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The unbalanced p53/SIRT1 axis may impact lymphocyte homeostasis in COVID-19 patients

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ABSTRACT

Background/objectives: A dysregulated inflammatory profile plays an important role in coronavirus disease-2019 (COVID-19) pathogenesis. Moreover, the depletion of lymphocytes is typically associated with an unfavourable disease course. We studied the role and impact of p53 and deacetylase Sirtuin 1 (SIRT1) on lymph-monocyte homeostasis and their possible effect on T and B cell signalling.
Methods: Gene expression analysis and flow cytometry were performed on peripheral blood mononuclear cells (PBMC) of 35 COVID-19 patients and 10 healthy donors (HD). Inflammatory cytokines, the frequency of Annexin+ cells among CD3+ T cells and CD19+ B cell subsets were quantified.
Results: PBMC from COVID-19 patients had a higher p53 expression, and higher concentrations of plasma proinflammatory cytokines (IL1β, TNF-α, IL8, and IL6) than HD. Deacetylase Sirtuin 1 (SIRT1) expression was significantly decreased in COVID-19 patients and was negatively correlated with p53 ($p = 0.003$ and $r = -0.48$). A lower expression of IL-7R and B Cell linker (BLNK), key genes for lymphocyte homeostasis and function, was observed in COVID-19 than in HD. The reduction of IgK and IgL chains was seen in lymphopenic COVID-19 patients. A significant increase in both apoptotic B and T cells were observed. Inflammatory cytokines correlated positively with p53 (IL-1β: $r = 0.5$ and $p = 0.05$; IL-8: $r = 0.5$ and $p = 0.05$) and negatively with SIRT1 (IL1-β: $r = -0.5$ and $p = 0.04$; TNF-α: $r = -0.4$ and $p = 0.04$).
Conclusions: Collectively, our data indicate that the inflammatory environment, the dysregulated p53/SIRT1 axis and low expression of IL7R and BLNK may impact cell survival, B cell signalling and antibody production in COVID-19 patients. Further studies are required to define the functional impact of low BLNK/IL7R expression during severe acute respiratory syndrome coronavirus-2 infection.

Introduction

In patients with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, excessive inflammatory responses are considered to play a major role in the pathogenesis of severe coronavirus disease-2019 (COVID-19) disease, leading to acute respiratory distress syndrome and multiple-organ failure (Moore and June, 2020). Dysregulated inflammatory profile, defective immune responses and lymphopenia have also been identified as

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important features of severe disease (Del Valle et al., 2020; Bordoni et al., 2020). Transcriptional profiling of peripheral blood mononuclear (PBMC) cells from SARS-CoV-2-infected patients show an increase in p53 signalling pathway suggesting the involvement of cell apoptosis in the pathogenesis of COVID-19 (Xiong et al., 2020). However, the impact of p53 on lymphocyte homeostasis in severe and mild cases of COVID-19 remains to be defined. We analysed p53 and deacetylase Sirtuin (SIRT1) transcriptional levels in PBMC and their possible effects on T and B cell signalling in 35 patients with COVID-19 disease and 10 healthy donors (HD). The level of apoptosis of T and B lymphocytes was also studied in COVID-19 patients.

Methods

Ethics

The study protocol was approved by the Institutional Review Board of the INMI "Lazzaro Spallanzani" (Protocol approval: N° 9/2020). All the patients signed a written informed consent before enrolment.

Study subjects

Thirty-five COVID-19 patients were enrolled. Inclusion criteria: the molecular diagnosis of SARS-CoV-2 infection by using RT-PCR on the nasopharyngeal swab; exclusion criteria: HIV, HBV, HCV infection, pregnancy and ongoing therapy with immunomodulatory drugs. Fourteen enrolled patients showed severe/critical COVID-19 that requires intensive care unit admission [5 female and 9 male patients; age median 68 years (IQR 50–77)]. Twenty-one patients (15 female and 6 male patients; age median 58.5 years (IQR 47.75–71)] showed mild COVID-19 (PaO_2/FIO_2 >300). Peripheral blood was collected soon after hospital admission. Aged matched HD were enrolled as controls.

Laboratory procedures

Peripheral Blood Mononuclear Cells (PBMC) isolation

PBMC were isolated from peripheral blood by density gradient centrifugation (Lympholyte-H; Cederlane).

