PATHOGENIC MONOCYTE, TH17 AND NK CELL IMMUNE NETWORKS IN THE LUNG FROM CRITICAL COVID-19 PATIENTS

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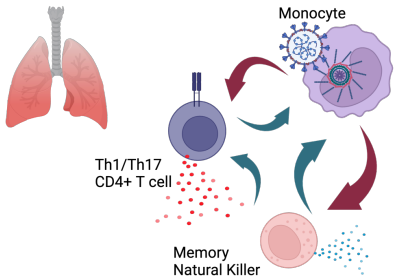
BACKGROUND

Pathogenic inflammation in the lung has been connected to non-classical monocytes (NC-Mo), Natural Killer (NK) cells and Th1/Th17 cell immune responses in critical COVID-19 patients. However, the pathogenic inflammatory networks in the pulmonary tissue of these patients, including interactions between specific innate immune cell subsets, the underlying molecular mechanisms involved in their activation and the functional impact on pathogenic Th1/Th17 cells is not completely understood and this knowledge may lead to new therapeutic targets.

METHODS

Frequency, activation and functional maturation of NC-Mo and memory NK cell subsets from n=17 Bronchoalveolar lavage (BAL) and n=16 peripheral blood (PB) samples from critical COVID-19 patients and n=9 healthy controls were assessed at baseline or after *in vitro* stimulation with SARS-CoV-2 Spike (S1) peptide pools. Histological distribution and activation of Mo and Th1/Th17 was also addressed by immunofluorescence in lung tissue from n=3 deceased critical COVID-19 patients. Induction of SARS-CoV-2 specific Th1 and Th17 cells in response to S1 peptides or S1-primed Mo was analyzed in PB and BAL from critical COVID-19 patients. Correlation network between immune, functional and clinical parameters from BAL samples was also assessed.

SCHEMATIC ABSTRACT



Activation of myeloid cells may associate with Th1/Th17 and memory NK cell activation and conform pathogenic immune cell networks in the lung of critical COVID-19 patients

RESULTS

SARS-CoV-2 S1 induced Mo differentiation into CD14lo CD16+ NC-Mo in BAL and PB samples from critical COVID-19 patients (**Figure 1A**) and induced NFκB p65 phosphorylation (**Figure 1B**). Moreover, we confirmed the differentiation of NC-Mo required NFκB activation (**Figure 1C**).

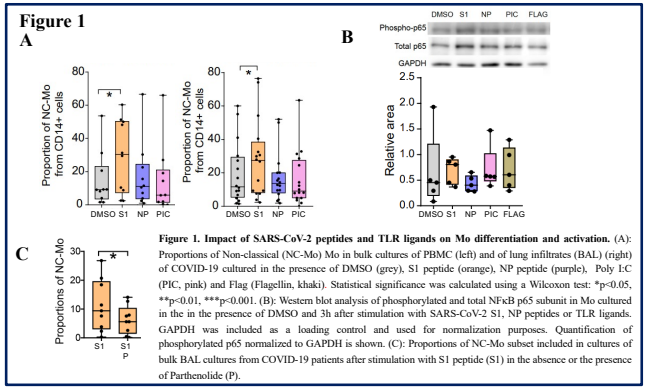


Figure 1. Impact of SARS-CoV-2 peptides and TLR ligands on Mo differentiation and activation. (A): Proportions of Non-classical (NC-Mo) Mo in bulk cultures of PBMC (left) and of lung infiltrates (BAL) (right) of COVID-19 cultured in the presence of DMSO (grey), S1 peptide (orange), NP peptide (purple), Poly I:C (PIC, pink) and Flag (Flagellin, khaki). Statistical significance was calculated using a Wilcoxon test: *p<0.05, **p<0.01, ***p<0.001. (B): Western blot analysis of phosphorylated and total NFκB p65 subunit in Mo cultured in the in the presence of DMSO and 3h after stimulation with SARS-CoV-2 S1, NP peptides or TLR ligands. GAPDH was included as a loading control and used for normalization purposes. Quantification of phosphorylated p65 normalized to GAPDH is shown. (C): Proportions of NC-Mo subset included in cultures of bulk BAL cultures from COVID-19 patients after stimulation with S1 peptide (S1) in the absence or the presence of Parthenolide (P).

