Immune monitoring of a child with autoimmune hepatitis and type 1 diabetes during COVID-19 infection

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Immunocompromised patients may be at increased risk to develop COVID-19 during the 2019 β-coronavirus infection. We present the unique opportunity we had to monitor the liver, IL-6 and immune cell course before, during and after COVID-19 in a boy with autoimmune hepatitis (AIH) and type 1 diabetes (T1D). $CD4^+$ and $CD8^+$ T cells frequencies decreased because of prednisolone, followed by a plateauing increase whereas $CD19^+CD20^+$ B cell increased strongly and was unaffected by COVID-19 infection. Moreover, the percentage of activated $CD8^+$ T cells expressing HLA-DR ($CD8^+$HLA-DR^+) increased during COVID-19 and subsided after its clearance. Total regulatory T cells (Tregs: $CD4^+CD25^+CD127^{low}FOXP3^+$) remained stable. Although activated Tregs ($CD4^+CD45RA^-FOXP3^{high}$) strongly increased upon prednisolone, it decreased afterwards. Furthermore, regulatory B cells (Bregs: $CD19^+CD20^+CD24^{high}CD38^{high}$) declined sharply owing to prednisolone. Serum IL-6 remained undetectable at all times. We demonstrated for the first time immune monitoring in a child with AIH and T1D before, during and after COVID-19. We hypothesize that continuing with low level of prednisolone without azathioprine may have abrogated activated Tregs, Bregs and IL-6 production and therefore permitting the activation of $CD8^+$ T cells, clearing the virus. Eur J Gastroenterol Hepatol 32: 1251–1255
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Introduction

Several studies are currently investigating the immune responses during the COVID-19, mediated by the 2019/2020 β-coronavirus (SARS-CoV-2) infection [1]. Most of these studies have already shown that, during the infection, patients develop an uncontrolled immune response, caused by the hyperactivation of innate immunity accompanied by substantial activation of peripheral lymphocytes [1–4]. The virus enters the cell via the angiotensin-converting enzyme-2 (ACE-2) expressed on including but not limited to the lung epithelia, kidney and liver [5–10]. This virus is a single-stranded RNA virus, which is recognized by toll-like receptor-7 (TLR7) mainly expressed by innate immune cells. The immune response exists out of a decrease of monocytes and eosinophils coinciding with an increase in neutrophils, C-reactive protein (CRP) and an increase in the secretion of cytokines such as interleukin-1β (IL-1β), IL-2, IL-4, IL-6, tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and in a decrease in the total number of lymphocytes [1–3], though these lymphocytes are activated, producing several pro- and anti-inflammatory cytokines. Severe disease course of COVID-19 has been considered as a failure of the immune system to control the virus. To that end, mounting a concerted antiviral innate and adaptive immunity without exhausting the immune system is paramount in the eradication of the virus.

Although COVID-19 mainly affects the respiratory system, deranged liver tests have been reported in up to 21% of admitted patients [11,12]. Additionally, previous reports demonstrated the increased risk of viral pneumonia in patients treated with immunosuppressive drugs [13,14]. Even more, it is yet to be clarified whether immunocompromised patients with or without cholangitis are at increased risk of cholestasis as ACE-2 is expressed on both hepatocytes and cholangiocytes [15,16]. Furthermore, the clinical manifestations, treatment and prognosis of COVID-19 infection for paediatric autoimmune hepatitis (AIH) or immunosuppressed children with liver transplantation may differ from those of the adult population. However, no relevant reports have been published yet. Herein, we report a case of mild COVID-19 infection in an immunosuppressed boy with AIH and type 1 diabetes mellitus (T1DM), who successfully recovered following a reduction of immunosuppressive drug (prednisolone/azathioprine). As the patient is being followed at our department since the diagnosis with AIH, we had the unique opportunity to monitor his liver enzyme tests and peripheral blood immune cell course prior to, during and after COVID-19. Additionally, the inclusion of serum IL-6 testing in the clinic laboratory testing panel permitted us to measure its level at two time points.

