B cells and progressive multifocal leukoencephalopathy: search for the missing link

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Progressive multifocal leukoencephalopathy (PML) is a devastating demyelinating disease caused by JC virus (JCV) replication in the brain. PML classically occurs in patients with severe immunodepression, and cases have recently been linked to therapeutic monoclonal antibodies such as natalizumab and also rituximab, which depletes B cells. B cells appear to play a complex role in the pathogenesis of PML. They may act as a viral reservoir and as a vector for viral dissemination in the central nervous system. Anti-JCV antibody responses appear to have a limited effect on JCV replication in the brain. However, accumulating evidence suggests that B cells may considerably influence T cell responses through their cytokine secretion. This immunomodulatory function of B cells may play an important role in the control of JCV infection and in the pathogenesis of PML, including rituximab-induced PML.

Keywords: progressive multifocal leukoencephalopathy, JC virus, B cells, immune regulation, T cells

Progressive multifocal leukoencephalopathy (PML) is a devastating demyelinating disease caused by JC virus (JCV) replication in the brain. PML classically occurs in patients with severe immunodepression due to disorders such as AIDS, hematological malignancies, and sarcoidosis, but is also a recognized adverse effect of therapeutic monoclonal antibodies such as natalizumab, efalizumab, and rituximab used to treat autoimmune diseases and hematological malignancies (1, 2). Specific CD4 and CD8 T cell responses appear to play a critical role in the control of JCV infection: for instance, the beneficial effect of highly active antiretroviral therapy (HAART) on AIDS-related PML is largely due to restoration of anti-JCV T cell immunity (3–5). The PML-promoting effect of rituximab, an anti-CD20 monoclonal antibody that specifically depletes B cells, suggests that B cells also contribute to the control of JCV infection (1). The incidence of PML in rituximab-treated patients depends on the underlying disease: it is about 2/8,000 in patients with systemic lupus erythematosus (SLE) and 1/25,000 in those with rheumatoid arthritis (RA) (6, 7). B cells have a dual role in PML: first, they can serve as a viral reservoir and may help disseminate the virus in the brain; second, they are an important component of the adaptive immune response and may play a significant role in JCV control.

B Cells are a Potential JCV Reservoir and a Vector for CNS Dissemination

JC virus infection usually occurs in childhood and persists throughout life. It generally remains clinically silent, despite active virus replication in the kidneys and urinary virus excretion in a
significant proportion of the general population. Severe, prolonged immunosuppression may lead to JCV dissemination to the central nervous system (CNS) from sites of persistence (kidney, bone marrow, lymphoid organs), or to reactivation of dormant virus already present in the CNS. In both cases, this may lead to productive infection of oligodendrocytes, followed by demyelination and development of PML (2, 8). Detection of JCV DNA in peripheral B lymphocytes and of JCV-infected B cells in brain tissue of PML patients suggests that B cells are directly involved in JCV dissemination to the CNS (9–12).

JC virus can infect CD34+ hematopoietic precursor cells and B cells, but not primary T cells (9, 13). Chapagain et al. showed that JCV can enter B cells and persist as intact virions (12). B cells are probably infected by JCV in lymphoid tissues such as the tonsils, spleen, and bone marrow (14–16). JCV-infected B cells may also derive from latently infected hematopoietic precursors in bone marrow (17–19). Nucleotide sequence analysis of JCV in peripheral blood mononuclear cells (PBMC), urine, and cerebral spinal fluid (CSF) of PML patients has revealed JCV sequence variations and rearrangements that influence viral pathogenicity and tropism (18, 20–25). JCV persists in at least two forms: a non-pathogenic form (archetypal virus) and a neurotropic form that contains a rearranged non-coding control region (NCCR) (20, 24, 26). B cells could serve for the generation, persistence, and dissemination to the CNS of the neurotropic form (27). Glial cells (the main targets of JCV in the brain) and B cells, but not T cells, both express nuclear DNA binding proteins that interact with the regulatory region of the JCV genome and may permit JCV replication (10, 28, 29). The NCCR is involved in transcriptional control of both early and late viral genes (25, 30–33). Two transcription factors (NF-1X and Spi-B) important for JCV genome transcription are upregulated in glial cells, B cells, and hematopoietic progenitor cells (25, 34, 35). Spi-B binding sites are present in the promoter/enhancer of JCV neurotropic variants but not in the archetypal virus. These sites are located in the region adjacent to TATA boxes, which are essential for the transcription of early and late viral genes (35–37). Rituximab modifies B cell homeostasis, and the reconstituted B cell pool after treatment consists mainly of immature (IgD<sup>+</sup>CD10<sup>+</sup>CD24<sup>-</sup>CD38<sup>-hi</sup>) and naive B cells (38–42). Rituximab depletes CD20<sup>+</sup> mature B cells in the periphery, probably leading to mobilization of pre-B and B cells from bone marrow and lymph nodes, along with an increase in CD34<sup>+</sup> progenitors in the periphery (17). Infected B cells arising from bone marrow and lymph nodes may transmit the infection to microvascular endothelial cells and, after crossing the blood–brain barrier, to glial cells (11, 12, 14). Also, natalizumab has been reported to inhibit VLA-4-dependent retention of CD34<sup>+</sup> hematopoietic precursor cells, B cell precursors, and B cells in bone marrow and lymphoid tissues, leading to increased circulation of pre-B and B cells (17, 43). However, it remains unclear how much its effects on B cells may contribute to natalizumab-associated PML.

