then $1{\cdot}10^5$ cells were transferred onto Transwell-24-plates (Corning Life Sciences®) collagen G coated. Cell monolayers were grown to reach confluence and to obtain a polarized epithelium as judged by transepithelial resistance (TER). Resistance up to a 200 value was necessary before starting the assay.

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