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MINI REVIEW

Do reduced numbers of plasmacytoid dendritic cells contribute to the aggressive clinical course of COVID-19 in chronic lymphocytic leukaemia?

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Abstract

Infections with SARS-CoV-2 have been unduly severe in patients with haematological malignancies, in particular in those with chronic lymphocytic leukaemia (CLL). Based on a series of observations, we propose that an underlying mechanism for the aggressive clinical course of COVID-19 in CLL is a paucity of plasmacytoid dendritic cells (pDCs) in these patients. Indeed, pDCs express Toll-like receptor 7 (TLR7), which together with interferon-regulatory factor 7 (IRF7), enables pDCs to produce large amounts of type I interferons, essential for combating COVID-19. Treatment of CLL with Bruton's tyrosine kinase (BTK) inhibitors increased the number of pDCs, likely secondarily to the reduction in the tumour burden.

1 | INTRODUCTION

Patients with haematologic malignancies have been severely affected by the SARS-CoV-2 infection, those with CLL in particular.[1-3] In the Stockholm region, Sweden, during the first wave of the COVID-19 pandemic, 32% of consecutively identified, hospitalized patients with CLL succumbed to the infection, whereas during the second wave, as many as 18% died, despite improved medical care.[3] There is currently no clear understanding of the underlying mechanism for the remarkably poor outcome in this patient population.

The risk of death from COVID-19 doubles every five years from childhood and onwards, and elderly men are particularly susceptible.[4] CLL is also a disease of the elderly with a male preponderance, but age and sex cannot fully account for the high mortality rates in CLL. While immunosuppressive treatments potentially impair the patients' ability to overcome the infection, many individuals with early-stage, untreated CLL had similar poor outcome, and some were in fact first diagnosed with CLL at the intensive care unit.[1,3]

2 | RATIONALE FOR A pDC DEFECT AS A CAUSE OF SEVERE COVID-19 IN CLL

Based on a series of observations explained beneath, we propose that an underlying mechanism for the aggressive clinical course of COVID-19 in CLL is a paucity of pDCs (Figure 1).

First, an international consortium of researchers has identified an essential role for the innate interferon (IFN) system in the protection from severe COVID-19. Autoantibodies against type I IFN or rare loss-of-function variants in genes implicated in viral sensing, type I IFN production, and signalling could explain up to 20% of severe COVID-19 cases.[4] Notably, damaging X-linked *TLR7* variants were identified in young, previously healthy males with life-threatening COVID-19 pneumonia.[5,6] Such rare *TLR7* inborn errors of immunity (IEI) account for as many as 1.8% of life-threatening COVID-19 cases among men under 60 years of age.[6] TLR7 is a receptor for single-stranded RNA and is predominately expressed by pDCs,[7] sensing infection by viruses such as SARS-CoV-2. Furthermore, autosomal recessive variants in *IRF7* have been described in several previously healthy adults with life-threatening COVID-19 pneumonia.[8] Interferon-regulatory factor 7 (IRF7) is a transcription factor constitutively expressed in pDCs, serving as a master regulator of type I IFN gene transcription.[9] It has also been shown that pDCs produce more Type I interferon than any other cell type in blood.[10,11] Thus, together, the demonstration that *TLR7* and *IRF7* deficiency causes severe COVID-19 provides a compelling link to pDCs as a critical source of type I IFN in protection from SARS-CoV-2.

