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BRIEF REPORT

Low levels of the key B cell activation marker, HLA-DR, in COVID-19 hospitalized cases are associated with disease severity, dexamethasone treatment, and circulating IL-6 levels

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Introduction

More than 1 year after the beginning of the coronavirus disease 2019 (COVID-19) pandemic, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a public health threat, despite the rollout of vaccination programmes. According to the World Health Organization (WHO), more than 219 million COVID-19 cases and over 4.5 million deaths have been reported worldwide, at the beginning of September 2021. Clinical data showed that 10–15% of hospitalized infected patients progress to severe complications [1].

During the last few months, many studies were performed on the kinetics of antibody responses in infected individuals, according to disease severity. For unclear reasons, the production of long-lasting antibodies against the SARS-CoV-2 appears to be compromised in a proportion of asymptomatic and symptomatic cases [2]. Our team recently evidenced that anti-SARS-CoV-2 Immunoglobulin G are rapidly vanishing in severe patients [3]. It is thus of paramount importance to quickly understand the cellular and molecular mechanisms responsible for the rapid decline of antibodies to SARS-CoV-2, especially in severe patients who are at greater risk of death. A deep immune profiling of COVID-19 patients revealed that there were no significant changes in the major B cell subsets related to disease severity [4]. However, little remains known about B cell activation status in severe versus non-severe cases. Major histocompatibility complex (MHC) class II proteins expressed by B cells are essential for their interaction with T cells and subsequent emergence of short-lived and long-lived antibody-producing cells [5]. As it is now well established that severe COVID-19 is associated with a loss of MHC class II molecules (HLA-DR) on monocytes [6], we evaluated surface expression of HLA-DR on circulating B cells.

Materials and methods

This study was conducted at the Teaching Hospital of La Réunion which is the reference center for infectious diseases in the Indian Ocean Region. The hospital takes care of SARS-CoV-2-infected individuals originated from La Réunion, metropolitan France and other territories, such as Mayotte. From the day of the first RT-PCR positive test, on March 11, 2020 until mid-August 2021, more than 46 000 COVID-19 cases were diagnosed on the island (i.e., around 5% of the population). We obtained leftover refrigerated EDTA blood samples from 59 hospitalized patients, as part of their care. These patients were classified as "mild," ($N = 14$) "moderate," ($N = 30$) or "severe" ($N = 15$) according to the WHO clinical progression scale [7]. The study was approved by the COVID-19 scientific committee of our hospital. As part of the validated COVID-19 research protocol initiated at the beginning of the epidemic (March 2020), patients were enrolled if they consented for the

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study (non-opposition and for research use only) at the time of entry at the hospital. Consents, given orally to the medical doctor in charge of the patient's care, were recorded in the medical file. Flow cytometry and ELISA experiments were carried out from the leftover blood taken to make the diagnostic.

All blood samples were collected between day 7 and day 13 after COVID-19 infection, a time frame which corresponds, in most cases, to day 2 to 8 after symptom onset, and when disease can turn into a severe form [8]. Of note, at the time of blood sampling, moderate and severe patients who were under dexamethasone ($N = 13$ for the moderate group; $N = 12$ for the severe group) treatment had received a dose of 6 mg daily for 72 to 96 h. This dose is in accordance with the RECOVERY trial performed in hospitalized patients with COVID-19 in the UK [9].

Unvaccinated healthy donors ($N = 12$, including 7 women and 5 men with an age range of 24 to 65 years) were recruited from the University and the Teaching Hospital of La Réunion under informed agreements and signed consents. They were all RT-PCR negative for COVID-19 and seronegative for nucleocapsid antibodies ("Human COVID-19 IgG antibody Elisa kit" from MyBiosource, reference: MBS3809906) at the date of flow cytometry analysis.

Flow cytometry

Flow cytometry experiments were performed within the first 4–5 h after blood sampling (at the beginning of each day). Whole blood samples could be stored at + 4°C up to 2 h during this time but samples were not frozen between sampling and staining. This process was standardized in our laboratory. For all patients, 100 µL of whole blood was mixed with 2.5 µL of monoclonal antibodies against CD19 (Beckman Coulter, reference: A07769) and HLA-DR (Beckman Coulter, reference: IM1638U). The appropriate isotype controls (Beckman Coulter, reference: A07794) were used to set the background staining levels. Samples were then incubated at room temperature in the dark, for 30 min. Red blood cells were then lysed with the Beckman ImmunoPrep™ Reagent System (Beckman Coulter, reference: 7,546,999). Flow cytometry analysis was performed with the Becton Dickinson C6 Plus Accuri™ flow cytometer and data were extracted by using the BD Accuri™ C6 software version 1.0. Viable lymphocytes were gated by using forward scatter versus side scatter and HLA-DR expression was assessed based on mean fluorescence intensity (MFI) analysis on a total of 10,000 CD19 + B cells.

