PERSPECTIVE

Role of CD20$^+$ T cells in multiple sclerosis: implications for treatment with ocrelizumab

Features of CD20$^+$ T cells: CD20 is a membrane-spanning hosphoprotein strongly expressed on the cell surface of B lineage cells and is widely regarded as a B cell specific marker. However, CD20 (**Figure 1**) is also expressed at a low level on a small subset of CD3$^+$ T cells which therefore are sometimes referred to as CD20^{dim}CD3$^+$ T cells in contrast to CD20^{bright}CD19$^+$ B cells which represent the majority of cells expressing CD20 (Hultin et al., 1993). The amount of the CD20 antigen has been assessed to be 25 to 50 times higher on CD20$^+$CD19$^+$ B cells compared to CD20$^+$CD3$^+$ T cells (Hultin et al., 1993). At first description of this cell population the frequency of these CD20$^+$ T cells has been described to represent an average of 2.4% of all peripheral blood lymphocytes (50 healthy controls) (Hultin et al., 1993). In the largest characterized cohort with 142 healthy individuals CD20$^+$CD3$^+$ T cells constituted a mean proportion of 1.6% (range from 0.1–6.8%) of all circulating CD3$^+$ T cells and in absolute numbers accounted for approximately 28 cells/μL (Wilk et al., 2009).

Since CD20-expressing T cells could not be detected in cord blood of newborn children but were detectable in thymus of young infants less than 3 months of age, it has to be assumed that this cell population develops in early childhood (Schuh et al., 2016; von Essen et al., 2019). The T cell origin of the CD20$^+$CD3$^+$ T cells was repeatedly demonstrated by detection of CD20-encoding mRNA by real-time RT-PCR in this cell population but not in CD20$^-$ T cells (Wilk et al., 2009; Schuh et al., 2016), the presence of other T cell markers, such as CD2, CD5, CD4, or CD8 (Hultin et al., 1993), the absence of other B cell markers, such as CD19 (Wilk et al., 2009; Palanichamy et al., 2014), and the evidence of intracellular Ca^{2+} influx after treatment with anti CD3 antibody, which was not detectable after application of anti-immunoglobulin (Ig) antibodies (Hultin et al., 1993).

CD20$^+$ T cells were found in primary and secondary lymphatic organs, such as thymus, bone marrow, lymph nodes and adenoids, as well as in the liver and in the cerebrospinal fluid (CSF) of healthy controls suggesting a ubiquitous dissemination of this cell population (Wilk et al., 2009; Schuh et al., 2016).

Although CD20$^+$ T cells are phenotypically heterogeneous, they display significant differences compared to the majority of T cells which do not express CD20. CD20$^+$ T cells encompass both CD4$^+$ helper as well as CD8$^+$ cytotoxic subsets. Notably, the ratio of CD4$^+$/CD8$^+$ cells is shifted towards a higher proportion of CD20$^+$ T cells co-expressing CD8 compared to CD20$^-$ T cells. CD4:CD8 ratios in the CD20$^+$ T cell subset between 0.6 and 1.2 have been described in contrast to CD4:CD8 ratios between 1.8 and 2.3 in CD20$^-$ T cells which resemble the common CD4:CD8 ratio in the overall CD3$^+$ T cell population (Hultin et al., 1993; Wilk et al., 2009; Schuh et al., 2016). CD20$^+$ T cells are more likely to express different activation markers, like CD49a and CD45RO, compared to CD20$^-$ T cells whereas the proportion of cells expressing the activation marker CD38 is significantly lower in CD20$^+$ T cells (Hultin et al., 1993; Wilk et al., 2009; Schuh et al., 2016).

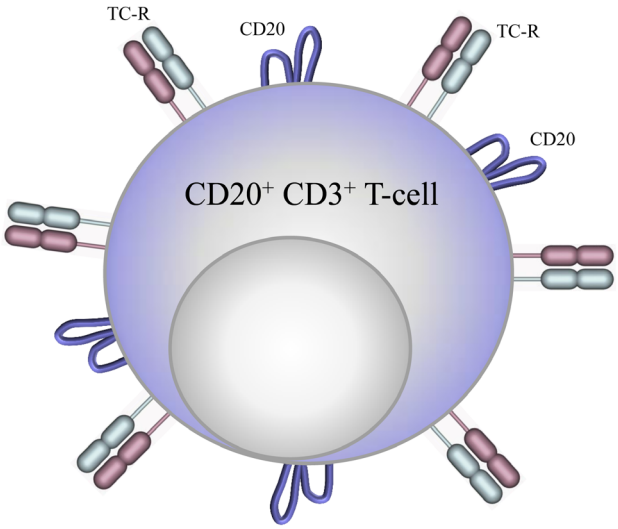

Figure 1 T lymphocyte expressing the T cell receptor (TC-R) and the marker CD20.

