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ERAPs Control In Vitro and Ex Vivo SARS-CoV-2 Infection by Triggering Antiviral Immune Responses

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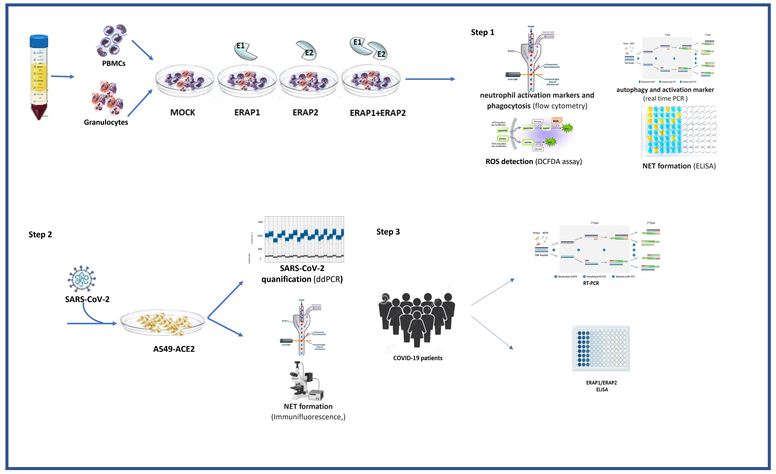
BACKGROUND

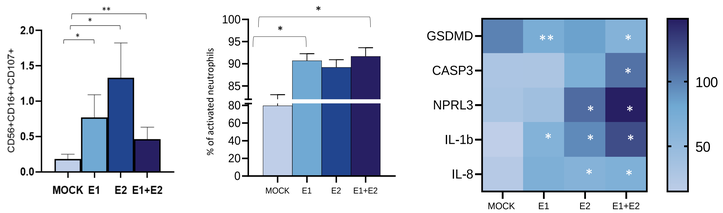
ERAP1 and ERAP2 (ERAPs) are two endoplasmic reticulum aminopeptidases which control susceptibility/progression of different immune related-human diseases ranging from autoimmunity, to cancer, preeclampsia, and viral infections. Beyond their canonical role in antigen processing and presentation, following inflammatory stimuli ERAPs can be secreted and modulate several features of both the acquired and natural immune response. In the extracellular milieu ERAP1 works as a robust innate immunity activator, inducing: 1) the activation of inflammatory processes by enhancing phagocytoses and nitric oxide (NO) synthesis by macrophages 2) the trimming of peptides with N-terminal arginine residues, favouring NO production; 3) inflammatory cytokine production in human PBMCs through NOD-like receptor, pyrin domain-containing (NLRP) 3 activation. Secreted ERAP2 fosters the monocyte/macrophage lineage activation, by increasing inflammasome assembly and phagocytosis. In addition, ERAP2 has been recently demonstrated to induce, through upregulation of unfolded protein response (UPR), the activation of autophagy in pancreatic stellate cells.

METHODS



RESULTS

rhERAPs induce innate immunity activation

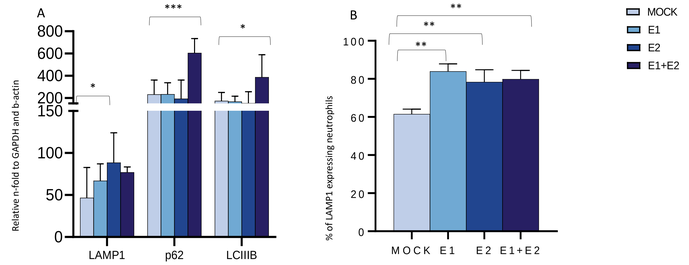


rhERAP stimulation prompts innate immune activation. Granulocytes and PBMCs isolated from 5 HCs were stimulated with 300 ng/ml of rhERAP1, rhERAP2, or rhERAP1+rhERAP2 for 3 h. **(A)** The percentage of total CD56+ natural killer expressing activation marker CD16++ and CD107 and (**B**) CD45$^+$ CD15$^+$ neutrophils expressing the activation markers CD11+ and CD66b+ was assessed by flow cytometry. **(C)** mRNA expression of activation markers was evaluated by RT-PCR the p value is relative to MOCK condition. Mean values and SEM are shown. *p < 0.05, **p < 0.01.

RhERAP-shaping of neutrophil homeostasis results into a reduced SARS-CoV-2 internalization and viral replication in A549-ACE2 cells.

