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Short Communication

The peripheral blood immune cell profile in a teriflunomide-treated multiple sclerosis patient with COVID-19 pneumonia

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A teriflunomide-treated multiple sclerosis patient with COVID-19 pneumonia was hospitalized and recovered in 15 days. The immunophenotyping analysis of peripheral blood cells was performed in two time points: the first was 1 month before (pre-infection) while the second was during COVID-19 pneumonia (infection). At the infection time point, no differences in the percentages of immune activation and immunesenescence of CD4+ and CD8+ T-cells were observed compared to the pre-infection time point. Our evaluation seems to confirm that teriflunomide controls T-cells immune activation and immunesenescence suggesting that teriflunomide should not be discontinued in MS patients with an active COVID-19 pneumonia.

1. Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is the cause of the coronavirus disease-2019 (COVID-19) pneumonia. The pathophysiology of SARS-CoV-2 infection closely resembles that of other coronavirus infection, with aggressive inflammatory responses strongly implicated in the resulting damage to the airways (Wong et al., 2004). Therefore, disease severity is not only due to the viral infection but also the host response. Patients with severe COVID-19 pneumonia show higher level of T-cell activation and high levels of inhibitory molecules associated with a reduction of T-cells functionality, suggesting an ineffective immune response (Qin et al., 2020; Zheng et al., 2020a; Zheng et al., 2020b). Moreover, an impaired cytotoxic immune response during SARS-CoV-2 infection was reported (Zheng et al., 2020a).

The COVID-19 pandemic has raised considerable concerns on the use of multiple sclerosis (MS) disease-modifying therapies (DMTs) which could increase the risk for acquiring the viral infection, potentially impair immune responses, and contribute to an unfavorable outcome (Raitas and Sepkowitz, 2012).

MS patient reports are now beginning to appear in the literature (Barzagari et al., 2020; Bowen et al., 2020; Maghzi et al., 2020; Novi et al., 2020; Sormani and Italian Study Group on COVID-19 infection in multiple sclerosis, 2020) and the most of them described mild COVID-19 pneumonia in line with the non-MS population. Although several clinical characteristics of patients with COVID-19 pneumonia were defined, the profile of the immune response was not well characterized and apparently focused on immunocompetent individuals. Moreover, some reports on teriflunomide-treated MS patients with COVID-19 pneumonia are now available but only clinical characteristics were described and the profile of the immune response during COVID-19 pneumonia was not well characterized.

Here, we report the peripheral blood immune cell profile in a teriflunomide-treated MS patient with mild COVID-19 pneumonia and compared findings with those obtained from the same patient 1 month before. Teriflunomide is a once-daily oral immunomodulatory disease-modifying therapy approved for relapsing remitting (RR) MS that selectively and reversibly inhibits dihydroorotate dehydrogenase, a key mitochondrial enzyme in the de novo pyrimidine synthesis pathway, thus reducing the proliferation of activated T and B lymphocytes through the arrest of the cell cycle in the S phase (Bar-Or et al., 2014).

2. Case presentation

At the end of January 2020, a 62-year-old RRMS woman (Expanded Disease Status Scale = 6), under teriflunomide treatment for the last 5
years, was evaluated at the neuroinfectious disease unit, Sapienza University of Rome, to assess the risk of infection before switching therapy. The patient was in good condition and no risk of infection was observed.

At the end of February 2020, the patient was admitted to the emergency room at the Policlinico Umberto I Hospital, Sapienza University of Rome, due to fever (38.4 °C). The first biochemical test showed high levels of C-reactive protein (CRP) (6.03 mg/dL), lactate dehydrogenase (LDH) (246 UI/L), and leukocytosis (10.670/mL, N 77.8%, L 13.3%). A chest X-ray showed small peripheral basal patchy infiltrates and bronchial wall thickening. *Legionella pneumophila*, flu, and respiratory syncytial virus tests were negative. Empiric antibiotic therapy (ceftriaxone 2 g plus azithromycin 500 mg/die) was started. After an initial improvement, the patient showed dyspnea, severe fatigue, dry cough, fever recurrence, and diarrhea. *Clostridium difficile* infection was suspected, and ceftriaxone was discontinued. The *clostridial* toxin test resulted negative.

Due to the previous chest X-ray pattern, clinical history, and COVID-19 outbreak, the patient was moved to the COVID-19 ward and a nasopharyngeal swab was performed, which resulted positive for SARS-CoV-2 on real-time reverse transcription-polymerase chain reaction (RT-PCR) (Panther Fusion® SARS-CoV-2 test, Hologic, USA). On examination, the patient’s body temperature was 37.4 °C, blood pressure was 130/80 mmHg, pulse was 78 beats per minute, respiratory rate was 20 breaths per minute, and oxygen saturation was 93% on ambient air. Blood gas analysis showed that the partial pressure of oxygen was 92 mmHg, the partial pressure of carbon dioxide was 33.0 mmHg, and the pH level was 7.45 with a PaO₂/FiO₂ (P/F) ratio of 438. The patient was started on chloroquine (500-mg tablet twice/day) and lopinavir/ritonavir treatment (200 mg plus 50 mg two tablets bid). After the diagnosis of COVID-19 pneumonia was confirmed, teriflunomide was discontinued in accordance with the attending neurologist. Clinical and laboratory conditions improved after 6 days of treatment (chloroquine plus lopinavir/ritonavir). After two consecutive negative nasopharyngeal swabs, the patient was discharged in good health and teriflunomide treatment was resumed. Since the patient did not undergo the accelerated procedure for teriflunomide elimination, we also evaluated teriflunomide plasma levels at discharge and found that teriflunomide plasma levels were > 10 mg/L.