Molecular analysis

RNA was extracted from PBMC by using Quick-RNA MiniPrep Kit (Zymo Research) and each cDNA sample was obtained using the reverse transcription kit (Promega) according to the protocol kits. Real time PCR reactions were performed with the Corbett Research Rotor Gene 6000 analyzer using FastStart Essential DNA Green Master (Roche Life Science) according to the manufacturer's instructions. The expression levels were normalized to the β2-microglobulin level using the equation 2-ΔCt. Primers used in this study were listed in Table 1.

Table 1
Sequences of primers.

Gene	Primer	Sequence
p53	Forward	GCCGTCCCAAGCAATGGATGATTT
	Reverse	TCTGGCATTCTGGGAGCTTCATCT
p21	Forward	TCAAAGGCCCGCTCTTACATCTTCT
	Reverse	TAGGAACCTCTCATTCAACCGCCT
SIRT1	Forward	GCAGATTAGTAGGCGGCTTG
	Reverse	TCTGGCATGTCCCACTATCA
BLNK	Forward	CGGACTCAGAGATGTACGTGAT
	Reverse	GGCTTGATCGATTGTCTATATACTC
IL-7R	Forward	CCCTCGTGGAGGTAAAGTGC
	Reverse	CCTTCCCGATAGACGACACTC
β2-microglobulin	Forward	GAGTATGCCTGCCGTGTG
	Reverse	AATCCAAATGCGGCATCT

Flow cytometry

To assess the apoptotic profile of T and B lymphocytes, we analysed the frequency of Annexin+ cells among CD3+ T cells and CD19+ B cell subsets in a selected group of COVID-19 patients (n = 15) as compared to HD, by Annexin V-FITC Apoptosis Detection Kit (eBioscience) according to the manufacturer's instruction. Briefly, PBMC was stained for 20 min at 4 °C with surface antibodies anti-CD3 PerCP Cy5.5 and anti-CD19 APC (BD). After washing (PBS 1X), cells were incubated for 10 min at room temperature with annexin V-FITC using the kit-binding buffer. Annexin V+ cells were evaluated in CD3+ cells and CD3–CD19+ cells.

Mature B cells were evaluated by cytoplasmic immunoglobulin κ/λ chain (Igκ and Igλ) mean fluorescence intensity using BD OneFlow™ PCST tube (BD Biosciences) according to the manufacturer's instruction. The percentage of CD127+ cells was quantified by flow cytometry using BD LyoTube 8-color CD4 and CD8 bundle (BD Biosciences). A total of 100,000 events were acquired on FACSCanto II flow cytometer and analysed by using DIVA software.

Inflammatory parameters

Plasma samples were obtained by the centrifugation of peripheral blood for 10 min at 1800 rpm and immediately stored at −80 °C until use. IL1-β, IL-6, IL-8, TNF-α, and IL-7 were measured in plasma samples by using an automated ELISA assay (ELLA microfluidic analyzer, Protein Simple, Bio-Techne, USA).

Statistical analysis

Quantitative variables were compared with a nonparametric Mann–Whitney test. A p value lower than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism v8.0 (GraphPad Software, Inc.). Spearman's rank test was used to determine correlations.

Results

To evaluate the impact of COVID-19 on lymphocyte homeostasis, we first compared the level of p53 expression in PBMC in COVID-19 patients (n = 35) and from (HD, n = 10). PBMC in COVID-19 patients showed a significant higher p53 expression (Figure 1A) with respect to HD, which confirms previous data (Xiong et al., 2020). The expression of the deacetylase SIRT1 was lower in COVID-19 patients (Figure 1B) and was negatively correlated with p53 (p = 0.003 and r = −0.48, Figure 1C). Furthermore, a significant higher level of p21, an essential mediator of p53-dependent cell-cycle arrest (Brugarolas et al., 1995), was observed in COVID-19 patients than in HD (Figure 1D).

To assess the apoptotic profile of T and B lymphocytes, we analysed the frequency of Annexin+ cells among CD3+ T and CD19+ B cells in a subset of COVID-19 patients (n = 15). As reported in Figure 1E–F, a higher apoptotic T and B cell percentage was observed in COVID-19 patients than in HD.