Enzyme-linked immunosorbent assay (ELISA)

Interleukin-6 (IL-6) levels were measured on plasma samples using commercially available ELISA kit (eBioscience, reference: 88–7066-88), according to the manufacturer's instructions. Plasma samples were prepared from whole blood (the same used for flow cytometry analysis) following centrifugation (20 min at 2000 g) and stored at −80°C until IL-6 analysis in a batch.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 software. Categorical variables were presented as frequency rates and percentages, and continuous variables were described using either means and standard deviations (SDs) for normally distributed data, or medians and interquartile ranges (IQRs) for data that were not normally distributed.

Proportions for categorical variables were compared using the Fisher exact test. Means for continuous variables were compared using independent group t tests when the data were normally distributed; otherwise, the Mann–Whitney test was used. Statistical significance was set at the 0.05 probability level.

Results and discussion

The mean of age of our study population was 57 years (SD, 17) and 51% were men. COVID-19 severity was associated with neutrophilia and lymphocytopenia, as well as increased plasma CRP levels (Table 1: see SI).

As demonstrated in other studies [10, 11], the frequency of CD19 + B cells was increasingly higher in our cohort of COVID-19 patients depending on disease severity. Healthy

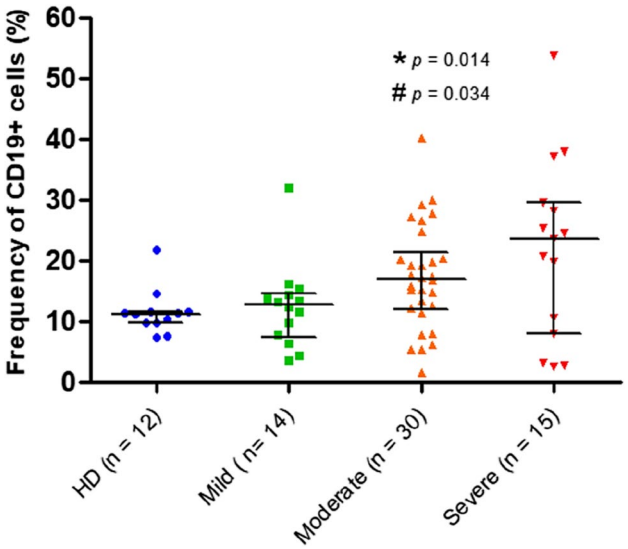

Fig. 1 Increased frequency of CD19 + B cells in moderate and severe COVID-19 hospitalized cases. For each patient, the percentage of CD19 + B cells was calculated among total lymphocytes by flow cytometry. Data are presented as median and IQR. Statistical significance ($p < 0.05$) is indicated as follows: (*) comparison versus the healthy donors (HD) group; (#) comparison versus the mild group

subjects, mild, moderate, and severe patients had, respectively, an average of 11.6%, 12.5%, 17.3%, and 20.7% of CD19 + B cells (Fig. 1). When absolute cell numbers were analyzed, we did not detect significant changes in the B cell compartment between healthy donors and COVID-19 cases of any category (data not shown).

A gradual and significant decline of B cell surface expression of HLA-DR was evidenced according to disease severity. Healthy donors had the highest average of MFI values (31,731 ± 10,027). This average significantly decreased by 36% in the moderate group and by 53% in the severe group compared to the value observed in the mild one (Fig. 2a). Of note, this significant drop in HLA-DR levels cannot be attributed to a potential depletion of HLA-DR positive plasmablasts due to long samples storage as flow cytometry experiments were carried out within the first 4–5 h after blood sampling [12].