Thus, the following conditions are required for JCV-induced PML to occur: changes in the NCCR that enhance viral transcription and replication; the presence of transcription factors that bind to the rearranged NCCR; immunodeficiency; and, likely, other factors such as an individual genetic predisposition.

The Specific Antibody Response Appears Insufficient to Control JCV Infection

Humoral immunity, and particularly the production of neutralizing antibodies, is an important line of defense against viral infections (44). Intrathecal antibody synthesis is observed in infections due to herpes simplex, varicella zoster, Epstein–Barr, cytomegalovirus, mumps, rubella, measles, dengue, and JCVs (45–48). Intrathecal synthesis of oligoclonal antibodies against VP1, the major structural protein of JCV, is found in PML patients, and a positive correlation has been found between the intensity of this response and the plasma cell count in PML brain tissue (45, 49). Between 67 and 78% of PML patients have an anti-VP1 intrathecal antibody response but its protective effect is unclear (45). Intrathecal synthesis of anti-VP1 antibodies with low affinity has occasionally been found in chronic CNS immune disorders such as multiple sclerosis (MS) and neuro lupus and infections (mumps meningitis and neuroborreliosis) (50–54). This low-affinity anti-VP1 antibody response may be related to reactivation of memory B cells already present in the CNS (45). T cell and IgG responses to JCV are significantly increased in HIV-infected PML survivors, and the IgG response correlates positively with the CD4 T cell count but negatively with HIV RNA load (55). Neither intrathecal nor serum JCV-specific antibodies prevent the onset or progression of PML in HIV-infected patients (56). A longitudinal study of an HIV-seronegative PML patient showed that the anti-VP1 antibody response increased with time, yet neurological status deteriorated and the patient died (45).

B Cells Modulate the Differentiation and Functions of CD4 and CD8 T Cells

The use of rituximab to treat autoimmune diseases has provided important clues to the regulatory effects of B cells on cellular immunity. In addition to B cell depletion, rituximab modulates the numbers and functions of peripheral blood lymphocyte subsets such as T, NK, and NKT cells in several autoimmune diseases, including RA, SLE, Evans’ syndrome, and MS (57–61). Rituximab treatment leads to substantial depletion of peripheral T cells, a decrease in the proportion of CD4 cells expressing the early activation marker CD69 and, conversely, an increase of the frequency of CD4<sup>+</sup>CD25<sup>hi</sup> regulatory T cells (58, 59, 61).

Lykken et al. demonstrated that acute and chronic B cell depletion by an anti-CD20 monoclonal antibody disrupts CD4 and CD8 T cell homeostasis and expansion in mice during acute viral infection (62). B cells appear to be required for optimal CD4 and CD8 T cell responses to acute and chronic viral infections in mice (62, 63). Immunoglobulin mu chain gene knockout (IgM<sup>−/−</sup> mice) have normal cytotoxic T cell responses to vesicular stomatitis virus (VSV), as well as to vaccinia virus and LCMV (acute Armstrong variant) (63). However, the initially normal CTL response to LCMV infection in IgM<sup>−/−</sup> mice disappears in the long term, leading to viral persistence (63). Adoptive transfer experiments show that naive and activated antiviral CD8 T cells from transgenic mice expressing an LCMV gp33-specific TCR are rapidly exhausted and disappear after trans infection into mice persistently infected by the LCMV–WE strain (63). Cotransfusion
of immune CD4 T cells or primed B cells from infected mice prevents this exhaustion, contrary to transfection of hyperimmune serum (63). This suggests that the positive effect of B cells on CD8 T cell antiviral functions is independent of antibody secretion. In B-cell-deficient mice, the CD8 T cell response is effective on acute LCMV and influenza virus infection but not on chronic LCMV infection (64–68). The absence of B cells results in increased death of activated CD8 T cells during the contraction phase, leading to poorer antigen-specific CD8 T cell memory (65, 69). CD4 T cells are required for the generation, long-term maintenance, and optimal reactivation of memory CD8 T cells (70–75). B cells are also required for the generation of CD4 T cell memory (68, 76–78). B-cell-deficient IgM−/− mice infected with a persistent LCMV variant have a profound CD4 help defect and secrete less interferon-gamma (IFN-γ) and interleukin 2 (IL-2) than normal mice, a defect mainly affecting CD8 T cells (76). In contrast to B-cell-deficient mice, transgenic mice that have normal proportions of B cells in the periphery but do not secrete LCMV-specific antibodies still have a functional CD4 T cell memory (68). This confirms that the effect of B cells on CD4 T cell memory is independent of antibody secretion. In mice depleted of B cells by anti-CD20 and infected by LCMV (Armstrong strain), primary virus-specific CD4 T cell effectors are generated but the CD4 memory precursor population is reduced and memory T cells show impaired cytokine production (79). These experiments suggest that B cells play a significant role in the generation of CD4 and CD8 T cell memory. As CD4 T cell help is required for CD8 memory T cell generation and maintenance, and as B cells influence CD8 T cell antiviral responses, an indirect effect via CD4 T cells appears likely. The effect of B cells on T cell responses may involve cytokine production (80). Indeed, cytokines secreted by B cells can modulate the differentiation and functions of several immune effectors, including CD4 and CD8 T cells, possibly explaining the antibody-independent immunoregulatory functions of B cells (80–84). The mechanisms that control cytokine production by B cells are therefore drawing increasing attention.