Secondly, there is clear evidence that the number of circulating pDCs is lowered in CLL, as reported by us and others.[12-14] In addition, pDC precursors were found to be functionally impaired.[15] Moreover, the best experimental model available for CLL, namely the TCL1-transgenic mouse, is also characterized by low numbers/frequencies of pDCs in the spleen, posited to explain an overall high infectious susceptibility in CLL.[12] What is the reason for the paucity of pDC in CLL? To answer this question, we describe several key observations that have been made. First, circulating pDCs decline with age.[16] Furthermore, differences in TLR7 expression may explain some of the male bias generally observed with respect to severe COVID-19 susceptibility.[17] Pioneering studies of mouse models linked gene dosage effects at the *Tlr7* locus to autoreactive B cell responses and autoimmunity.[18] *TLR7* belongs to the

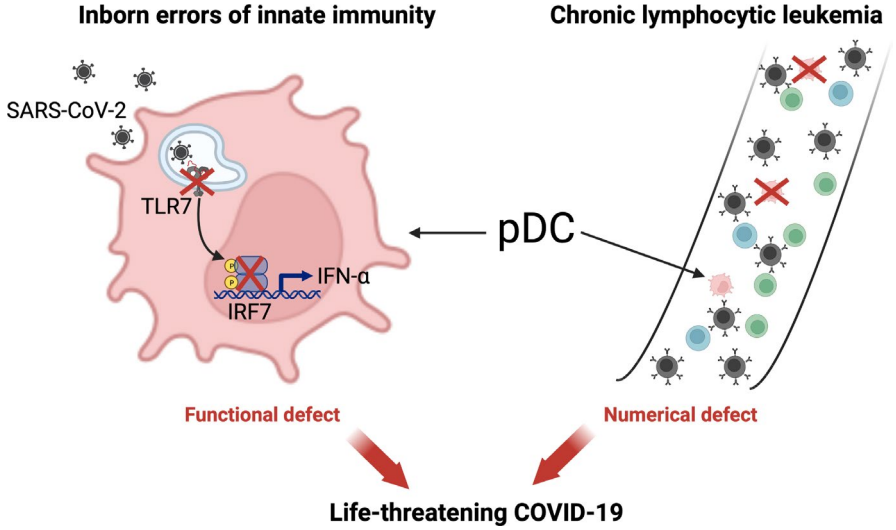

FIGURE 1 Plasmacytoid dendritic cell (pDC) and susceptibility to severe COVID-19. Several lines of evidence have highlighted the importance of pDC-derived type I IFN production for protection from severe COVID-19. pDC *functional* impairment of TLR7-mediated viral sensing in endosomes, or of IRF7-driven transcription, blocks subsequent IFN-α production and occurs by genetic variants causing TLR7 and IRF7 loss-of-function (left). Alternatively, as we propose, in the case of CLL, severe COVID-19 is caused by a *reduced number* of circulating pDC (right), secondary to the tumour burden (CLL cells are depicted as lymphocytes with antibodies on their surface)

selected group of genes that do not undergo lyonization,[19] with higher levels of TLR7 being expressed in females as compared to males.[20] Moreover, women seem to have a higher frequency of pDCs, and oestrogen was reported to increase TLR7 activity.[21] However, among patients with CLL, there is no overt sex-dependent difference in survival among SARS-CoV-2 infected individuals.[2,3] Thus, the preferential depletion of pDCs in CLL remains enigmatic. We speculate that the tumour burden in CLL, and not the treatment, is what may cause the striking reduction in pDCs and susceptibility to severe COVID-19. Even if other haematopoietic cell lineages are also lowered in CLL, as mentioned, multiple lines of evidence suggest that pDCs are essential for the early, innate defence against SARS-CoV-2.[5-15]

It is unclear to what extent the tumour burden in CLL inhibits pDC development. In both CLL patients and in the TCL1 mouse model, pDC precursors were found in normal amounts in the bone marrow. In the periphery, pDC numbers were reduced only in patients with progressive disease and linked to decreased FMS-like tyrosine kinase 3 receptor (FLT3) expression.[12] Furthermore, levels of TLR9, another Toll-like receptor highly expressed in pDCs, were reduced in mature pDCs obtained from the TCL1 mouse and from patients with advanced CLL, leading to a reduced IFN-α response to TLR9 agonists. In the mouse model, inhibition of TNF or TGF-β could increase FLT3 expression and restore pDC numbers.[12] Other experimental models have demonstrated that both IFN-α and IFN-γ can promote pDC development and differentiation synergistically with FLT3.[22-24] The mentioned insights into these cytokine networks also provide clues to how pDC numbers might be enhanced in CLL patients to strengthen viral immunity.