As glucocorticoids have been reported to reduce HLA-DR expression on peripheral B cells in vivo [13], we evaluated this contribution in patients treated with dexamethasone (DXM). Our results showed that, in moderate patients intravenously injected with DXM, MFI was significantly reduced by 34% compared to the value observed in patients without glucocorticoid treatment (Fig. 2b). We argued that DXM may not be the sole factor involved in the reduced expression of HLA-DR on B cells. Indeed, in patients without DXM treatment, HLA-DR levels were significantly lessened by 32% in moderate versus mild patients (Fig. 2c). Of note, when comparing severe and moderate patients with DXM treatment, HLA-DR expression was 21% lower in the severe group, although this result did not reach statistical significance (Fig. 2d).

We thus sought to further investigate other mechanisms which may affect HLA-DR expression on B cells. IL-6 is known to inhibit HLA-DR expression on CD14 + monocytes of severe COVID-19 patients [6]. We measured the plasma concentration of this cytokine in the different group of patients. Healthy donors and mild cases had low levels of IL-6 (< 20 pg/mL). As expected, IL-6 levels were significantly increased according to illness severity and reached an average of 74 pg/mL in the severe group (Fig. 3a). Moreover, we found significant higher levels of IL-6 in moderate versus mild patients without DXM therapy (Fig. 3b), also in severe versus moderate patients with DXM therapy (Fig. 3c). In agreement with these results, a negative significant correlation was found between plasma amounts of IL-6 and levels of B cell HLA-DR expression in COVID-19 cases

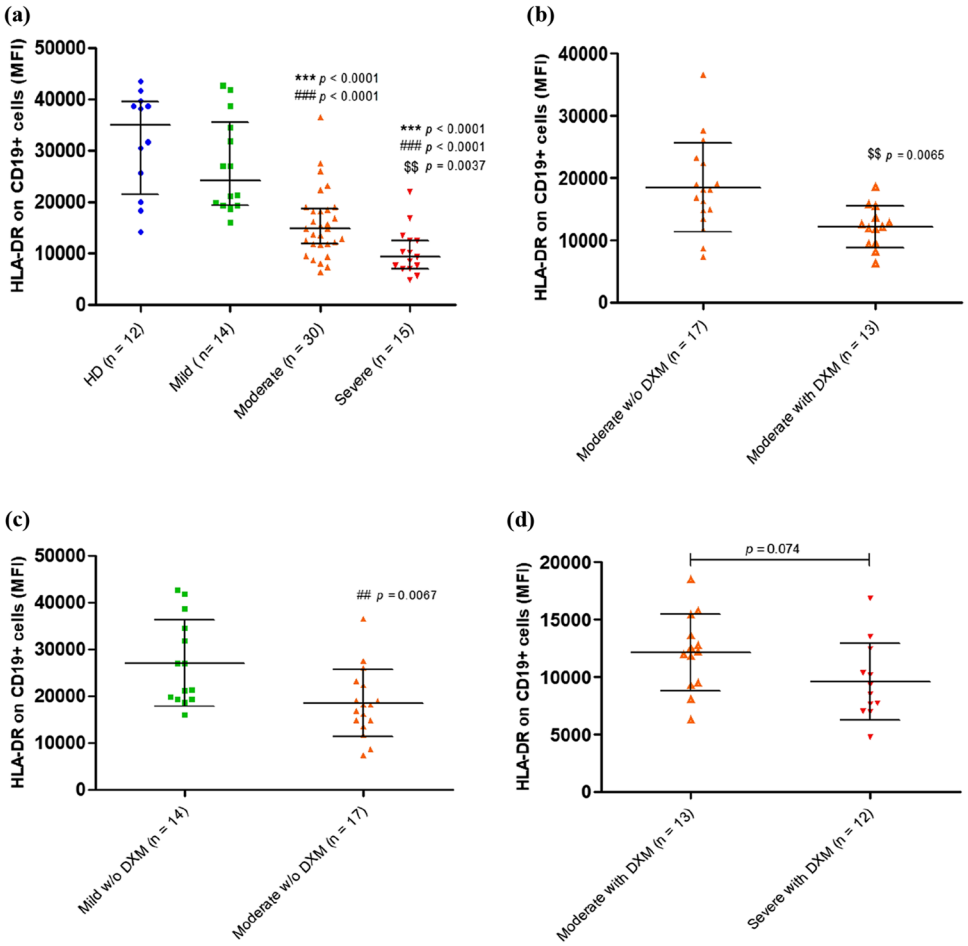

Fig. 2 Decreased HLA-DR surface expression on B cells of COVID-19 hospitalized cases: the impact of disease severity and DXM treatment. For each patient, HLA-DR expression was evaluated on CD19 + B cells by flow cytometry (MFI), according to disease severity (**A**) and comparing patients with or without (w/o) DXM treatment (**B**), (**C**) and (**D**). Data are presented as median and IQR (**A**) and mean and SD (**B**), (**C**) and (**D**). Statistical significance ($p < 0.05$) is indicated as follows: (*) comparison versus the healthy donors (HD) group; (#) comparison versus the mild group; ($) comparison with the moderate group