Interestingly, CD20-expressing T cells displayed a higher expression of CD95 (Fas/APO-1) and a considerable higher susceptibility to apoptosis under resting conditions as well as after stimulation by contrast with T cells which did not express CD20 (Wilk et al., 2009). Upon stimulation with CD3, CD20$^+$ T cells showed a significantly lower proliferation rate compared to CD20$^-$ T cells (Wilk et al., 2009). Strikingly, under resting conditions, an increased proportion of CD20$^+$ T cells displayed production of various cytokines compared to CD20$^-$ T cells which did not show a relevant percentage of cytokine-producing cells. Among others, expression of interferon-γ (IFNγ), interleukin (IL)-1β as well as other ILs, IL-2, IL-4, IL-8 and IL-10, chemokine (C-C motif) ligand 2, transforming growth factor β and tumor necrosis factor α was significantly increased in CD20$^+$ T cells compared to CD20$^-$ T cells. After stimulation, the amount of cytokine-producing T cells was strongly upregulated in both the CD20$^+$ as well as in the CD20$^-$ T cell population, but cytokine production remained significantly higher in CD20$^+$ T cells (Wilk et al., 2009). Under resting conditions, no relevant IL-17 production has been found in CD20$^+$ T cells (Wilk et al., 2009), but, upon stimulation, a small proportion (< 5%) of CD20-expressing T cells showed production of IL-17 in contrast to CD20$^-$ T cells, where significantly less cells were positive for IL-17 (Schuh et al., 2016).

In conclusion, CD20$^+$ T cells constitute a small subset of ubiquitously distributed T cells with an increased proportion of cytotoxic CD8$^+$ T cells, which represent a highly activated subpopulation with considerably enhanced cytokine production even during resting conditions. These findings are suggestive that CD20$^+$ T cells might play a crucial role in pro-inflammatory processes.

CD20$^+$ T cells in multiple sclerosis (MS): MS is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Although the exact pathophysiological mechanisms are far from being fully understood a vast body of evidence demonstrates that the disease is to a large extent driven by autoreactive T cells (Sospedra and Martin, 2016). Consequently, the subpopulation of CD20$^+$ T cells has also been investigated in patients with MS.

The CD4:CD8 ratio and the frequency of different subpopulations, e.g., naive, central memory, effector memory and memory T cells in the CD20$^+$ T cell subset was comparable to the CD20$^+$ T cell population in healthy controls (Palanichamy et al., 2014; von Essen et al., 2019). Also, other known properties of CD20$^+$ T cells, such as enhanced production of various cytokines (e.g., IFNγ, IL-4 or tumor necrosis factor α) or a higher rate of apoptosis, in contrast to CD20$^-$T cells were similarly observable in MS patients (von Essen et al., 2019). Interestingly, the frequency of CD20$^+$ T cells in peripheral blood is significantly elevated in patients with relapsing-remitting MS (RRMS) as well as in patients with primary progressive MS (PPMS) by contrast with healthy controls (Palanichamy et al., 2014; von Essen et al., 2019).

A considerably higher proportion of CD20$^+$ T cells in peripheral blood displayed expression of chemokine receptors and adhesion molecules as C-C chemokine receptor type 2 (CCR2), CCR5, CCR6 or melanoma cell adhesion molecule 1 in RRMS patients as well as in healthy controls compared to CD20$^-$ T cells. In line with the finding of an enhanced migratory potential of CD20$^+$ T cells, the proportion of CD20$^+$ helper and cytotoxic T cells of the general CD4$^+$ and CD8$^+$ T cell subpopulation was considerably higher in the CSF compared to peripheral blood in patients with RRMS (von Essen et al., 2019). While one study found similar frequencies of CD20$^+$ T cells and CD20$^+$ B cells in the CSF of RRMS patients (Schuh et al., 2016), another study described a significantly higher amount of CD20$^+$ T cells compared to B cells in the CSF of untreated RRMS patients (von Essen et al., 2019). CD8$^+$ CD20$^+$ T cells vary from CD4$^+$ CD20$^+$ T cells by showing a higher proportion of proliferating cells, a smaller percentage of cells prone to apoptosis, a greater share of IFNγ producing cells and differences in composition of individual T cell subtypes and expression of chemokine receptors (von Essen et al., 2019). However, functional distinctions between these two

cell populations have not been investigated in detail. Strikingly, the percentage of CD20$^+$ T cells in the CSF of RRMS patients correlated with clinical disability of MS patients, measured by the expanded disability status scale (von Essen et al., 2019). Interestingly, CD20$^+$ T cells in MS patients as well as in healthy controls showed increased antigen reactivity in response to the myelin antigens as myelin oligodendrocyte glycoprotein and myelin basic protein compared to CD20$^-$ T cells (von Essen et al., 2019). Consistent with these results, CD20-expressing CD4$^+$ and CD8$^+$ T cells were also found in chronic white matter lesions of MS patients (Holley et al., 2014).