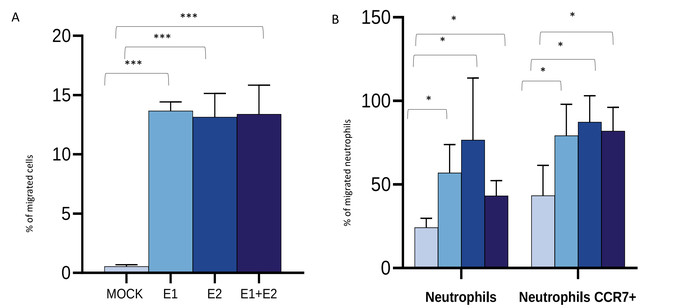
ERAP production is associated with a better prognosis to COVID-19.

rhERAP administration alters neutrophil autophagy



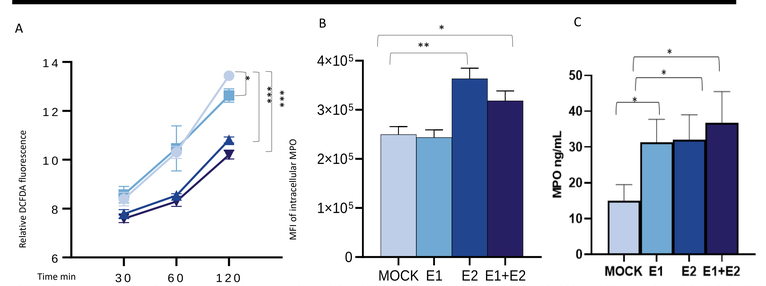
rhERAPs stimulation favors neutrophil autophagy. Granulocytes isolated from 5 HCs were stimulated with 300 ng/ml of rhERAP1, rhERAP2, or rhERAP1+rhERAP2 for 3 h. (**A**) mRNA expression of autophagy markers was evaluated by RT-PCR. **(B)** The percentage of total CD45$^+$ CD15$^+$ neutrophils expressing the autophagy marker LAMP1+ was assessed by flow cytometry. Mean values and SEM are shown. *p < 0.05, **p < 0.01, ***p < 0.001.

Neutrophil migration is prompted by rhERAP administration



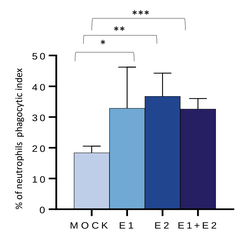
rhERAPs administration prompts neutrophil migration. Transwell migration assays were performed to assess neutrophil migration 3h after rhERAPs administration**. (A)** Bars represent the mean percentage of cells recovered in the lower chamber compared to the number of cells added to the top chamber at the beginning of the assay. **(B)** The percentage of neutrophile migration was assessed by counting the CD15+ and CCR7+ percentage of neutrophils in the lower chamber**.** Each bar represents the mean of 5HC experiments ± SEM. *p < 0.05, ***p < 0.001.

rhERAP administration triggers neutrophil cytotoxic activity



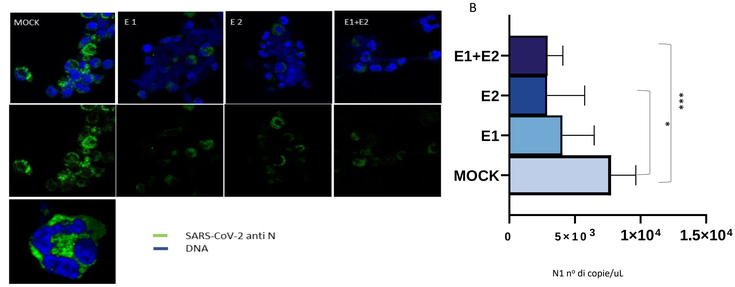
rhERAPs treatment regulates neutrophils cytotoxic activity. (**A**) Granulocytes were cultured with 300ng rhERAP1, 2 or COMBO for 24h, ROS accumulation was measured using a 2',7'-dichlorofluorescein (DCFDA) assay at different time points after DCFDA infiltration. DCFDA fluorescence was normalized to DAPI fluorescence and expressed compared to the number of cultured cells. (**B**) The percentage of neutrophils expressing intracellular MPO+ following rhERAP stimulation was assessed by flow cytometry (**C**) while the MPO released was assessed by ELISA. Mean values and SEM are shown. *p < 0.05, **p < 0.01, ***p < 0.001.

