SARS-CoV-2 spreads to lymph nodes and strongly expands CD4+ T_{EMRA} cells in a patient with mild COVID-19

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Abstract

A woman with mild Covid-19 developed cervical adenopathy, being diagnosed of Epstein–Barr virus infectious mononucleosis. After a FNAP we demonstrate that SARS-CoV-2 is found in lymph nodes (LNs) even in mild disease along with a strong expansion of terminally differentiated effector memory CD4+T-cells , a cell population that is practically absent in LN.

Key words: SARS-CoV-2, lymph node, CD4+ T_{EMRA} cells, co-infection

INTRODUCTION

A novel coronavirus (severe acute respiratory syndrome-coronavirus-2; SARS-CoV-2) has caused a global pandemic of respiratory illness called coronavirus infectious disease 2019 (COVID-19). Most frequent COVID-19 symptoms include mild, self-limiting respiratory tract illness up to severe progressive pneumonia, multiorgan failure, and death.[1] Although lymphadenopathy is a common manifestation in many viral diseases, radiology analysis has thus far concluded that lymphadenopathies are characteristically absent in most SARS-CoV-2 patients who were investigated using computerized tomography (CT).[2] To date, COVID-19 has not been reported in superficial, palpable lymph nodes (LN). The underlying reason for the frequent lymphocytopenia observed in patients with severe disease remains unclear because it is typically not feasible to sample LN .[3]

We report the case of a young woman who experienced, mild classical Covid-19 symptoms. After 3 weeks, she had a torpid evolution with reappearance of fever and cervical node enlargement, with singular immunophenotypic LN findings.

Case report

Clinical presentation

On first days of March 2020, 21-year-old Caucasian woman experienced symptoms of fever, malaise, frontal headache, and cough. She was monitored by phone, and suspected COVID-19 was diagnosed. Twenty-five days later, our patient contacted again with her family caregiver because slight persistent fever, odynophagia, and right cervical node enlargement. Antibiotics were prescribed without improvement. Six days later, she attended our hospital emergency room. She had no relevant medical history. On physical examination it was observed 12 breaths/minute and arterial oxygen saturation of 98%. The right and left

cervical submandibular regions showed palpable LN (diameter, 2–3 cm and <1 cm, respectively), which were not painful or adhered to deep tissue. A blood test including hemogram, biochemistry, and serologies (Supplementary Material Table 1) and an X-ray showed normal results, except for slightly elevated C-reactive protein (6.6 mg/L).. RT-PCR nasopharyngeal SARS-CoV-2 swab was positive. Purulent tonsillitis was noted next days, with associated development of additional new lymphadenopathy and enlargement of existing lymph nodes. To rule out a malignant etiology or the possible involvement of SARS-COV-2, a fine needle aspiration (FNA) puncture was performed after informed consent. ymptoms persisted for >10 days and then progressively improved. By first of May, most symptoms had resolved, and the LN decreased in size.

METHODS

Molecular and serological assays for virus detection

The nucleic acids were extracted from the samples (serum, node FNA, and nasopharyngeal swab) using NucliSens easy-Mag platform (bioMériux, **Boston, Massachusetts (MA), USA**). For SARS-CoV-2 detection, the TaqMan 2019-nCov Assay Kit (Thermo Fisher, Waltham, MA USA) was used, amplifying ORF1ab, S and N proteins, sequences unique to SARS-CoV-2. Serological or molecular tests for the diagnosis of EBV, CMV and neurotropic viruses were performed with commercially available kits.

FNA cytology

FNA was performed with four passes using a 25g needle attached to an aspiration device (Cameco Enebyberg, Sweden). ISH was performed using the BOND EBV probe ISH kit (Leica Biosystems, Wetzlar, Germany), according to the manufacturer's instructions, in formalin-fixed and paraffin-embedded cell blocks.

Flow cytometry

All conjugated antibodies were obtained from BD Biosciences (San Jose, CA, USA). Antibodies were added to peripheral blood (PB) or FNA samples and incubated for 20 min. Samples were acquired using a FACSCanto II flow cytometer (BD Biosciences).

All tests have negative controls.