Prior to the COVID-19 outbreak, we and, during the pandemic, others reported that pDCs increase in number in CLL patients under treatment with BTK inhibitors (BTKi), as a likely consequence of the drug-mediated reduction in the tumour burden.[13,14] Conversely, we observed no changes in plasma IFN-γ,[25] one of the cytokines influencing the generation of pDCs. BTKi act by inhibiting the intracellular signalling molecule BTK through the binding to its catalytic domain.[26] BTKi have during recent years revolutionized the treatment of CLL and other haematopoietic malignancies and, to a great extent, replaced chemotherapy and monoclonals.[27-29] A majority of all clinical trials has been performed using the first approved BTKi, ibrutinib (Imbruvica); both effects and adverse effects may differ depending on the compound, as reviewed.[30] Presumably, all BTKi which reduce the tumour burden could promote the generation of pDCs. However, in order to achieve a profound reduction in the tumour mass, combinatorial treatment with other targeted therapies such as BCL-2 inhibitors may be necessary.

3 | BTK INHIBITOR TREATMENT IN CLL AND COVID-19 SUSCEPTIBILITY

Apart from the effect on pDCs, treatment with BTKi strongly impairs the antibody response to SARS-CoV-2 vaccine.[31,32] This is presumably due to the drug affecting not only the tumour population, but also non-malignant, naïve B lymphocytes. From this, it could be deduced that the primary humoral immune response is affected by BTKi not only during vaccination, but likely also in the course of COVID-19.

The overall importance of humoral immunity in COVID-19 is, however, unclear. Treatment of CLL with B cell depleting anti-CD20 monoclonals may aggravate the viral infection, but conclusive evidence is lacking.[2] However, such treatment increases the risk of severe COVID-19 and death in patients with rheumatoid disease.[33] Similar to the effect of anti-CD20 therapy, patients with another IEI, X-linked agammaglobulinemia, XLA, an inherited defect in the *BTK* gene,[34] have essentially no B lymphocytes and cannot mount humoral immune responses.[29,34] The outcome of COVID-19 in patients with XLA has varied from uneventful to more severe.[35,36]

Based on all these findings, the increased numbers of pDC during BTKi treatment[12,13] would improve the prognosis and BTKi could potentially also act as general suppressant of the COVID-19 hyperinflammation itself. To this end, BTKi have been investigated in several clinical trials outside of CLL to elucidate their potential ameliorating effect on severe COVID-19 pneumonia, but without consensus on the outcome.[37,38] Nevertheless, the time course may be crucial, and there could be a major difference between being on BTKi therapy prior to being infected with SARS-CoV-2 vs being treated after the onset of viral disease. Regarding patients with CLL, it was recently found that continued BTKi treatment may have had a potential, although not statistically significant, clinical benefit upon contracting COVID-19, as compared to those who stopped the drug.[2]

4 | CONCLUDING REMARKS AND A HYPOTHESIS

In conclusion, based on the existing evidence, we propose that an underlying mechanism for the severe course of COVID-19 reported in CLL is the reduced number of pDCs. Indeed, since these cells are key producers of type I IFN

and, hence, essential for combating COVID-19 at an early stage, any impairment, whether functional or numeric, will negatively affect the individual's capacity to clear an infection with SARS-CoV-2. It could be hypothesized that lack of pDCs also may contribute to the severe COVID-19 observed in other hematological conditions than CLL, as well as following conditioning regimens prior to hematopoietic stem cell or Chimeric Antigen Receptor (CAR) T-cell therapy. Furthermore, it could be envisaged the transfer of pDCs during the early phase of the viral infection may ameliorate COVID-19 in both patients with CLL and those with an impaired pDC function.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

CIES involved in conceptualization and writing original draft. RZ, AÖ, MP, PB and YB involved in hypothesis analysis. All authors involved in reviewing and editing.

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