rhERAP administration fosters neutrophil phagocytosis



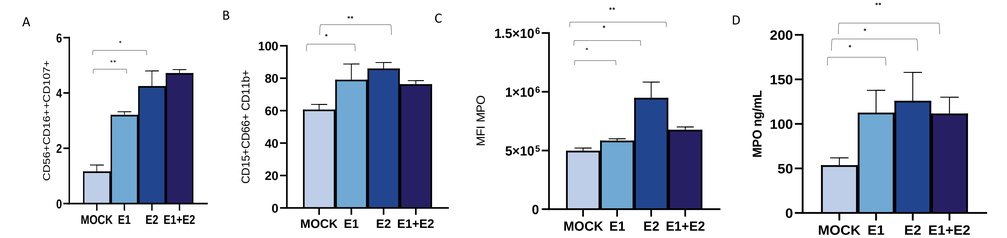
rhERAP stimulation increases neutrophil phagocytosis. The % of neutrophil phagocytic index was evaluated by flow cytometry. Mean values and SEM are shown. *p < 0.05 , **p < 0.01, ***p < 0.001.

rhERAP treatment affects SARS-CoV-2 internalization and viral replication



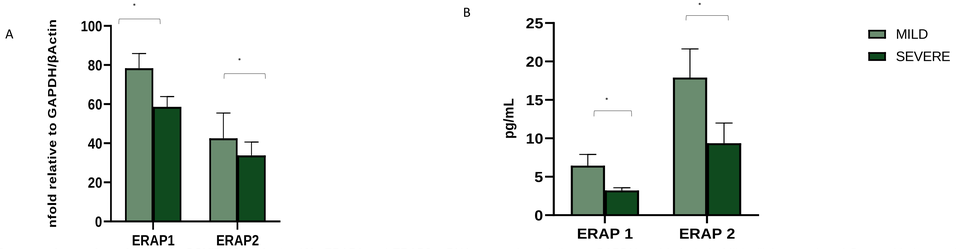
rhERAP stimulation reduces SARS-CoV-2 internalization and replication in mixed cell culture. (**A**) Confocal fluorescence images of SARS-CoV-2 anti N (green), and DAPI (blue) of neutrophils 6h after rhERAPs treatments; scale bars: 20 µm. (**B**) SARS-CoV-2 N1 concentration in supernatant of mixed cell culture (A549 expressing ACE2 and Neutrophils treated with rhERAP proteins) at 72h post SARS-CoV-2 infection. *p < 0.05, ***p < 0.001.

rhERAPs treatment induces innate immunity activation in SARS-CoV-2 infection



rhERAP stimulation prompts neutrophil immune activation in SARS-CoV-2 infected A549-ACE2 cells. Granulocytes and PBMCs isolated from 5 HCs were pre-stimulated with 300 ng/ml of rhERAP1, rhERAP2, or rhERAP1+rhERAP2 for 3 h co-cultured with A-549 and infected with SARS-CoV-2. (**A**) The percentage of total CD56+ natural killer expressing activation marker CD16++ and CD107 and (**B**) CD45+ CD15+ neutrophils expressing the activation markers CD11+ and CD66b+ as well as neutrophil cytotoxic activity (**C**) was assessed by flow cytometry. (**D**) MPO released by neutrophils was assessed by ELISA. Mean values and SEM are shown. *p < 0.05, **p < 0.01.

Severe patients display low intracellular and plasmatic concentration of ERAPs



ERAP expression and production in COVID-19 patients. (**A**) ERAP1 and ERAP2 mRNA expression relative to GAPDH and b-Actin in Milde (light green) and Severe (dark green) patients . (**B**) ERAP1 and ERAP2 plasmatic concentration in Milde (light green) and Severe (dark green) patients. Mean values and SEM are shown. *p < 0.05.

CONCLUSIONS

RhERAPs trigger several cellular/antiviral mechanisms in neutrophils suggesting that their anti-SARS-CoV-2 potential is not limited to their canonical role in antigen presentation and CD8+ T cell activation. In particular, we observed:

1) NK and Neutrophil activation in uninfected and infected conditions;
2) The induction of cell autophagy verified through LC3B and LAMP1 increased expression;
3) Increased number of neutrophil migration;
4) Decreased ROS intracellular accumulation;
5) Augmented neutrophil extracellular traps formation and phagocytosis.

Dissecting the molecular and cellular mechanisms that can be triggered by rhERAP treatments is crucial in the light of new emerging ERAP-based personalized treatments to treat different immune-based diseases including viral infections.