RESULTS

Cytological and immunophenotypically FNA analysis

Cytologically, there was abundant polymorphous lymphoid cellularity, as follows: scattered germinal center component with dendritic–lymphocytic aggregates and tangible body macrophages, and conspicuous plasma cells, plasmacytoid lymphocytes, and large atypical cells with an immunoblastic appearance (some resembling binucleated reed Stenberg-cells). To rule out the neoplastic population, immunophenotypic flow cytometry study was performed. Pan-T or pan-B markers and CD4/CD8 or kappa/lambda ratios were not altered. Similarly, no aberrant antigens were present. These results suggested a reactive LN. The high suspicion of acute EBV-related infectious mononucleosis was suggested by FNA cytology and confirmed by intensively positive ISH results for EBV in the cell block FNA. Consistent with this finding, high serum VCA-IgM levels and no EBNA antibodies indicated a primary EBV infection (Supplementary Material Table 1).

Molecular FNA SARS-CoV-2 analysis

RT-PCR of a FNA diluted right cervical node sample showed a positive result for SARS-CoV-2 (cycle threshold for SAR-CoV-2N gene=37).

FNA and PB naive and memory T-cell subsets analysis

We analyzed CD4+ and CD8+ T-cell naive and memory phenotypes in matching LN and PB samples from our patient. Results were compared with patients who were diagnosed previously with reactive adenopathy related to EBV or other infectious diseases. Each subset was gated for CD45RA and CD197 (CCR7) antigen expression. Functional subsets were defined as naive CD45RA+CD197+, central memory (T_{CM}) CD45RA-CD197+, effector memory (T_{EM}) CD45RA-CD197-, and CD45RA+ effector memory (T_{EMRA}) CD45RA+CD197-. Naive or central memory cells, which are the two main CD4+ subsets that are usually detected in normal or reactive LN that are or are not infected by EBV (Fig. 1B), switched almost completely to effector memory and especially to T_{EMRA} T-cells (Fig. 1A). The latter population was virtually absent in normal or reactive lymphoid tissues. However, these changes were much less pronounced in LN CD8+ functional subsets (Fig. 1C–D).

A matched blood sample from our patient was analyzed. T_{EMRA} CD4+ T-cells were practically absent, with all four functional subsets distributed in a similar manner to other viral reactive processes (Fig. 1F) or healthy donors (data not shown).

DISCUSSION

This study shows, for the first time, that SARS-CoV-2 infects from the initial infection site (respiratory tract) to the locoregional LN, such as cervical LN, even in mild disease. Additionally, changes in the T lymphocyte immune profile that were observed in the LN are distinct from that observed in PB.

Several reports reveal that only 6% of patients who were admitted to hospital for COVID-19 had lymphadenopathies.[4] Feng et al.[5] showed that SARS-CoV-2 directly infects macrophages in LNs and the spleen from six autopsies using immunofluorescent staining and electron microscopy. Consistent with these results, one could hypothesize that SARS-CoV-2 belongs

to a group of viruses that does not normally induce extensive adenopathies, although its detection in LN necropsies from COVID-19 patients is the rule rather than the exception. Additionally, palpable lymphadenopathies have not been reported in COVID-19 patients. However, we present a case of a palpable adenopathy that was caused by co-infection with SARS-CoV-2 and EBV. The findings strongly suggest that the enlarged LN was a consequence of EBV rather than SARS-CoV-2 infection, but this co-infection was an excellent opportunity to assess the presence of coronavirus in LNs from patients with mild symptoms. Our patient was laboratory-confirmed positive for the SARS-Cov-2 virus using quantitative RT-PCR on an FNA sample. This case suggests that virus reaches LNs , regardless of disease severity.The other relevant finding of this study is an unexpected expansion of CD4+ T_{EMRA} in the patient's cervical LN. To date, SARS-CoV-2-induced cellular immunity has been poorly characterized in anatomical compartments other than PB or lung. The first data regarding changes in lymphocyte PB populations in patients with severe symptoms showed T-lymphopenia, an increase in naive CD4+ T-cells, and a decrease in memory CD4+ T-cells.[6] However, as mentioned previously, we observed a large increase in the percentage of CD4+ T_{EMRA} cells in LN, a cell subset virtually absent in LN from healthy[7] or infected individuals, including EBV infection.[8]