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Methods and subject

As described before [17], we isolated peripheral blood mononuclear cells (PBMC) by gradient centrifugation (Percoll) and stained PBMCs with a live/dead Fixable Viability Dye (Invitrogen, USA) and with antibodies (Biolegend, USA) against cell surface immune cell markers such as CD3, CD4, CD8, CD19, CD20, CD24, CD25, CD38, CD56, CD127, CD45RA and HLA-DR for 30 minutes at 4°C covered from light. Following cell surface staining, intracellular and or intranuclear immune markers CD152 (cytotoxic T-lymphocyte associated protein 4: CTLA-4) and FOXP3 antibody (eBioscience, USA) staining was performed after an incubation with FOXP3 fixation/permeabilization buffer and 1× permeabilization buffer (Treg staining kit, eBioscience, USA) as per supplier protocol. Stained cells were analysed by a 14-colour flow cytometry (Attune NxT). A minimum of 100 000 gated events were recorded. Written informed consent was obtained by parent/patient. Monitoring of lymphocyte subsets is as per standard (Koc University Ethics: 2019.255.IRB2.077). IL-6 and liver enzyme levels were collected from patient records.

Case

A 5-year-old boy with a history of T1DM presented (17 January 2020) with hepatomegaly and elevated aminotransferase levels while his diabetes was well-controlled with insulin and diet. Laboratory work up showed hypergammaglobulinemia and anti-nuclear antibody (ANA) positivity of 1/320 titre, whereas anti-liver-kidney-microsomal antibody (anti-LKM), anti-smooth muscle antibody (ASMA) and anti-soluble liver antigen antibody (anti-SLA) were negative. His liver biopsy histology was compatible with AIH. He received 2 mg/kg/day prednisolone for 2 weeks (29 January 2020) then steroid was tapered while 50 mg azathioprine was initiated. His liver test normalized at the end of month one and IgG level decreased (Table 1). On the third month of therapy (8 April 2020), he presented with fever, cough and earache. His mother stated that they had been in contact with a COVID-19 person. His fever was 38°C and oropharyngeal and left tympanic membrane hyperaemia were noticed during his physical examination. Throat swab sample was taken to perform a PCR testing assay for COVID-19 virus. The test came back positive whereas his thorax CT was unremarkable. Laboratory tests revealed lymphopenia (Table 1) with elevated CRP. Azathioprine (50 mg/day) was discontinued, continuing with prednisolone (8 mg/day) with close follow up at outpatient clinic. His symptoms improved significantly within 1 week and COVID-19 PCR was negative on 7th day (14 April 2020) and 14th day (21 April 2020). IL-6 levels remained below the detection level of 1.5 pg/ml at all time points. For one month, his liver enzymes were unchanged and within normal limits.