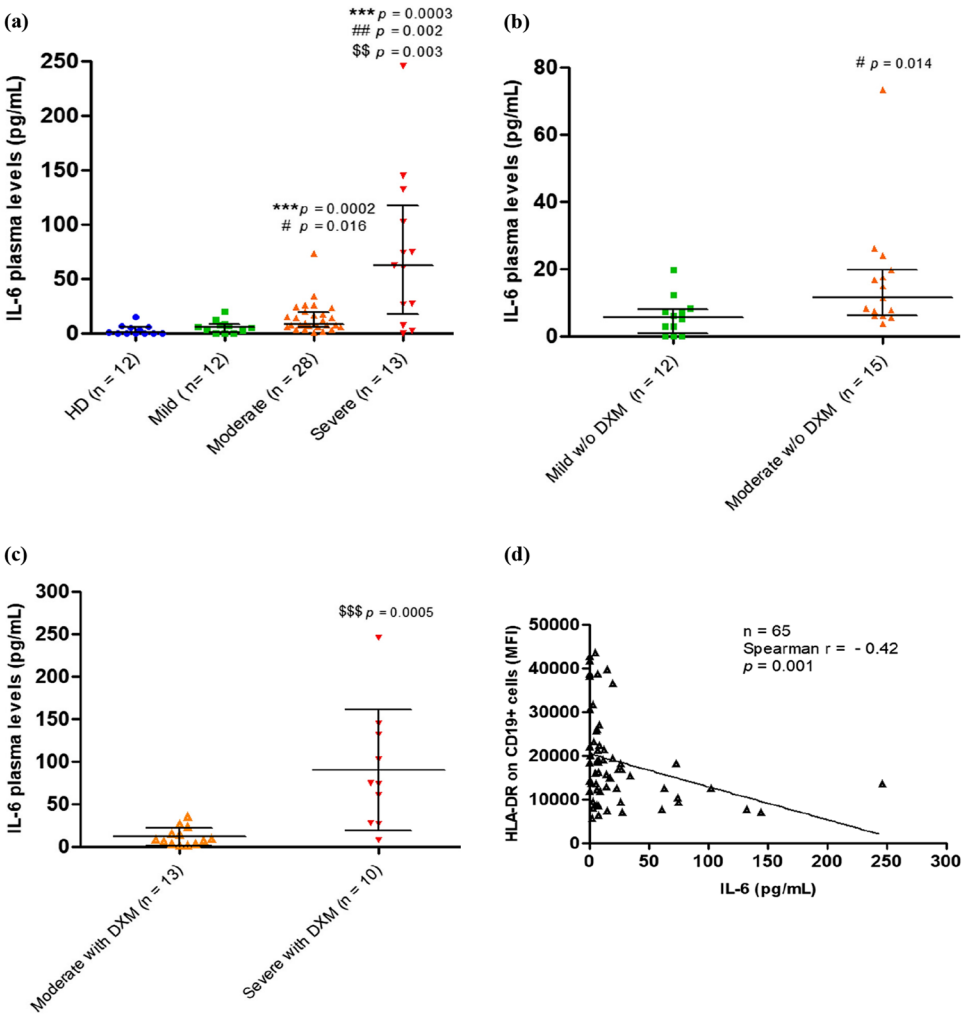

Fig. 3 DXM-independent reduction of HLA-DR expression on B cells is correlated with IL-6 plasma levels. For each patient, IL-6 plasma levels were measured by ELISA, according to disease severity (**A**) and comparing different groups of patients (**B**) and (**C**). Of note, the analysis could not be performed for two patients of each category (**A**) due to insufficient plasma samples. Data are presented as median and IQR (**A**) and (**B**), and mean and SD (**C**). Statistical significance ($p < 0.05$) is indicated as follows: (*) comparison versus the healthy donors (HD) group; (#) comparison versus the mild group; ($) comparison with the moderate group. A negative correlation was found between B cell HLA-DR and IL-6 levels (D). Linear regression (solid line), Spearman correlation coefficient (r) and *p*-value are depicted. Of note, the correlation was made with the data of patients for whom IL-6 plasma levels could be measured ($N = 53$), but also with those of healthy donors ($N = 12$)