To evaluate the possible role of inflammation in modulating the apoptotic processes, we quantified the plasma level of IL1β, IL-6, IL-8 and TNF-α, confirming a higher level of inflammation in COVID-19 than in HD (Figure 1G). Of note, the inflammatory cytokines were correlated positively with p53 (IL-1β: r = 0.5, p = 0.05 and IL-8: r = 0.5, p = 0.05 and Figure 1H) and negatively with SIRT1 (IL1-β: r = −0.5, p = 0.04 and TNF-α: r = −0.4, p = 0.04 and Figure 1I).

We thus analysed the impact of p53 upregulation on the expression of important positive regulators of T and B lymphocytes development and homeostasis [IL-7/IL-7R and B cell linker (BLNK), respectively]. Although a high plasmatic IL-7 level (Figure 2A), we found a significant lower IL-7R expression in PBMC from COVID-19

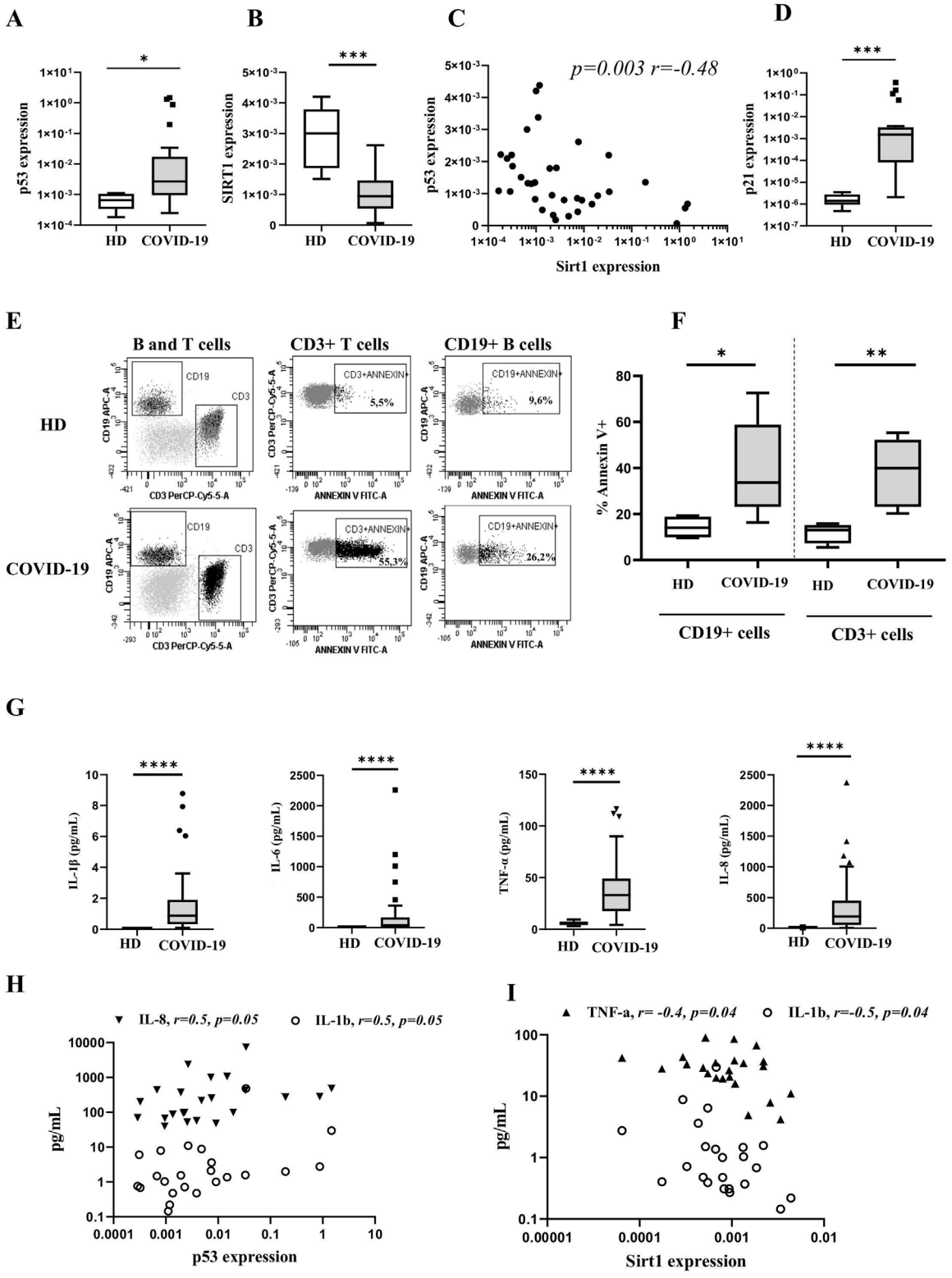

Figure 1. p53/Sirt-1 axis expression, apoptosis and inflammation in COVID-19 patients. (A, B, D) Expression of p53, Sirt-1 and p21 was quantified in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 35). Results are shown as Box and Whiskers graphs. (C) The correlation between p53 and SIRT1 expression is shown in COVID-19 patients (n = 35). (E, F) Representative cytometry plots show the Annexin staining on CD3 and CD19 cells from COVID-19 patients and HD. The median percentage of Annexin+ CD3 and CD19 cells in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 15) are shown as a Box and Whiskers graph. (G) The level of inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) was quantified in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 23). (H, I) The correlations between p53 and inflammatory cytokines (IL-8 and IL1-β), and between SIRT1 and inflammatory cytokines (IL-1β and TNF-α) are shown in COVID-19 patients (n = 23).
Differences were evaluated by Mann–Whitney test; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ and ****: $p < 0.0001$.