In this case report, these results had to be interpreted based on EBV pathogenesis, since it should not be forgotten that most EBV-specific CD8+ or CD4+ T cells during symptomatic primary infection have a CD45RA-/CD45RO+ phenotype in PB and lymphoid tissues.[9] EBV does not significantly affect T-cell subset distribution in lymphoid organs.[8] In contrast to EBV infection, we observed a remarkable redistribution of CD4+ T-cells in a cervical LN that was co-infected with SARS-CoV-2 and EBV, including the almost total disappearance of naive and central memory CD4+ T-cells and a very intense expansion of CD4+ effector memory cells. These striking differences make it unlikely that EBV contributes directly to the described CD4+ T_{EMRA} phenotype. However, we cannot exclude that an unknown interplay between

active EBV infection and SARS-CoV-2 may affect the huge LN CD4+ T_{EMRA} lymphocyte expansion. The magnitude of the expansion is so great that it could result, at least partially, from bystander activation.[10]

In contrast to CD4+ cells, our results showed that the percentages of LN CD8+ T-cell subsets remained essentially unchanged compared to normal lymphoid tissue. This finding may initially seem surprising because CD8+ cells are usually the main cell population involved in the control of viral infection. An increasing amount of evidence suggests that an increase in CD8+ T-cells with an activated phenotype in the blood is related to SARS-CoV-2 clearance,[11] and that a high CD8+ T-cell count in the lungs is associated with better control of COVID-19 progression.[12] However, CD4+ T-cells provide necessary help for antiviral function of CD8+ T-cells, and a subset of CD4+ T_{EMRA} cells exhibits potent effector functions including secretion of proinflammatory cytokines and cytotoxic molecules.[13]

Further studies are needed to confirm the CD4+ T_{EMRA} lymphocyte expansion in LN from COVID-19 patients and to understand their precise role in either the control of infection or the promotion of the immunopathogenesis that leads to cytokine storm and the role of viral co-infection in these findings.

NOTES

Authors Contribution ERS: immunophenotype analysis and manuscript writing, literature search; ABB: FNA analysis, cytology interpretation, contribution to manuscript writing; LMG: microbiology analysis, contribution to manuscript writing; CQ: clinical management, contribution to manuscript writing; ERM: Immunophenotype analysis, contribution to manuscript writing PPE: clinical management, contribution to manuscript writing; J.M. López-Pintor microbiology analysis, contribution to manuscript writing, PEWD Immunophenotype analysis, contribution to manuscript writing; AMZ clinical management contribution to manuscript writing, JIFV: immunophenotype analysis, contribution to manuscript writing PGA immunophenotype analysis, contribution to manuscript writing; RBG immunophenotype analysis, contribution to manuscript writing. , LMV data interpretation, contribution to manuscript writing MJPE clinical management, manuscript conception and contribution to writing, data interpretation, literature search.

Funding. No funding sources were used for this work.

Disclosure Statement MJPE reports grants and personal fees from VIIV Health care, grants and personal fees from Gilead Science, grants and personal fees from MSD, outside the submitted work; AMZ reports grants and personal fees from VIIV Health care, grants and personal fees from Gilead Science, outside the submitted work. All other authors have nothing to declare.

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Figure 1 (A, B, C, D, E, F). Impact of SARS-CoV-2 on the distribution of CD4+ and CD8+ T-cell subsets in LN and PB. Functional CD4+ and CD8+ T-cell subsets that were classified based on CD45RA and CD197 expression were studied in LN and PB from a patient who was co-infected with SARS-CoV-2 and EBV (A, C, E), from a patient infected with EBV (B, D) and from a patient infected with CMV (F). Open arrow indicates the strong expansion of CD4+ T_{EMRA} cells in a cervical LN from the SARS-CoV-2+, EBV+ patient. Dot plots were gated on live CD4+ or CD8+ T-cells. Antigens are identified on the *X*- and *Y*-axes. Numbers in plots represent the percentage of cells in each quadrant; quadrants were set according to isotype control antibodies.

Figure 1 Footnote

SARS-CoV-2, severe acute respiratory syndrome- coronavirus-2; LN, lymph node; PB, Peripheral blood; EBV, Epstein–Barr virus

Figure 1