

MANNOSE-BINDING LECTIN (MBL) INHIBITS SARS-COV-2 INFECTION AND REPLICATION IN VITRO

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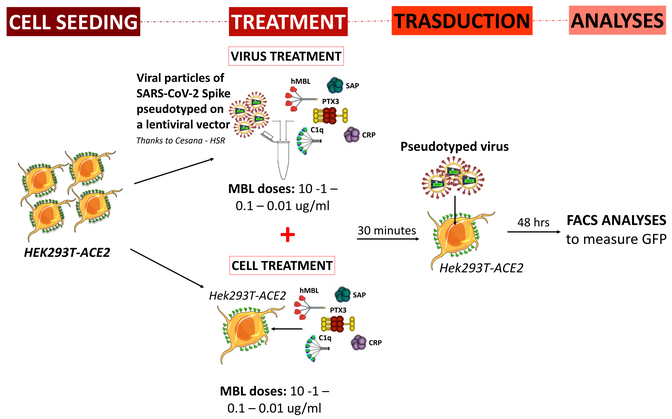
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BACKGROUND

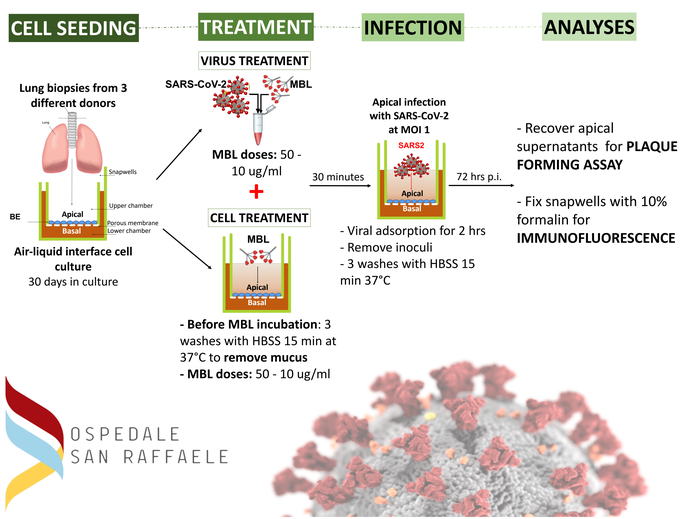
Humoral innate immunity consists of a limited, but diverse, set of humoral fluid phase pattern recognition molecules (PRMs) that represent a first line of resistance against microbial invaders by promoting pathogen disposal by phagocytosis, complement activation and inflammation. These factors encompass complement, ficolin, collectin and pentraxin family of proteins. Aim of our study was to test a panel of PRMs for anti-SARS-CoV-2 activity.

METHODS

- Spike pseudotyped virus neutralization assay



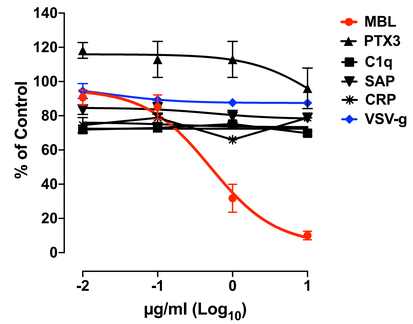
- Infection of human bronchial epithelial cell



The humoral arm of innate immunity includes diverse molecules with antibody-like functions, some of which serve as disease severity biomarkers in COVID-19. Here, we conduct a systematic investigation of the interaction of human humoral fluid phase pattern recognition molecules (PRMs) with SARS-CoV-2. Out of 12 PRMs tested, Mannose-Binding Lectin (MBL) binds to the Spike protein, including the variants of concern (VoC), in a glycan-dependent way and it inhibits SARS-CoV-2 infection in primary human bronchial epithelial cells (HBEC). These results suggest that MBL can play an important role in resistance to, and pathogenesis of COVID-19, a finding with potential translational implications.