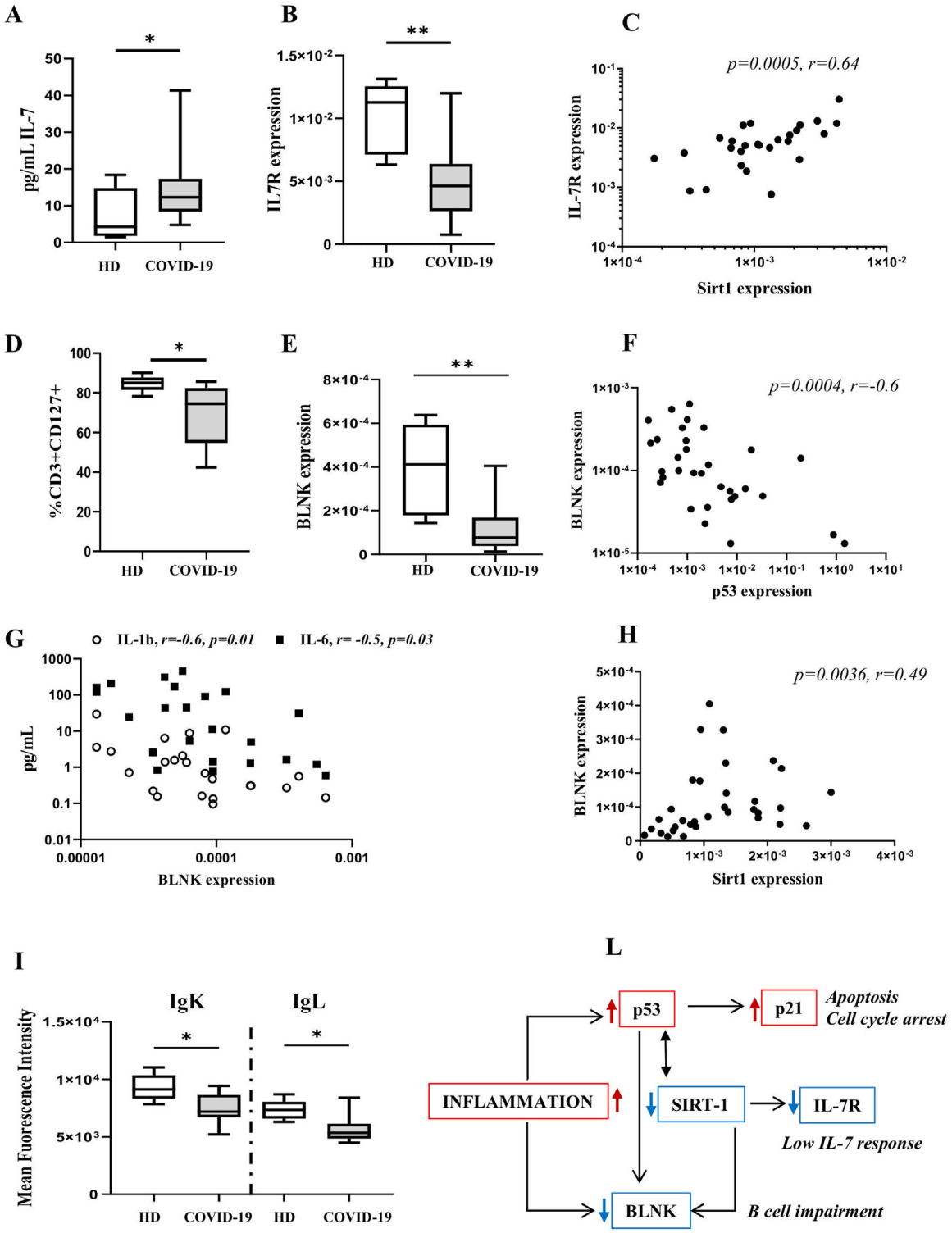

Figure 2. T and B cell regulator expression and IgG level in PBMC from COVID-19 patients. (A) The level of plasmatic IL-7 was quantified in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 35). (B) The gene expression of IL-7 receptor in PBMC was evaluated in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 27). (C) The correlation between SIRT1 and IL-7R is shown in COVID-19 patients (n = 27). (D) The percentage of CD127+ cells among T cells was analysed by flow cytometry in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 27). (E) The gene expression of BLNK in PBMC was evaluated in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 35). (F) The correlation between BLNK and p53 is shown in COVID-19 patients (n = 35). (G, H) The correlation tests between BLNK and IL-1β, IL-6 (n = 23) and between BLNK and SIRT1 are shown in COVID-19 patients (n = 35). (I) The mean fluorescent intensity of IgK and IgL was evaluated on B cells by flow cytometry in HD (white bars, n = 10) and in COVID-19 patients (grey bars, n = 15).

than HD (Figure 2B), that was positively correlated with SIRT1 (p = 0.0005, r = 0.64 and Figure 2C). This result was confirmed by the analysis of the percentage of CD127 (IL-7 receptor α) positive cells among CD3+ T cells. Flow cytometry analysis showed a significantly