Immune cell subsets course

We found the frequency of $CD4^+$ and $CD8^+$ T cells initially decreased under the influence of prednisolone, followed by a plateauing increase (Fig. 1a). As opposed to T cells, B cell ($CD19^+$ $CD20^+$) population increased strongly upon prednisolone treatment and seemingly was unaffected by COVID-19 infection as the frequency dropped back (Fig. 1a). Furthermore, we investigated the activation status of $CD8^+$ and $CD4^+$ T cells. We observed, as opposed to $CD4^+$ T cells, the frequency of activated $CD8^+HLA\text{-}DR^+$ T cells increased from 2.18% to 4.47% during COVID-19. However, $CD8^+HLA\text{-}DR^+$ T cell frequency dropped to 2.15% and 1.14% after the clearance of the virus and at the last time point of the follow up. The frequency of natural killer (NK) ($CD56^+CD3^-$) and NKT ($CD56^+CD3^+$) cells remained stable over the whole observation period (Fig. 1a). However, the frequencies of immunoregulatory T, B and NK cells demonstrated major fluctuations after initiating immunosuppressive (IS) treatment. Total regulatory T cell (Tregs) ($CD4^+CD25^+CD127^{+/-}FOXP3^+$) frequency increased slightly when compared to the treatment naïve condition and fluctuated afterwards. Nevertheless, Sakaguchi Tregs subsets [18], the activated Tregs ($CD4^+CD45RA^-FOXP3^{high}$) and naïve Tregs ($CD4^+CD45RA^+FOXP3^{dim}$) demonstrated a sharp increase and a substantial decrease upon treatment initiation, respectively (Fig. 1b). COVID-19 infection did not have a major impact on these subsets as their frequencies went back to similar values observed in the first sample. Similarly, the frequency of CTLA-4, an immunoregulatory marker expressed by suppressive Tregs, showed an initial increase from 25.8% to 36% upon prednisolone treatment but decreased to 19.4% and 23.1% at second- and third-week post SARS-CoV-2 infection, respectively. Additionally, we found that regulatory NK cells ($CD56^{high}CD3^-$) decreased first but recovered subsequently (Fig. 1c). Next, we also wondered whether $CD19^+CD20^+CD24^{high}CD38^{high}$ regulatory B cell (Bregs) frequency was affected by treatment or COVID-19. We found that IS treatment almost eradicated Bregs, but COVID-19 infection had no obvious effect (Fig. 1d). The frequency of Bregs remained low at all follow-up time points compared to the initial frequency.

Table 1. Biochemistry and virology.

Parameters	17-01-20	29-01-20	08-04-20	14-04-20	21-04-20
Lymphocyte count (4000–10 500/µl)	3200	3200	1300	3400	1700
Monocyte count (0-100/µl)	500	500	500	400	300
Neutrophil count (1500–8500/µl)	2600	9000	3300	1800	8100
ALT (<39 U/l)	97	29	30	20	20
AST (<52 U/l)	74	20	33	23	28
GGT (<18 U/l)	17	22	26	19	19
IgG (5.84–15.09 g/l)	16,1	17	11,1	9,1	9,49
CRP (<5.0 mg/l)	<0.3	<0.3	7.8	0.5	<0.3
COVID-19 viremia status	–	–	+	–	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase; COVID-19, coronavirus 2019 (SARS-Cov-2); CRP, C-reactive protein; GGT, gamma-glutamyl transferase; IgG, immunoglobulin G.