**Effector B Cells as Amplifiers of Th1-Type Responses to Viral Infections**

B cells produce cytokines in response to a broad array of stimuli, including microbial products, antigens, and T cell-derived signals (80, 85). Under appropriate conditions in vitro, B cells differentiate into effector subgroups 1 and 2 (Be1 and Be2), which produce cytokines associated with Th1 and Th2 responses, respectively (86–89). In mouse experiments, differentiation into Be1 cells is induced by Th1 lymphocytes and mediated by IFN-γ and antigenic activation through B cell receptors (88). Like IFN-γ, IL-12 plays a key role in Be1 polarization, but the initial trigger of Be1 commitment is likely type-I interferons (IFN-α/β) (89, 90). These interferons initiate a cascade of molecular events that induce B cell differentiation into Th1-like cells (89, 90). Similarly, naive B cell differentiation into Be2 cells is dependent on IL-4 (88). Be1 and Be2 cells, by producing polarizing cytokines such as IFN-γ and IL-4, induce the differentiation of naive CD4 T cells into Th1 and Th2 cells (86). Spatiotemporal interactions between B cells, CD4 T cells, and dendritic cells (DCs) are critical during early viral infection and likely determine the orientation and nature of the immune response. Immediately after VSV infection in mice, antigen-specific B and CD4 T cells interact at the T cell–B cell zone border (91). During initiation of the immune response, intact antigens are presented to B cells by DCs (especially follicular DCs), and then B cells present them in the form of peptides to T cells (92–94). Be1 commitment may be initiated by IFN-α/β and then by IL-12 produced by DCs. After antigen priming, T cells migrate toward the B cell area of lymph nodes where they interact with B cells, which, by secreting Th1-like cytokines, may stabilize Th1 differentiation of CD4 T cells. IFN-γ-secreting Be1 and Th1 cells may positively influence each other, thereby creating a Th1 amplification loop between B and T cells.

As Th1 cells are involved in the control of intracerebral JCV infection (95), the Th1-type amplification loop created by B–T cell interactions might be important for the development of effective anti-JCV immune responses. Withdrawal of natalizumab therapy in multiple sclerosis patients who develop PML leads to an immune reconstitution inflammatory syndrome (IRIS) in the brain, due to massive afflux of autoimmune and JCV-specific T cells (96, 97). In MS patients with PML–IRIS, brain-infiltrating anti-JCV CD4 T cells are largely IFN-γ-secreting cells. Bi-functional Th1-2 cells (secrating both IL-4 and IFN-γ) are also present, while IL-17-producing cells are barely detectable (98). Histopathologic analysis of brain tissue from patients with IRIS has revealed the prominent presence of not only CD4 and CD8 T cells but also B/plasma cells and monocytes (98). The regulation of B cell activation by antigen sequestered within the CNS is unclear. Despite the lack of draining lymphatic vessels in the CNS, antigen-bearing DCs can migrate from the CNS to cervical lymph nodes, preferentially reaching B-cell follicles rather than T cell-rich areas (99). B cells activated by antigen-bearing DCs may interact with T cells and favor Th1 differentiation. Thus, by disrupting Th1 responses (100), rituximab may impair the cellular immune response to JCV.