Fig. 1. (A) Gating strategy for flow cytometry immunophenotyping analysis (a) After gating for single cells in forward scatter area (FSC-A) versus height (FSC-H) plots, polymorphonuclear cells and lympho-monocytes were identified in FSC-A SSC-A plot. In the lympho-monocyte gate, NK, NKT and T cells were defined according to CD3 and CD56. After gating for CD3 + CD56- lymphocytes, CD3 + CD4+ and CD3 + CD8+ cells were identified. Immune activated T-cells were defined as HLA-DR + CD38+ while immunosenescent T-cells as CD28-CD57+. Maturation T-cell subsets were defined as follows: naive (N, CD45RO-CD27+), central memory (CM, CD45RO + CD27+), effector memory (EM, CD45RO + CD27-) and effector (E, CD45RO-CD27-) cells. Only for CD3 + CD8+ T-lymphocytes we identified an intermediate maturation subset (I, CD27lowCD45RO+). After gating for NK cells as CD3-CD56+, NK maturation subsets were defined according to CD56 and CD16 expression as CD56bright, CD56dim. In the lympho-monocyte gate, after exclusion of CD56 + CD14- cells and HLA-DR-CD14- cells, monocyte maturation subsets were defined according to CD14 and CD16 expression as atypical monocytes (CD14-CD16+), intermediate monocytes (CD14 + CD16+), and classical monocytes (CD14 + CD16+). (B) Evaluation of immunophenotyping analysis at the two time points. A representative healthy donor (HD) matched for sex and age is reported.
Table 1
Immunophenotyping analysis of peripheral blood cells at the two time points.

|                   | Pre-infection | Infection | HD   |
|-------------------|---------------|-----------|------|
| T cells (%)       | 54.8          | 50.2      | 62.2 |
| CD4 (%)           | 69.6          | 70.2      | 65.7 |
| CD4 HLA-DR + CD38+ (%) | 1.03      | 0.91      | 3.8  |
| CD4 CD28-CD57+ (%) | 1.5         | 1.7       | 0.04 |
| CD4 naive (%)     | 46.2          | 53.3      | 36.4 |
| CD4 central memory (%) | 44.0      | 39.6      | 52.3 |
| CD4 effector memory (%) | 9.8       | 6.3       | 10.2 |
| CD4 effector (%)  | 7.9           | 7.6       | 28.1 |
| CD8 (%)           | 1.1           | 1.3       | 2.8  |
| CD8 HLA-DR + CD38+ (%) | 3.8       | 4.7       | 6.6  |
| CD8 naive (%)     | 45.8          | 53.3      | 49.7 |
| CD8 central memory (%) | 19.6      | 16.9      | 17.2 |
| CD8 effector memory (%) | 8.3       | 5.8       | 18   |
| CD8 effector (%)  | 3.6           | 2.6       | 13   |
| CD8 intermediate (%) | 2.8       | 1.3       | 1.7  |
| NK cells (%)      | 8.0           | 8.5       | 15.2 |
| CD56dim NK cells (%) | 95.3      | 97.5      | 95.8 |
| CD56bright NK cells (%) | 3.9       | 2.5       | 3.6  |
| NKT cells (%)     | 2.9           | 2.5       | 7.6  |
| B cells (%)       | 12.9          | 16.8      | 8.7  |
| Monocytes (%)     | 27.5          | 23.6      | 44.1 |
| Classical monocytes (%) | 21.1     | 21.0      | 32.8 |
| Intermediate monocytes (%) | 1.7     | 1.1       | 4.1  |
| Atypical monocytes (%) | 8.0       | 4.7       | 7.1  |

A representative healthy donor (HD) matched for sex and age is reported.

3. Methods

Blood samples were collected in heparin tube and peripheral blood immune cell profile was evaluated by flow cytometry. Immunofluorescence staining was performed as previously described (Iannetta et al., 2016). We investigated the expression of T-cell (CD3, CD4 and CD8, CD27 and CD45RO), natural killer (NK) cell (CD56 and CD16), NKT (CD56 and CD3) and monocyte markers (CD14 and CD16).

Since co-expression of CD38 and HLA-DR is the key phenotype of the activation of CD4+ and CD8+ T-cells in response to viral infections, we analyzed co-expression of CD38 and HLA-DR. Finally, the immunosenescence T-cell phenotype was evaluated as a lack of CD28 expression and the expression of the senescence marker, CD57 (Merino et al., 1998). Two time points were considered: the first was 1 month prior to SARS-CoV-2 infection (pre-infection time point) while the second was during COVID-19 pneumonia (12 days after symptom onset) (infection time point). The gating strategy is shown in Fig. 1A. Timeline is shown in Fig. 1C.