lower frequency of CD127-expressing T lymphocytes in COVID-19 patients than in HD (Figure 2D), suggesting a reduced IL-7 response. Accordingly, a reduced lymphocyte count was observed in COVID-19 patients [COVID-19: median

1.26 cells/mmc (IQR: 0.74–1.78) vs HD: median 2.01 cells/mmc (IQR: 1.8–2.14)].

We finally explored the B cell signalling and found a significant lower BLNK expression in COVID-19 patients than in HD (Figure 2E), which was negatively correlated with p53 ($p = 0.0004$, $r = -0.6$ and Figure 2F) and with inflammatory cytokines ((IL1-β: $r = -0.6$, $p = 0.01$ and IL-6: $r = -0.5$, $p = 0.03$ and Figure 2G) and positively with SIRT1 ($p = 0.0036$, $r = 0.49$ and Figure 2H). As BLNK has an effect on antibody production, we investigated a possible relationship between BLNK and Ig chains expression. The expression of both IgK and IgL was lower in B cells of COVID-19 patients than in HD (Figure 2I), which suggests that the signal from BCR may be defective in COVID-19 patients.

Discussion

Dysregulated inflammatory profiles, defective immune response and lymphopenia have been identified as critical factors involved in the pathogenesis of SARS-CoV-2 infection (Del Valle et al., 2020; Bordoni et al., 2020). In this work, we found a dysregulated p53/SIRT1 axis that results in an unbalance of T and B cell homeostasis and function.