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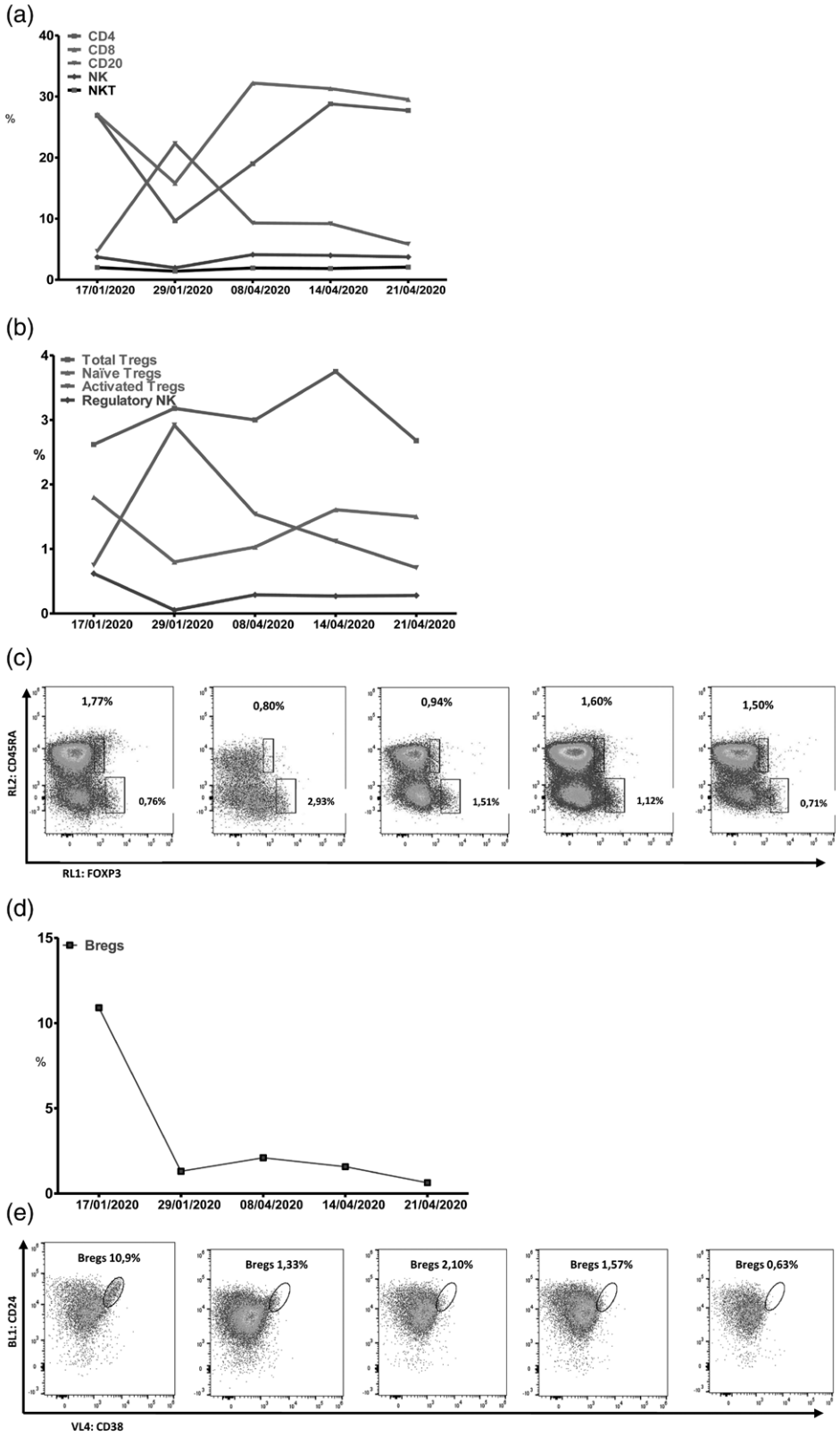


Fig. 1. Blood lymphocyte cell subset frequencies. (a) This depicts the frequencies of $CD4^+$, $CD8^+$ T cells, $CD19^+CD20^+$ B cells, $CD56^+CD3^-$ natural killer (NK) cells and $CD56^+CD3^+$ natural killer T (NKT) cells at serial sampling time points before, during and after COVID-19. (b) This provides the course of regulatory immune cells such as total regulatory T cells (Tregs, $CD4^+CD25^+CD127^{low}FOXP3^+$), naïve Tregs ($CD4^+CD45RA^+FOXP3^{dim}$), activated Tregs ($CD4^+CD45RA^-FOXP3^{high}$) and regulatory NK cells ($CD56^{high}$ $CD3^-$). (c) This illustrates the dot plots of (naïve/activated) Tregs at above mentioned time points. (d) and (e) demonstrate the course of regulatory B cells ($CD19^+CD20^+CD24^{high}CD38^{high}$) and dot plots of these cells, respectively.

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Discussion

IS treatment is the cornerstone for the management of AIH. Steroid and azathioprine significantly suppress T cell immune responses, which are essential in the effective control of all viral infections. But there is no prospective study related to paediatric AIH course and treatment modalities for COVID-19 [16]. We stopped azathioprine and the patient continued on steroid treatment in order to prevent a secondary reactivation of AIH and adrenal failure [16]. Interestingly, we did not observe any progression of his liver-related symptoms or worsening of his laboratory parameters. This may be the result of the anti-inflammatory effect of corticosteroids, which relieve systemic symptoms (such as fever or fatigue) caused by the storm of inflammatory cytokines [19]. Additionally, the level of GGT remained stable during COVID-19. This is interesting as cholangiocytes express the ligand, ACE-2, which SARS-CoV-2 uses to infect cells.

