Innate Immune Response Through NOD1 Agonists Prevents SARS-CoV-2 Infection in Lung Epithelial Cells

Edurne Garcia-Vidal[1], Ignasi Calba[2], Eva Riveira-Muñoz[1], Elisabet García[1], Bonaventura Clotet[1], Pere Serra-Mitjà[3], Cecilia Cabrera[2], Ester Ballana[1], and Roger Badia[1,2].

[1]IrsiCaixa Institute for AIDS Research, Badalona, Spain, [2]Institute for Health Science Research Germans Trias i Pujol (IGTP), Badalona, Spain, [3]Hospital Sant Pau, Barcelona, Spain

E-mail: egvidal@irsicaixa.es
Phone: +34 934656374

402

CROI Conference on Retroviruses and Opportunistic Infections

BACKGROUND

The lung is susceptible to infections caused by respiratory viruses such as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Difficulty to exert a directed antiviral activity at the lung mucosal tract is one of the major roadblocks for the management of respiratory infections. Improving the capacity of the respiratory mucosa to trigger potent immune responses at early stages of the infection, leading to an early viral clearance, is a potential alternative intervention for respiratory infections.

Here, we explored the capacity of reported immunomodulatory compounds to boost innate immune response in lung epithelial cells with the final objective to identify novel therapeutic targets.

METHODS

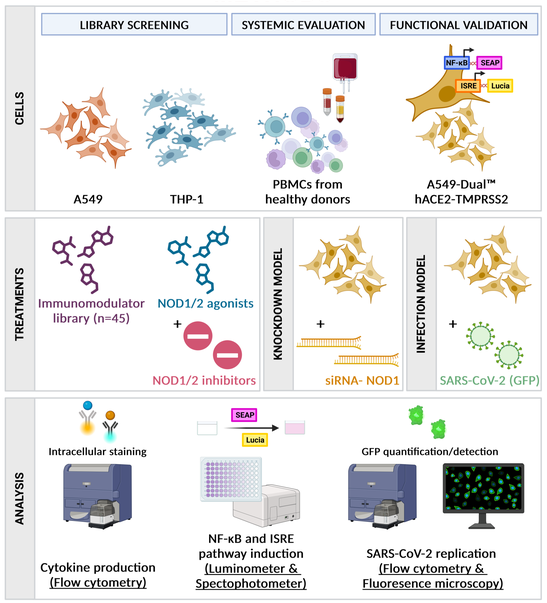


Figure 1. Materials and methods. Cytokine production was evaluated in lung epithelial (A549) and monocytic (THP-1) cell lines. Systemic effect of the hit compounds resulting from the library screening (NOD1 and NOD2 agonists) was tested in PBMCs. NF-κB and ISRE pathway induction was studied in a reporter cell line (A549-Dual™hACE2-TMPRSS2). Target specificity was assessed with NOD1/2 inhibitors and in a knockdown cell model. Anti-SARS-CoV-2 activity was evaluated in an *in vitro* infection model.

RESULTS

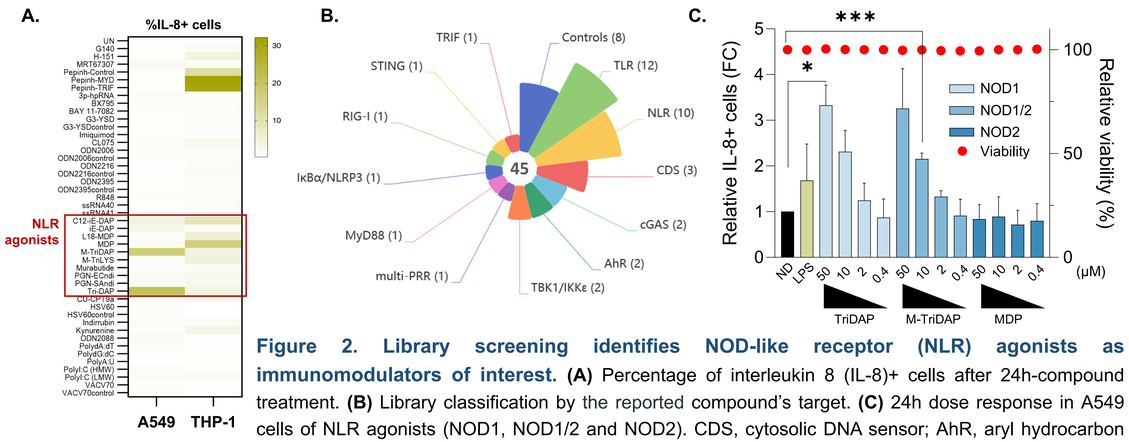


Figure 2. Library screening identifies NOD-like receptor (NLR) agonists as immunomodulators of interest. (A) Percentage of interleukin 8 (IL-8)+ cells after 24h-compound treatment. **(B)** Library classification by the reported compound's target. **(C)** 24h dose response in A549 cells of NLR agonists (NOD1, NOD1/2 and NOD2). CDS, cytosolic DNA sensor; AhR, aryl hydrocarbon receptor; PRR, pattern recognition receptor; FC, fold change; ND, no drug. *p<0.05; ***p<0.001.