The significant higher p53 expression in PBMC of COVID-19 patients confirms previous observations by Xiong et al. (2020). We showed that the high p53 expression correlates with a significant reduction in the expression of the deacetylase SIRT1 and was associated with a higher expression of p21 in COVID-19 patients. SIRT1 is a pleiotropic protein able to target several transcription factors, thus it orchestrates several intracellular pathways associated with cell death/survival (Mendes et al., 2017; Kwon and Ott, 2008). A direct interaction between SIRT1 and p53 is well known, as the p53 deacetylation through SIRT1 can inhibit p53 activity (Yi and Luo, 2010). Therefore, our data suggest that COVID-19 can be characterized not only by an increase in p53 transcript in circulating lymphocytes but also by a persistently activated p53 form, possibly due to the low level of SIRT1. Accordingly, the high level of p21 observed in COVID-19 patients may also participate in the cell-cycle arrest (Brugarolas et al., 1995), thus it contributes to lymphopenia in severe COVID-19.

The lymphopenia described in COVID-19 patients confirmed previous data (Huang et al., 2020 395: 497–506; Bordoni et al., 2020) and can be at least partially due to an increase in apoptotic processes. Accordingly, we found an increase of apoptotic T and B lymphocytes in COVID-19 patients. This observation was in line with reports on SARS-CoV-1 infection during the 2003 SARS outbreak, and it seems to be mediated by indirect mechanisms rather than by direct viral infection/replication pathways (Chan and Chen, 2008).

These data bring forth the discussion regarding the actual impact is of p53 upregulation on the expression of positive regulators of T and B lymphocytes development and homeostasis [IL-7/IL-7R and BLNK, respectively] (Barata et al., 2019; Shengli and Kong-Peng, 2002; Minegishi et al., 1999). Whilst higher plasma IL-7 levels were observed, as shown by De Biasi et al. (2020), we found a lower IL-7R expression in PBMC from COVID-19 than HD, which was positively correlated with SIRT1, suggesting a reduced responsiveness to IL-7 that may contribute to the dysregulation of T-cell homeostasis and lymphopenia.

BLNK bridges B cell receptor-associated kinase activation with downstream signalling pathways. A lower BLNK expression in COVID-19 patients than in HD was observed, which was negatively correlated with p53, suggesting an interplay between p53 and functional pathways in B cell response. Accordingly, B cells from COVID-19 patients expressed a lower level of both IgK and IgL than in HD, highlighting a defect in the BCR signal in COVID-19 patients; thus, impairing activation, proliferation and differentiation of mature B lymphocytes.

Collectively, our data indicate that the inflammatory environment, the unbalanced p53/SIRT1 axis and the low expression of key genes of lymphocytes homeostasis (IL7R and BLNK) may impact T and B cell survival, B cell signalling and antibody production in COVID-19 patients (Figure 2L). Further studies are required to define the functional impact of BLNK/IL7R expression during SARS-CoV-2 infection.

Conflict of interest

All authors declare no conflicts of interest.

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References

Barata JT, Durum SK, Seddon B. Flip the coin: IL-7 and IL-7R in health and disease. Nat Immunol 2019;20(12):1584–93.
Bordoni V, Sacchi A, Cimini E, Notari S, Grassi G, Tartaglia E, et al. An inflammatory profile correlates with decreased frequency of cytotoxic cells in COVID-19. Clin Infect Dis 2020;71(16):2272–5.
Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannonet GJ, et al. Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature 1995;12(377):552–7.
De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun 2020;611(1) 3434 1–17.
Chan PKS, Chen GG. Mechanisms of lymphocyte loss in SARS coronavirus infection. Hong Kong Med J 2008;4:21–6.
Del Valle DM, Schulze KS, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med 2020;26(10):1636–43.
Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
Kwon HS, Ott M. The ups and downs of SIRT1. Trends Biochem Sci 2008;33 (11):517–25.
Minegishi Y, Rohrer J, Coustan-Smith E, Lederman HM, Pappu R, Campana D, et al. An essential role for BLNK in human B cell development. Science 1999;3 (286):1954–7.
Moore BJB, June CH. Cytokine release syndrome in severe COVID-19. Science 2020;368(6490):473–4.
Mendes KL, de Farias Lelisc D, Sousa Santos SH. Nuclear sirtuins and inflammatory signaling pathways. Cytokine Growth Factor Rev 2017;38:98–105.
Shengli X, Kong-Peng L. Delayed cellular maturation and decreased immunoglobulin k light chain production in immature B lymphocytes lacking B cell linker protein. J Exp Med 2002;196(2):197–206.
Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020;9(1):761–70.
Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. Biochim Biophys Acta 2010;1804(8):1684–9.