The effect of virulent factor on mechanical and structural properties of epithelial respiratory cells

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Background/Aims: During quite breathing, adults inhale around 12 000 liters of air containing particles, bacteria, virus and dust, meaning that the airway epithelium is permanently exposed to various aerocontaminants. In that frame**,** respiratory infections due to bacteria remain a major public health issue. Studying the effect of virulent factors is an appropriate way to model these respiratory infections. Accordingly, we study epithelial cell response to bacterial infection by using airway specific virulent factor with the ultimate aim to find new therapeutic targets. We presently explore the cellular and molecular mechanisms of virulent invasion in a cellular model of respiratory epithelial cells exposed to the adenylate cyclase (CyaA), a toxin produced by *Bordetella pertussis*, the causative agent of the wooping cough. Furthermore, CyaA is able to invade a wide range of eukaryotic cells using a unique mechanism that involves direct translocation of its catalytic domain to produce supraphysiological levels of cAMP [1]. By studying the changes in cell functions and cell mechanical properties in the course of an initial cell invasion by CyaA, we expect to assess the potential alterations of mechanotransduction processes induced by bacterial toxins.

Methods: First, we examined the functional effects of CyaA and more precisely, its toxicity on culture of A549 human alveolar epithelial cells by viability test. Then, we estimated the role of the toxin on repair and migration phenomenon by wound healing experiment. To better understand the behavior of the cytoskeleton (CSK) during the invasion of the toxin, we evaluated the role of CyaA on one of major components of CSK and adhesion of A549 cells by staining of actin fibers and focal adhesion (FA) points. The effects of CyaA on mechanotransduction was studied on A549 cells by a new force spectroscopy method which enables to assess the strength of multiple integrin bonds created in the early phase of cell adhesion by an Atomic Force Microscopy (AFM) [2]. Spherical probes of 6.6 µm in diameter coated with RGD peptide are used. This method provides complementary information on mechanical properties of CSK and adhesion properties between cells and matrix molecules. All these experiments are conducted with two monomeric species of CyaA [3], [4] (the active form CyaA and an enzymatically inactive form CyaAE5, i.e, unable to produce cAMP) to assess the role of cAMP on host cells after the translocation of the toxin [5].

Results: Concerning the viability test, we observe that CyaA affects the cell viability in a dose-dependent manner for short time exposure (from 15 to 60 min). At high CyaA concentration (> 0.5nM), the cell viability rate decreases of almost 30% after 60min of exposure to the toxin compared to the control condition. This result is especially marked for long time exposure (24 or 48h). In the case of an exposure to the inactive variant CyaAE5, no significant difference with the control condition was found, even for long time exposure. In the control case, the wound repair is complete after 36 h. In the case of pathogenic exposure, CyaA affects repair in a dose-dependent manner. Indeed, above 0.5nM, the cell monolayer fails to close. Regarding actin fibers and FA, we performed a quantification of the actin levels of fluorescence and the number of the FA points. We note that the levels of fluorescence and the number of FA points decrease drastically in dose-dependent manner. Indeed, after 60 min of exposure to CyaA at more than 0.5 nM, the actin level of fluorescence and the number of FA points decrease of almost 40% compared to the control. This method performed on A549 cells for 3 different CyaA concentrations (0.5, 5 and 10 nM) demonstrates that CyaA toxin significantly affects both cell adhesion (detachment forces are decreased) and cell mechanics (Young's modulus is increased). CyaA toxin (at 0.5 nM) assessed at three indentation/retraction speeds (2,5 and 10 µm/s) significantly affects global detachment forces, local rupture events and Young modulus compared with control conditions, while the enzymatically inactive form CyaAE5 has no effect.

Conclusion: Our results suggest that CyaA induces changes in cell function (viability and repair) and adhesion and mechanical properties of A549 are deeply modified by exposure to the active form of the toxin but not its enzymatically inactive variant. We find that CSK structure, mechanics and adhesion properties are significantly affected after cell exposure to CyaA. Altogether, our results show that the cAMP molecule plays a crucial role in the structural and mechanical properties during intoxication of respiratory cells.

References: 1. Ladant et al, Trends Microbiol, 7:172-76, 1999 ; 2. Nguyen et al, Biol Cell, 109(7) 255-272, 201 ; 3. Karst at al.n JBC, 2014; 4. Cannella et al, Scientific reports, 2017; 5. Angely et al. Biol. Cell, 109, 7-19.

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