Are bronchoalveolar lavages a good source for microbial profiling? Differences between throat and bronchoalveolar lavage microbiomes

Bronchoalveolar lavage (BAL) is a common diagnostic method used to investigate the immune status in the lower respiratory tract. Besides its application in clinical routine, BAL is the method of choice to investigate microbial profiles of the lower respiratory tract in medical research. However, BAL has been criticized as being prone to contaminations, since the bronchoscope passes the throat during the procedure.

Latest advancements in sequencing technology have enabled culture-independent studies of bacterial communities at deep resolution. However, despite a number of recent studies (Charlson et al., 2011; Erb-Downward et al., 2011; Morris et al., 2013), the composition of the healthy human lower respiratory microbiome remains to be defined, and it is still unclear whether BAL generates reliable data in the context of possible contaminations via the oral cavity. To add to this important discussion, here we present for the first time to our knowledge a comparison of the microbial communities from throat swabs (TS) and BAL from healthy individuals.

For analysis, TS and BAL were taken from five healthy individuals who gave their written consent as approved by the local ethics committee of the University Hospital Freiburg (ID 03/10). In order to minimize BAL contamination from the upper respiratory tract (e.g. mouth, throat, larynx, pharynx), BAL was taken with a wedge and only the third return of lavage washing was used. For equal sample conditions, throat swabs were diluted in saline. DNA was extracted with a Molysis Complete 5 kit that includes the degradation of human DNA. Sequencing was performed using 16S rDNA analysis based on 454 FLX technology as previously described with 30 cycles of PCR (Stratil et al., 2013). Analysis of the sequences was carried out using mothur, QIIME and R (Borcard et al., 2011; Caporaso et al., 2010; Schloss et al., 2009). After strict quality control including chimera check with uchime (implemented in mothur), the median sequence number was 9092 ± 1137 sequences for the BAL group and 6993 ± 2982 sequences for the TS group. The sequencing output was normalized to 1000 sequences per sample (Hamady & Knight, 2009). The 16S rDNA sequences were clustered into OTUs (operational taxonomic units) at the 97% similarity level, leading to a total of 17 highly abundant OTUs (> 1%) that were included in the subsequent analysis. The coverage for all samples was over 99%.

Comparison of the bacterial composition on the genus level (top nine genera) of the 10 samples is shown in Fig. 1a. Although clinically healthy, proband no.5 had a strongly deviating taxonomic profile (Fig. 1a, b) and was therefore excluded from statistical testing. Nevertheless, this sample is informative regarding the fact that the bacterial profile of the TS cannot be recovered in the corresponding BAL sample.

Statistical analysis of the remaining data revealed significant differences between BAL and TS (ADONIS; P value = 0.009), with high similarity within the two sample types (ANOSIM, R = 0.472, P = 0.019). These differences are mainly caused by an unequal distribution of the genera Streptococcus and Prevotella (P<0.05). While the TS samples were dominated by Streptococcus, which is consistent with previous studies (Dewhirst et al., 2010), the BAL samples contained mainly Prevotella, Streptococcus and Veillonella. In line with a previous report (Charlson et al., 2011), the sample-wise investigation of shared OTUs between BAL and TS showed that 28–50% of the OTUs appeared in the lung exclusively, suggesting a lung-specific microbiome. To further characterize the lung-specific microbiome, each OTU was tested for an unequal distribution between BAL and TS using a non-parametric t-test. Out of 17 highly abundant OTUs, five differed significantly (uncorrected P value < 0.05). Of these, three OTUs were more abundant in BAL than in TS, representing Prevotella pallens (OTU 33), Veillonella dispar (OTU 34) and Prevotella histicola (OTU 48; Fig. 1c), with OTU 33 being unique to BAL samples.

Even though many OTUs were shared between BAL and throat swabs, the results presented indicate substantial differences in the microbial profiles of the two sampling types, including a Prevotella species that was unique to the lower respiratory tract. This finding confirms a recent report that compared mouse and BAL samples (Morris et al., 2013), although the specific species differ. This points to a highly variable composition of the lung microbial profile in healthy individuals. Further sources of variation stem from the specific sampling procedures and analysis methods. In summary, our study demonstrates that, given the applied methods, BAL sampling is capable of detecting a distinct human lower respiratory microbiome.

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Fig. 1. For analysis of microbial communities, five BAL and five TS samples were taken from healthy individuals. The figure shows (a) relative taxa abundances at the genus level, (b) RDA of the BAL (red dots) and TS samples (blue dots) and (c) raw abundances of the highly abundant OTUs (≥1%). Less frequently occurring species were summarized into rare OTUs. Proband 5 was regarded as an outlier and excluded from statistical testing.
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