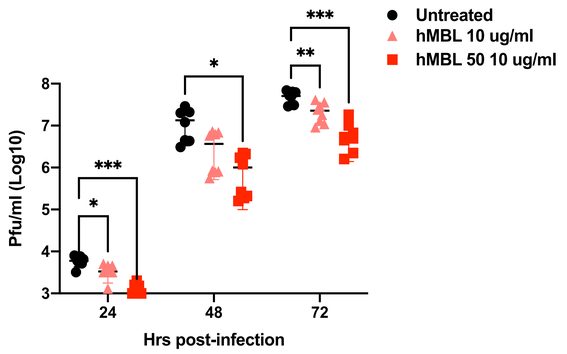
RESULTS

1. MBL inhibits entry of Spike pseudotyped virus



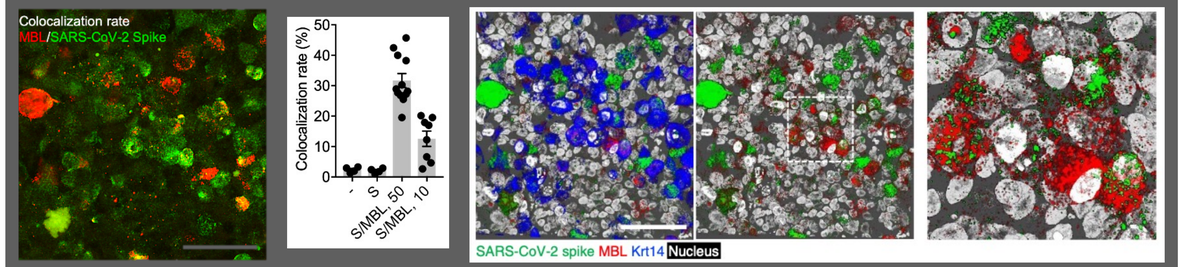
1. Entry of lentiviral particles pseudotyped with SARS-CoV-2 Spike protein in 293T cells overexpressing ACE2 in the presence of ten-fold serial dilutions of humoral innate immunity PRMs (from 0.01 to 10 μg/ml). As control, entry of lentiviral particles pseudotyped with the VSV-g glycoprotein was tested in parallel in the presence of MBL. Percentage of control was calculated as ratio of GFP-positive cells in the presence of humoral PRMs and the GFF-positive cells in the absence of humoral PRMs. Data are means ± SEM, n=3 independent experiments in triplicates with the curves representing a three-parameter dose response model.

2. MBL Inhibition of SARS-CoV-2 infection in human bronchial epithelial cells (HBEC)



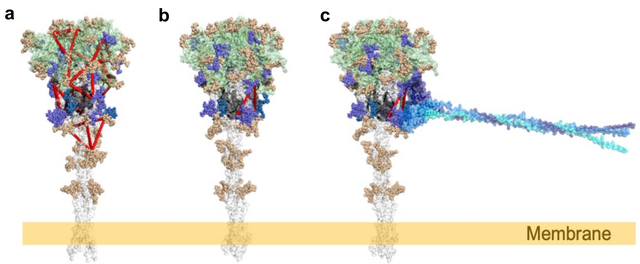
2. SARS-CoV-2 release from the HBEC apical surface after 24-48-72 hrs PI, in the presence of MBL at 10 or 50 μg/ml (34 or 170 nM) MBL. Mean values, n=3 independent experiments performed in triplicate (2 donors) or duplicate (1 donor) cell cultures are shown. P values were determined by one-way ANOVA with Bonferroni's correction. ***: P value = 0.0002 , **: P value = 0.005 , *: P value = 0.01.

3. Colocalization of SARS-CoV-2 Spike protein and MBL in infected HBEC



3. Confocal microscopy analysis of the localization of SARS-CoV-2 Spike protein (green), MBL (red) in HBEC cultures infected by SARS-CoV-2 in presence or not of MBL (50 μg/ml -170 nM). Left panel: extracted signals of SARS-CoV-2 Spike protein and MBL. Bar, 30μm. Middle panel: colocalization rate between SARS-CoV-2 Spike protein and MBL (10 or 50 μg/ml). Each spot corresponds to a single XYZ image presented as MIP. Right panel: 3D rendering reconstruction showing a blended reconstruction of the localization between SARS-CoV-2 Spike protein (green) and MBL (red) in the apical side of the cytokeratin 14 (blue) HBEC cultures. Bar, 30μm.

4. Interaction of MBL with SARS-CoV-2 S protein through its Carbohydrate Recognition Domain (CRD)



4 (a). 14 mannose-binding sites (red triangles), imposed onto Spike protein, where S1 region is green, beginning of S2 region is black, and S2' region is white. **(b)** Putative binding site of MBL2. Pose with the highest site-specific probability to be glycosylated with oligomannose; **(c)** Spike-MBL complex. Glycosylation sites are coloured according to the oligomannose content: Gold < 60%. Purple > 80% up until S2' region. Blue > 80% in the S2' region.

CONCLUSIONS

We found that MBL interacts with Spike and has antiviral activity with an EC50 of approximately 0.08 μg/ml (0.27 nM) and an affinity of 34 nM. These concentrations are well in the range of those found in the blood of normal individuals (up to 10 ug/ml), which increase 2-3-fold during the acute phase response. MBL directly interacts with SARS-CoV-2 Spike and this interaction required a trimeric conformation of the viral protein. The hypothesized MBL binding site spans across the S1 and S2 region of the Spike protein, suggesting a possible neutralization mechanism. However, the mechanisms responsible for the antiviral activity of MBL remain to be fully defined.

MBL has been safely administered to patients with cystic fibrosis and chronic lung infections in which MBL deficiency contributes to pathogenesis. Therefore, these results have translational implications in therapeutic approaches.

ADDITIONAL KEY INFORMATION

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