In summary, the frequency of CD20$^+$ T cells is significantly higher in patients with RRMS and PPMS compared to healthy controls. CD20$^+$ T cells show a greater migratory capacity towards the CSF than CD20$^-$ T cells and their amount in the CSF correlates with disease severity. These results indicate that CD20$^+$ T cells might be important players in the pathophysiology of MS.

Ocrelizumab in the treatment for MS: As CD20 is widely regarded as a B cell specific marker, the aforementioned concept of T cells taking center stage in the pathophysiology of MS has been questioned in recent years, since anti-CD20 addressing therapies have shown an impressive impact on reducing disease activity in MS patients. Phase I and phase II studies with rituximab, a chimeric monoclonal anti-CD20 antibody, revealed rapid and pronounced reduction of inflammatory brain lesions and clinical relapses in patients with RRMS (Hauser et al., 2008). These convincing results of anti-CD20 therapy in the treatment of MS culminated in the development and finally approval of ocrelizumab, a humanized monoclonal anti-CD20 antibody, for the treatment of RRMS and PPMS. Ocrelizumab treatment resulted in a strong decrease of gadolinium-enhancing lesions already 4 weeks after administration of the first dose (Kappos et al., 2011). Phase III trials for ocrelizumab treatment showed lower rates of disease activity and progression in patients with RRMS and PPMS (Hauser et al., 2017; Montalban et al., 2017).

Impact of ocrelizumab on CD20$^+$ T cells – missing puzzle piece? It has previously been shown that rituximab depletes CD20$^+$ T cells in MS patients (Palanichamy et al., 2014). Since ocrelizumab exerts its cytotoxic effects in a different way (Kappos et al., 2011) and the binding site to CD20 is not identical with rituximab, it was unclear whether ocrelizumab might efficiently deplete CD20$^+$ T cells. We therefore analyzed blood samples of 21 patients with RRMS and PPMS before and 14 days after first administration of 300 mg ocrelizumab by multicolor flow cytometry (Gingele et al., 2018). We were able to confirm previous results of a predominant co-expression of CD8 by CD20$^+$ T cells. CD20$^+$ T cells were found in peripheral blood of every untreated MS patient and amounted to a mean frequency of 2.4% of CD45$^+$ lymphocytes or in absolute numbers to 42.5 cells/μL. Remarkably, CD20$^+$ T cells accounted for an average of 18.4% of all CD20$^+$ cells, also encompassing CD19$^+$ B cells. Taking into consideration that in MS patients nearly a fifth of all CD20$^+$ cells, which are addressed by ocrelizumab, are highly reactive T cells should lead to the conclusion that anti-CD20 directed therapies cannot be regarded as B cell specific. Flow cytometry analysis of blood samples 14 days after first application of ocrelizumab revealed that alongside CD20$^+$CD19$^+$ B cells also CD20$^+$CD3$^+$ T cells were nearly completely depleted.

This striking result of swift and efficient depletion of CD20$^+$ T cells, which represent a highly activated T cell subset with pro-inflammatory capabilities and a relevant proportion of all CD20$^+$ cells, is likely to be one of the missing puzzle pieces explaining the compelling clinical effectiveness of anti-CD20-directed therapies and ocrelizumab particularly.

Clinical trials have shown rapid and pronounced reduction of gadolinium-enhancing lesions already 4 weeks after administration of the first dose of ocrelizumab or rituximab, which was the earliest time point measured (Hauser et al., 2008; Kappos et al., 2011). Since total antibody levels were not altered, clinical effects of treatment with anti-CD20 therapies are not explainable by reduction of pathogenic autoantibodies. Instead, the lack of B cells serving as antigen presenting cells for T cells or the absence of B cells to produce cytokines to activate T cells have been discussed as possible effect mechanisms of ocrelizumab and rituximab in the treatment of MS.

When investigating the mode of action of anti-CD20-directed therapies, instead of solely focusing on possible effects of B cell depletion, the role of CD20$^+$ T cells should be moved into the spotlight. They represent a unique cell population with a highly activated phenotype, pro-inflammatory and migratory properties and considerable evidence exists that they play an important role in the pathophysiology of MS. Further clinical and basic research is needed to elucidate the role of CD20$^+$ T cells in MS.

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