Pertaining to the case discussed in this report, the clinical course is intriguing and warrants further clinical and immunological follow up of these patients.

Recently, Li *et al*. demonstrated that the profile of peripheral blood lymphocyte subsets and serum cytokines in a cohort of 40 children with COVID-19 pneumonia and 19 with respiratory syncytial virus (RSV) [20]. They observed that effective $CD8^+$ T cell activation might be one of the reasons why the symptoms of pneumonia in most children with COVID-19 pneumonia were mild to moderate and the percentage of $CD8^+$ T cells was significantly higher compared to RSV patients. Interestingly, in our serial observation, we found $CD8^+$ and $CD4^+$ T cell frequency to be increased while he had COVID-19. Even more, we demonstrated that $CD8^+$ T cells but not $CD4^+$ T cells were more activated, expressing HLA-DR, during COVID-19 as compared to prior to and after the SARS-CoV-2 infection. NK percentages did not alter between their COVID-19 and RSV patients. Similarly, we found that NK cells but also NKT cells remained stable over the entire observation period. We also demonstrated a decrease in the percentage of B cell, which is in concordance with their finding. However, in adults, the immune phenotype is rather different. The numbers of all major cell subsets appeared to be much lower compared to healthy controls and in severely affected COVID-19 patients compared to ones with mild symptoms [1–3]. This may advocate that impaired immunity is the main culprit in developing severe pneumonia in adults.

As immune regulation is key in mounting an appropriate immune response, we investigated the course of the major immune-regulatory cell types such as Tregs (naïve/activated), Bregs and regulatory NK cells. The percentage of total Tregs remained stable when observing the period prior to the infection and during COVID-19. Qin *et al*. found that patients with mild disease had more Tregs, whereas naïve/activated Tregs subsets did not alter [3]. However, in our patient, the course of the percentages of naïve/activated Tregs subsets were opposed to each other likely explaining why the total Tregs frequency and function did not have major fluctuations. Furthermore, in our case report, we found that regulatory NK cells remained more or less stable, unaffected by COVID-19. To date, there is no data pertaining the role of regulatory NK cells in the outcome of this ailment.

Most remarkable were the dynamics of Bregs in our patient. Its sharp decline mediated by the initiation of IS drugs perpetuated at later time points. Knowing that Bregs are one of the major sources of IL-10 production, it may support the notion that mild pneumonia is favoured by the paucity of IL-10. Indeed, a study about neonatal-specific regulatory B (nBreg) cells in human neonates with RSV infection showed that the RSV-infected nBregs increased the severity of the infection by producing IL-10 and weakening Th1 cell responses [21]. Even more, Ouyang *et al*. described the serum level of IL-10 to correlate with IL-6 [2]. IL-6 is known to have a worsening effect on pneumonia. It is increased in patients with severe COVID-19 [2,3]. Even though IL-6 is crucial in the germinal centre T–B interaction mediated formation of long-lived plasma cells, producing highly specific antibodies, it equally impairs CD8 T cells by down regulating gamma-interferon, upregulating PD-1 and suppression of cytokine signalling-3 [22–24]. Therefore, it is not unconceivable that lower Bregs resulted in less IL-10 and IL-6 production. For IL-6, we were indeed able to demonstrate its lack in our patient both during and after COVID-19.

In conclusion, we demonstrated for the first time immune monitoring in a child with AIH and T1DM with COVID-19. We hypothesize that continuing low level of steroids in this patient abrogated activated Tregs, Bregs and consequently IL-6 production and therefore permitting an increased activation status specific for $CD8^+$ T cells to clear the virus. Albeit, as this is a case report, further research is warranted to investigate whether these results can be reproduced and to clarify the role of immune regulatory cells in COVID-19.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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