(Fig. 3d). Although our investigation suggests that IL-6 may, at least, in part, control HLA-DR levels on B cells, other complementary experiments using IL-6 blockers should be performed to confirm this finding. Interestingly, in the context of other diseases (cancer), IL-6 also reduces HLA-DR expression on dendritic cells in a STAT3-dependent manner, thus impairing the activation of CD4 + T cells [14]. Mechanisms by which DXM could further downregulate B cell HLA-DR expression are the potentiation of the IL-6/STAT3 pathway, and the inhibition of the transcription factor NF-κB, as previously described [15–17]. Other cytokines such as IL-10 that trigger STAT3 phosphorylation may also modulate HLA-DR levels on B cells. In this regard, Qin et al. demonstrated that IL-10 levels were negatively correlated with HLA-DR expression on monocytes of COVID-19 cases [18]. Of note, several other cytokines and chemokines, including, among others, IL-2, TNF-α, IFN-γ, and CCL2, are increased in patients with severe COVID-19 and could be also correlated with a lower quantitative expression of HLA DR on B cells [18,19

Another way by which SARS-CoV-2 could cause the decrease of HLA-DR surface expression in moderate and severe patients is the direct infection of B cells. Indeed, it is well described that SARS-CoV-2 viremia is associated with COVID-19 severity and that the virus can infect lymphocytes through spike interaction with CD147 protein that is highly expressed on various immune cells, including activated lymphocytes [20–22]. Accumulating evidence has suggested that an impaired type I interferon response occurs in severe subjects [23]. This may compromise HLA-DR expression enhanced by STAT1 activation.

Our data are consistent with those obtained by Kazancioglu et al. showing that the percentage of activated T cells (CD3 + HLA-DR +) and B cells (CD19 + CD38 +) is lower in severe COVID-19 patients [24]. It is thus likely that a low activation status of B cells precedes a decline in specific

antibody response to SARS-CoV-2, particularly in severe cases.

In conclusion, our data indicate that low expression levels of HLA-DR on circulating B cells can be attributed to both DXM intravenous therapy and COVID-19 severity on its own (correlation found with IL-6). This report emphasizes the need to have major follow-up studies regarding moderate and severe COVID-19 cases given their low B cell activation status and, thus, their higher risk to develop a weak long-term humoral response. This is of paramount importance since it is now well established that SARS-CoV-2 reinfection is possible. Further studies should also be conducted in vaccinated individuals. Indeed, some investigations pointed out that the antibody response induced by spike mRNA vaccines starts to wane at around 2–3 months, and, moreover, the presence of SARS-CoV-2 spike protein in epithelial cells can promote IL-6 release [25–27]. Passive immunization that does not require the activation of immune cells might be an alternative approach for critical cases who do not develop persistent humoral immunity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12026-022-09269-w.

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Author contribution Conceptualization: Philippe Gasque, Anthony Dobi; Methodology: Anthony Dobi, Arthur Dubernet, Mahary Lalarizo Rakoto, Anne-Laure Sandenon Seteyen, Damien Vagner, Grégorie Lebeau, Loïc Raffray; Formal analysis and investigation: Anthony Dobi, Arthur Dubernet, Mahary Lalarizo Rakoto, Damien Vagner, Grégorie Lebeau; Writing—original draft preparation: Anthony Dobi; Writing—review and editing: Anthony Dobi, Arthur Dubernet, Mahary Lalarizo Rakoto, Anne-Laure Sandenon Seteyen, Damien Vagner, Grégorie Lebeau, Loïc Raffray, Philippe Gasque; Funding acquisition: Philippe Gasque; Resources: Philippe Gasque; Supervision: Philippe Gasque.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and informed consent The procedures used in this study adhere to the tenets of the Declaration of Helsinki. All patients consented ("not opposed to" and for research only) to be included in this study and as part of the institutional care program of COVID-19 patients attending the Teaching Hospital of La Réunion.

Conflict of interest The authors declare no competing interests.

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