# CD20$^+$ T Cells as Pathogenic Players and Therapeutic Targets in MS

Success of anti-CD20 therapy has placed B cells center stage, to an extent that T cells are becoming perceived as minor players in the pathogenesis of multiple sclerosis (MS). However, anti-CD20 therapy not only depletes CD20$^+$ B cells, but also a very distinct subset of CD20$^+$ T cells which has pro-inflammatory features.[1–6] In this issue of the *Annals of Neurology*, Quendt et al show that different approved therapies affect this subset of T cells differently.[5] They show that DMF reduces expression of chemokine receptors, such as CXCR3 as well as the cytokine-producing ability of CD20$^+$ T cells. Fingolimod reduces these cells in the blood as part of its overall T-cell depleting effect. Natalizumab increases the level of circulating CD20$^+$ T cells, in line with the concept that blocking α4 integrin prevents the exit of encephalitogenic T cells from blood. This confirms and extends previous observations describing effects of fingolimod, DMF, and natalizumab,[2] as well as alemtuzumab[4] on CD20$^+$ T cells. Quendt et al further show that these CD20$^+$ T cells produce increased amounts of the encephalitogenic cytokines GM-CSF, IFN-γ, IL-17, and TNF-α as well as the regulatory cytokines IL-4 and IL-10.[5] This extends previous reports about the cytokine profile of CD20$^+$ T cells[2,4] and their expression of the cytotoxic markers perforin and GzmB (Table).[7]

CD20$^+$ T cells constitute about 5% of all circulating T cells. The intensity of CD20 membrane expression on this T cell subset is lower than that on B cells, therefore they are sometimes designated as CD20$^{dim}$ T cells. These cells are heterogenous, but enriched for memory T cells[2,5] and CD8$^+$ T cells.[1,2,4] CD4$^+$CD20$^+$ and CD8$^+$CD20$^{+4}$ T cells, or only CD8$^+$CD20$^+$ T cells,[1] were reported to be enriched in the blood of patients with MS.

CD20$^+$ T cells pervade tonsils, bone marrow, and thymus as seen by fluorescent activated cell sorting (FACS).[2] Imaging mass cytometry in a primate study localized CD3$^+$CD8$^+$CD20$^+$ T cells in lymph nodes at the margin of follicles.[8] CD20$^+$ T cells express a higher level of adhesion molecules and chemokine receptors than CD20$^-$ T cells, indicating an increased migratory potential to the central nervous system (CNS).[4,5] In the cerebrospinal fluid (CSF), CD20$^+$ T cells constitute about 5 or more percent of total immune cells, so they are about as abundant as B cells.[2,4] A positive correlation between the percentage of CD20$^+$ T cells in the CSF with the level of myelin basic protein (MBP) in the CSF and also with the Expanded Disability Status Scale (EDSS) has been described,[4] an intriguing observation awaiting confirmation and elaboration. CD20$^+$ T cells were also detected

**TABLE. Properties of CD20$^+$ T Cells and Evidence for their Role in the Pathogenesis of MS**

| Feature | Detailed properties |
|---|---|
| Abundance | About 5% of blood cells, pervade lymphatic organs |
| Phenotype | Heterogeneous, enriched for CD8$^+$ T cells and memory T cells |
| Cytokine profile | Largely pro-inflammatory |
| Expression of adhesion molecules and chemokine receptors | Enhanced, strong migratory capacity |
| Antigen-specificity | Increased reactivity to myelin self-antigens |
| Response to MS therapeutics: | Differentially targeted by different therapies |
| Presence in CSF | Enriched; Correlated to MBP content |
| Presence in MS lesion | Enriched in cells with a phenotype of tissue-resident T cells |

CSF = cerebrospinal fluid; MBP = myelin basic protein; MS = multiple sclerosis.

 

by immunostaining of MS lesions in the perivascular space, and by FACS analysis of fresh autoptic material.[9] The expression of CD20 on T cells was associated with a phenotype of tissue resident memory T cells; further, expression levels of CXCR6, Ki-67, and granzyme B were higher on CD20$^+$ when compared to CD20$^-$CD8$^+$ tissue resident memory T cells.[9]

Using peptide:MHC I tetramers, an increased proportion of myelin-specific CD8$^+$ T cells in patients with MS exhibited a memory phenotype and expressed CD20 compared to control subjects.[10] Myelin oligodendrocyte glycoprotein and MBP induced a higher proliferation in CD20$^+$ than in CD20$^-$ blood-derived T cells from patients with MS.[4]

All these properties - cytokine profile, adhesion molecule expression, antigen-specificity, localization in CSF and MS lesions along with the effects of MS therapeutics - support the view that CD20$^+$ T cells contribute to the pathogenesis in MS. Most likely, however, the efficacy of anti-CD20 in MS is not entirely dependent on depletion of CD20$^+$ T cells, because first evidence has been provided that B cell depletion with anti-CD19 is also beneficial in MS.[11] This would be independent of CD20$^+$ T cells as they lack CD19 transcripts and surface protein.[1,2,9]

Whereas anti-CD20 therapy almost completely depletes CD20$^+$ B cells and T cells in the blood,[1–3, 6,10] depletion in lymphatic tissue is far less complete. In a primate model, low dose ofatumumab depletes B cells and CD20$^+$ T cells in blood and lymphatic organs, but apparently spares marginal zone B cells.[8] This sparing of marginal zone B cells still needs to be elaborated, but it may contribute to the safety profile of anti-CD20 therapy.

The function of CD20 on B cells has remained unclear until it was recently reported that CD20 is an organizer of the IgD-class nanocluster on the B cell membrane.[12] The loss of CD20 or engagement with rituximab results in a dissolution of the IgD-class nanocluster and a transient B cell activation inducing a B cell-to plasma cell differentiation. It was concluded that CD20 functions as gatekeeper of the resting state of naive B cells.[12]

What is the origin of CD20 on this subset of T cells? Quendt et al refer to a yet unpublished paper of their own group stating that CD20$^+$ T cells emerge from B/T cell interactions during which membrane embedded CD20 is transferred from B cells to T cells via trogocytosis.[5] This is intriguing, but previous studies reported low levels of transcripts for CD20 in human CD20$^+$ T cells in blood[1,2] and also in MS lesions.[9] The relative contribution of transcription in T cells versus trogocytosis from B cells as well as potential species differences remain to be resolved.

Broadening the view from MS to other diseases might enhance our understanding of CD20$^+$ T cells in MS. In Sjögren's syndrome, a presumably pathogenic CD20$^+$ fraction of IL-17 producing T cells was reduced by rituximab.[13] In a patient with nephrotic syndrome caused by minimal change disease, a relapse was successfully treated with rituximab; remarkably, this patient had no detectable B cells, but CD20$^+$ T cells in blood when rituximab was administered.[14] The success of rituximab in this case could be explained by depletion of CD20$^+$ T cells or of CD20$^+$ B cells in lymphatic organs. The importance of CD20$^+$ T cells is not restricted to autoimmune diseases but extends to a role in replication of HIV: CD20 is expressed by productively HIV-infected T cells and the HIV reservoir could be reduced by anti-CD20 treatment.[15]

In conclusion, there is growing evidence that CD20$^+$ T cells importantly contribute to the pathogenesis of MS and serve as therapeutic targets not only for anti-CD20 antibodies, but also for several additional MS therapies.

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## Potential Conflicts of Interest

None of the authors has a conflict of interest related to this invited commentary.

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