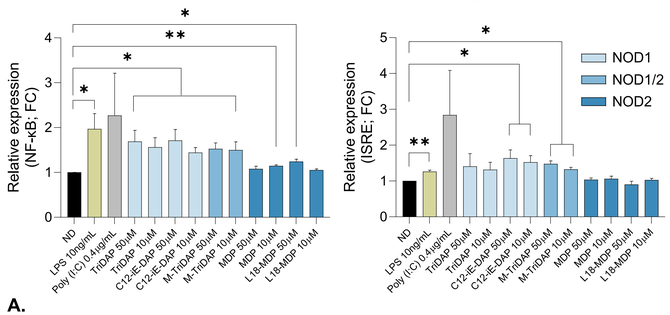
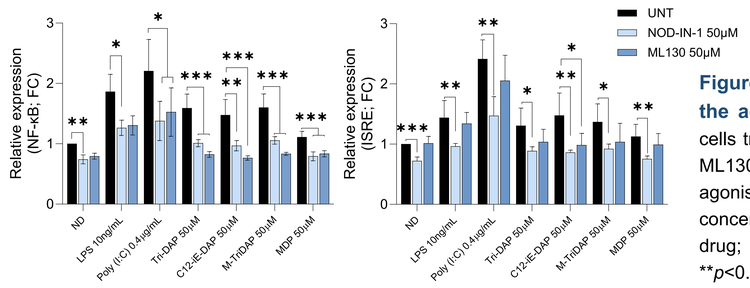


Figure 3. NOD1 agonists activate NF-κB and ISRE pathways. Reporter expression in A549-Dual™ cells incubated for 24h with NOD1 (TriDAP, C12-iE-DAP), NOD1/2 (M-TriDAP) and NOD2 (MDP, L18-MDP) agonists. ND, no drug; FC, fold change. *p<0.05; **p<0.01.



B.

IC50	NOD1	NOD2
ML130	0.56μM	>20μM
NOD-IN-1	5.74μM	6.45μM

Figure 4. NOD1/2 selective inhibitors impair the activity of NOD1 agonists. (A) Reporter cells treated for 2h with the inhibitors (NOD-IN-1, ML130) followed by a 24h incubation with the agonists. **(B)** Half maximal inhibitory concentration (IC50) of the inhibitors. ND, no drug; FC, fold change; UNT, untreated. *p<0.05; **p<0.01; ***p<0.001.

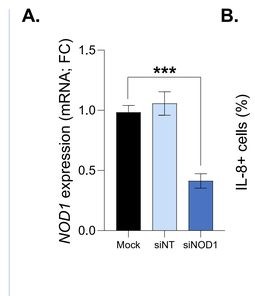


Figure 5. Immunomodulatory activity is dependent on *NOD1* expression. (A) Effective inhibition of *NOD1* mRNA expression by small interfering RNA (siRNA) in A549-Dual™ cells. **(B)** IL-8 production induced by the agonists is inhibited in the *NOD1* knockdown model. ND, no drug; NT, non-targeting; FC, fold change. **p<0.01; ***p<0.001.

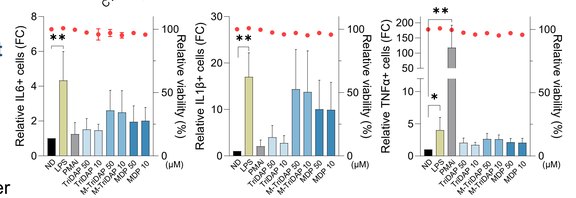
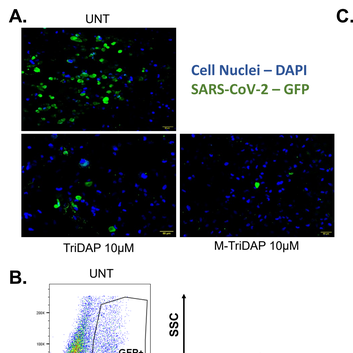


Figure 6. NOD1 agonists do not elicit innate immune activation in PBMCs. Induction of IL-6, IL1β and TNFα is not observed in PBMCs after 24h incubation with the NOD1 agonists. ND, no drug; FC, fold change. **p<0.01.



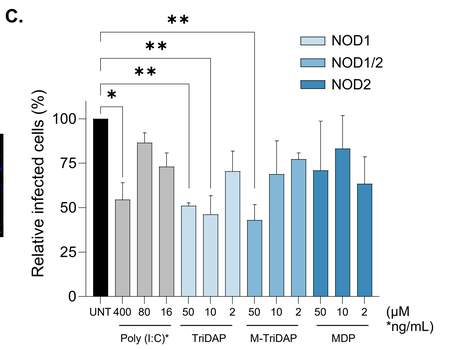


Figure 7. NOD1 agonists inhibit SARS-CoV-2 infection *in vitro* in lung epithelial cells. Fluorescence microscopy **(A)** and flow cytometry **(B)** images of infected cells. **(C)** NOD1 agonists pretreatment blocks viral replication (%GFP) 48h post-infection. UNINF, uninfected; UNT, infected untreated. *p<0.05; **p<0.01.

NOD1 agonists are able to induce an **immune response** with antiviral potential. This innate immune activation is **specific of epithelial lung cells** and residual in immune-derived cells.

In vitro activation of **NOD1** signaling protects **against SARS-CoV-2 infection** in lung epithelial cells.

CONCLUSIONS

- The innate immune response is activated by NOD1 agonists in human lung epithelial cells with the production of IL-8 and the activation of the NF-κB and ISRE pathways.
- NOD1 expression is required for the agonists' activity, demonstrating specificity of the effect.
- NOD1 (TriDAP) and dual NOD1/2 (M-TriDAP) agonists promote an antiviral state that prevents SARS-CoV-2 replication in lung epithelial cells.

This study has been funded by Instituto de Salud Carlos III, Fondo de Investigación Sanitaria (FIS) through the project PI22/01575 (co-funded by European Regional Development Fund/European Social Fund) "Investing in your future"). EGV (SLT017/20/000090, PERIS, Departament de Salut is research fellow from Generalitat de Catalunya and Fons Social Europeu. RB, and EB are research fellows from ISCIII-FIS (CP19/00011 and MSII19/00012, respectively).