4. Results

At the infection time point, the immunophenotyping profile showed a slight reduction in the percentages of total T-cells and monocytes and a slight increase in the percentages of B cells. No differences were found in the percentage of total NK and NKT cells. Among T-cell subsets, no differences were found in the percentages of CD4+ and CD8+ T-cells as well as in the percentages of immune activation (HLA-DR + CD38+) and immunosenescence (CD28-CD57+) of CD4+ and CD8+ T-cells between the two time points. Notably, the evaluation of T-cell subsets according to the expression of CD45RO and CD27, showed an increase in the percentages of CD4 and CD8 naive T-cells and a reduction in the percentages of CD4 and CD8 memory and effector subsets at the infection time point compared to pre-infection time point. For NK subsets, no differences in the percentages of CD56dim and CD56bright NK cells were observed. Finally, at the infection time point a slight reduction of atypical monocytes was observed compared to pre-infection time point. All the results expressed as percentages are reported in Table 1 and shown in Fig. 1.

5. Discussion

Here we reported the peripheral blood immune cell profile in a teriflunomide-treated MS patient with mild COVID-19 pneumonia and compared the findings with those obtained from the same patient 1 month before. The most relevant findings are that no changes in the percentages of immune activation and immunosenescence of T-cells were found.

The viral infection can result in a strong antigenic stimulation leading to increased number of immune activated cells in both CD4+ and CD8+ T subsets. Recent reports showed an increase of CD38 and HLA-DR co-expression on T-cells in peripheral blood of patients with COVID-19 pneumonia (Thevarajan et al., 2020; Xu et al., 2020). Moreover, previous report suggested that beside lymphopenia, the function exhaustion of cytotoxic lymphocytes in patients with COVID-19 pneumonia could be a potential risk factors for severity of disease (Qin et al., 2020). In our case no differences in the percentages of immune activated and immunosenescent T-cells were observed between the two time points. Furthermore, our 62-year-old RRMS patient was at a significantly high risk of development severe COVID-19 pneumonia due to age. However, she recovered completely from COVID-19 pneumonia.

We speculate that this finding is possibly due to the effects of teriflunomide on cells with high proliferative rates, such as activated lymphocytes. Indeed, teriflunomide decreases activated T-cell proliferation through reversible inhibition of the mitochondrial enzyme dihydroorotate dehydrogenase (Oh and O’Connor, 2014) and induces a regulatory status in the innate immune response (Medina et al., 2019). Indeed, teriflunomide targets selectively activated immune cells without cell lysis and reduces the level of immune activation without the major immunosuppression. Moreover, teriflunomide does not significantly compromise protective immunity, and its anti-inflammatory activity primarily affects the pathogenic immune processes associated with MS activity (Bar-Or et al., 2014).

Consistent with a previous report (Medina et al., 2019), in our case the evaluation of the T-cell subsets showed a lower percentages of terminally differentiated CD4+ and CD8+ T-cells and higher percentages of naive T-cells in both two time points. As reported, teriflunomide was shown to inhibit the proliferation of activated T and B cells leaving the homeostatic proliferation (self-renewal) of resting cells unchanged (Mills and Mao-Draayer, 2018). A delicate balance may be necessary in the host immune response to successfully confront COVID-19 pneumonia. Indeed, either overactivation of the immune response or an inadequate immune response could lead to a poor outcome (Mehta et al., 2020).

Finally, at the infection time point, a slight reduction in the percentages of atypical monocytes was found compared to pre-infection time point. As described by other authors, the reduction of atypical monocytes of peripheral blood could be due to a mechanisms of migration of these cells in the lungs (Thevarajan et al., 2020). To date, no sufficient data are available on teriflunomide effects on monocyte subsets. However, Medina et al. reported an increase in total monocytes expressing inhibitory programmed cell-death protein 1 ligand (PD-1 L), a ligand of PD-1, a cell surface receptor with an important role in down-regulating the immune system and promoting self-tolerance by suppressing T-cell inflammatory activity (Goodman et al., 2017).

In summary, our finding underlines that teriflunomide may prevent an excessive host immune response by T-cells hyperactivation in COVID-19 pneumonia.

Despite the main limitation of this study which includes a single case only, our evaluation of peripheral blood cell profile before and during COVID-19 pneumonia supports recent clinical indication that teriflunomide should not be discontinued in MS patients.

These preliminary data could stimulate additional researches on larger cohorts of MS patients to characterize the peripheral blood cells to better understand the efficacy of teriflunomide in preventing the
development of acute respiratory distress syndrome associated with COVID-19 pneumonia.

Ethical approval

This study was approved by the ethics committee of Policlinico Umberto I of Rome (protocol number 130/13) and the patient provided a written informed consent.

Declaration of competing interest

All the authors report no disclosures relevant to the manuscript.

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