Bronchial Smooth Muscle of Severe Asthmatic Patients Decreases Rhinovirus Replication Within the Epithelium Through a CCL-20/PkR/eIF2alpha-Dependent Pathway

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Viral infection of the bronchial epithelium (BE), particularly with rhinovirus (RV) has been implicated in the vast majority of exacerbations in severe asthma. Preventing these exacerbations remains a major unmet need. Bronchial remodeling in severe asthma has been characterized by increased bronchial smooth muscle (BSM) mass, which had a poor prognostic value, since higher BSM mass was associated with increased exacerbation rate. However, the role of asthmatic BSM in RV infections of the BE has never been demonstrated. We thus hypothesized that asthmatic BSM increased both BE susceptibility and response to RV infection. We then enrolled 19 severe asthmatic patients from the COBRA cohort, and 37 control subjects. We designed cell biologic, transcriptomic and proteomic approaches both in vitro using co-culture model of BE with BSM cells and ex vivo using patients’ biopsies. BE cells were cultured and differentiated in air-liquid interface. RV particle number within BE cells was measured using digital PCR. Proteomic and transcriptomic data were analyzed using IPA (Qiagen). Cytokines concentrations in the supernatant were assessed using ELISA assays. Finally, PkR pathway was analyzed by western blotting. Severe asthmatic patients were similar to control subjects in terms of sex ratio, age and BMI. Not surprisingly asthmatic patients presented a lower FEV-1. We found that asthmatic BSM cells co-cultured with BE cells increased RV replication and level of IL-6 in the supernatant compare to that co-cultured with control BSM cells. Large scaled proteomic and transcriptomic analysis highlighted CCL-20 production by asthmatic BSM cells, as a potential candidate to specifically increase the RV infection of the BE cells. Blocking CCL-20 in the co-culture supernatant with asthmatic BSM cells reduced the number of RV particles within the BE cells. Moreover, such a co-culture decreased the PkR pathway activation after RV infection. Indeed, we showed a decreased level of the activated form of PkR and eIF2-alpha. Finally, a direct stimulation of BE cells with CCL-20 reproduced both the increased number of RV particles and the down-regulation of the PkR pathway. In conclusion, these results clearly demonstrated that asthmatic BSM increased the BE response to RV, through an increase of CCL-20 production by BSM, which in turn, down-regulated PKR response of the BE leading to increase RV replication and particles number in BE. To the best of our knowledge, it is the very first time that such a direct bottom-up effect of asthmatic BSM on BE has been demonstrated.

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