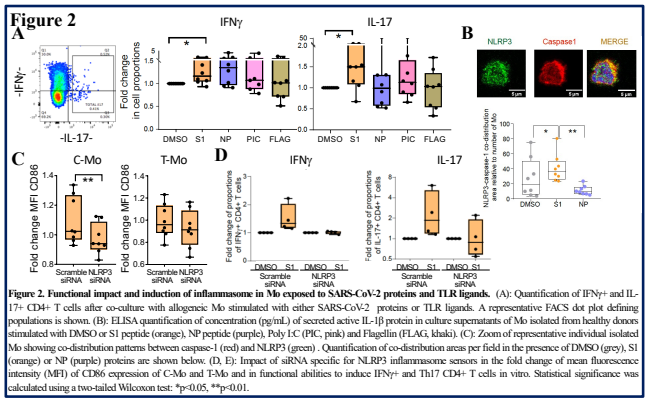


Figure 2. Functional impact and induction of inflammasome in Mo exposed to SARS-CoV-2 proteins and TLR ligands. (A): Quantification of IFNγ+ and IL-17+ CD4+ T cells after co-culture with allogeneic Mo stimulated with either SARS-CoV-2 proteins or TLR ligands. A representative FACS dot plot defining populations is shown. (B): ELISA quantification of concentration (pg/mL) of secreted active IL-1β protein in culture supernatants of Mo isolated from healthy donors stimulated with DMSO or S1 peptide (orange), NP peptide (purple), Poly I:C (PIC, pink) and Flagellin (FLAG, khaki). (C): Zoom of representative individual isolated Mo showing co-distribution patterns between caspase-1 (red) and NLRP3 (green) . Quantification of co-distribution areas per field in the presence of DMSO (grey), S1 (orange) or NP (purple) proteins are shown below. (D, E): Impact of siRNA specific for NLRP3 inflammasome sensors in the fold change of mean fluorescence intensity (MFI) of CD86 expression of C-Mo and T-Mo and in functional abilities to induce IFNγ+ and Th17 CD4+ T cells in vitro. Statistical significance was calculated using a two-tailed Wilcoxon test: *p<0.05, **p<0.01.

In addition, activation of NC-Mo in response to S1 was associated with higher ability to induce IFNγ+ and IL-17+ CD4+ T cells (p=0.0156) (**Figure 2A**). In addition, stimulation of Mo with S1 protein induced the NLRP3 inflammasome (**Figure 2B**), and this was required for CD86 upregulation (**Figure 2C**). Moreover, functional ability to induce Th1/Th17 cells after S1 stimulation was dependent on NLRP3 inflammasome (**Figure 2D**). Importantly, NLRP3+Caspase1+ Mo, IFNγ+ and IL-17+ T cells were detected in lung infiltrates from deceased COVID-19 patients (**Figure 3**).

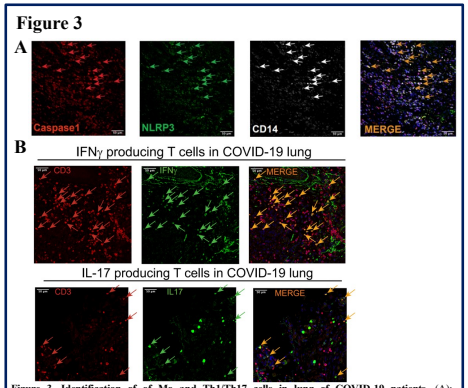


Figure 3. Identification of Mo and Th1/Th17 cells in lung of COVID-19 patients. (A): Representative 40X magnification confocal microscopy images showing analysis of expression of Caspase-1 (red), NLRP3 (green), CD14 (white) and merged images including DAPI (orange) from lung tissue from critical n=3 COVID-19 patients. (B): Representative confocal microscopy images showing analysis of IFNγ (upper panel, green) or IL-17 (bottom panel, green) expression on CD3+ T (red) cells present in the lung tissue of three critical n=3 COVID-19 patients tested.

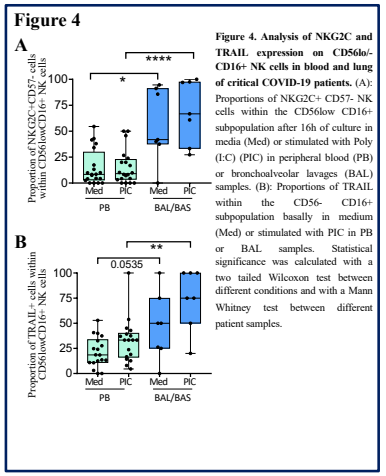


Figure 4. Analysis of NKG2C and TRAIL expression on CD56lo/-CD16+ NK cells in blood and lung of critical COVID-19 patients. (A): Proportions of NKG2C+ CD57- NK cells within the CD56low CD16+ subpopulation after 16h of culture in media (Med) or stimulated with Poly (I:C) (PIC) in peripheral blood (PB) or bronchoalveolar lavages (BAL) samples. (B): Proportions of TRAIL within the CD56- CD16+ subpopulation basally in medium (Med) or stimulated with PIC in PB or BAL samples. Statistical significance was calculated with a two tailed Wilcoxon test between different conditions and with a Mann Whitney test between different patient samples.

Additionally, memory NKG2C+ NK cells were enriched in BAL and PB in critical COVID-19 patients and increased expression of TRAIL on this subset correlated with higher frequencies of NC-Mo in the lung (p=0.016) (**Figure 4**), and increased levels of systemic inflammation biomarkers such as procalcitonin (p=0.017) C-reactive protein (p=0.0027) and ferritin (p=0.016) in these subjects (**Figure 5**). Consistently, higher levels of the inflammasome target ferritin correlated with increased detection of Th17 cells in these individuals (p=0.037) (**Figure 5**).

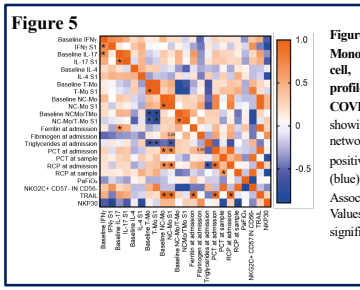


Figure 5. Association between Monocyte subsets with CD4+ T cell, NK cell and clinical profiles in the lung of critical COVID-19 patients. Heatmap showing Spearman correlation network. Colors represent positive (orange) or negative (blue) R values of association. Associations reaching (*p<0.05). Values close to statistical significance are also highlighted.

CONCLUSIONS

Interconnection between inflammasome activation in Mo, induction of Th1/Th17 and NKG2C+ memory NK cells feed pathogenic immune cell environments during critical COVID-19 and may represent promising therapeutic cellular targets.

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