**B Cells as Regulators of Cellular Immune Responses to Viral Infections**

The regulatory effects of B cells on immune responses are complex and not only restricted to Th1- or Th2-like responses: some B cells, described as B regulatory cells (Bregs), also have T regulatory-like activities (80, 101, 102). Besides pro-inflammatory cytokines, many B cell subsets also secrete IL-10, a cytokine that suppresses both the activities of T cells (CD4 and CD8) and innate cell-mediated inflammatory responses, while also being involved in Treg maintenance (81–84, 101–104). Breg function is mainly but not exclusively dependent on IL-10 (80, 101, 102). Mice and human plasma cells, in addition to their Ig production, could contribute to immune regulation by producing IL-10, like Bregs (105, 106). Interestingly, B cell depletion with rituximab has an inducing effect on Tregs (107–111). In SLE, RA, lupus nephritis, and idiopathic thrombocytopenic purpura patients, and particularly in good responders, the Treg frequency and response are restored or enhanced by rituximab (107–111).

B cell homeostasis is modified after rituximab treatment, and the reconstituted B cell pool consists mainly of immature (IgD+CD10+CD24hiCD38hi) and naive B cells with increased CD38 and CD5 expression (112–114). Besides immature and...
naive B cells, plasma cells are also prominent in the reconstituted B cell population (112, 114). However, CD27+ memory B cells recover more slowly than naive B cells and remain below baseline values for about 2 years (112). It has been demonstrated that the cytokine profile (anti- or pro-inflammatory) depends on the B cell differentiation stage (naive, memory, etc.) (113). Indeed, IL-10 is produced almost exclusively by naive B cells, while the pro-inflammatory cytokines lymphotoxin (LT) and tumor necrosis factor (TNF-α) are mainly produced by memory B cells (113). Therefore, rituximab-induced changes in the reconstituted B cell population may also affect the overall B cell cytokine profile (113). Naive B cells predominate in the post-rituximab B cell population; in addition, IL-10 production is enhanced and LT and TNF-α production is downregulated as compared to the pretreatment situation (113). The impact of cytokine changes induced by B cell depletion is evident in myasthenia gravis patients who respond well to rituximab: indeed, these patients exhibit rapid repopulation by IL-10-producing B cells and a sustained increase in the circulating Treg frequency, contrary to non-responders (115, 116). The immunosuppressive effect of rituximab could result from the disappearance of Be1 cells leading to failure of effector T cell activation, and also from the selective survival and

**FIGURE 1** | Regulation of anti-JCV T cell responses by different B cell subsets and the impact of therapeutic B cell depletion on this regulation. In this model, naive and memory B cells and plasma cells play distinct roles in the regulation of antiviral immune responses through the release of different cytokines. Following therapeutic B cell depletion, there is a shift towards regulatory-like cytokine secretion by the B cell pool. Before therapeutic B cell depletion, IFN-γ-secreting Be1 and Th1 cells mutually enhance each other’s functions and favor a CD8 T cell response, which effectively controls JCV infection. B cell depletion disrupts the Th1 amplification loop and thereby impairs T cell responses to JCV. In contrast to anti-CD20, anti-CD19 depletes also plasma cells. After therapeutic B cell depletion, the B cell pool is mainly reconstituted by naive B cells and plasma cells (IL-10- and IL-35-producing cells), which may promote Treg-like responses. CD1d+ CD6+ regulatory B cells may exhibit some resistance to anti-CD19-mediated depletion. Enhanced Breg and Treg responses disrupt T cell-mediated control of JCV infection and may favor the emergence of PML. Abbreviations: Mem B, memory B cell; Be1, effector B cell subgroup 1 (Th1-like B cells); Breg, B regulatory cells (Treg-like B cells); Th1, Th helper 1 cells, Treg, regulatory T cells.
repopulation of Breg-like subsets. It has been shown that human CD19hiCD24hiCD38hi B cells have regulatory effects that include inhibition of the differentiation of naïve T cells into Th1/Th17 cells and the conversion of CD4+ CD25+ T cells into Tregs by IL-10 (102, 117). In addition to CD19hi B cells, it has recently been found that plasmablasts and plasma cells are important IL-10 producers and that they can inhibit the effects of DCs on the generation of effector T cells (118, 119). In addition to IL-10, plasma cells also produce IL-35 (119). IL-35, which induces Tregs, also regulates the expansion and activity of IL-10-producing Bregs (119–122). Wang et al. have shown that IL-35 induces B cell differentiation into a Breg subset that produces IL-35 as well as IL-10 (122). Mice that lack IL-35 or are defective in IL-35 signaling produce fewer Bregs and develop severe experimental autoimmune uveitis (122). Together, these results suggest that naïve B cells, memory B cells, and plasma cells have distinct roles in regulating immune responses by secreting cytokines with pro- or anti-inflammatory effects, and that rituximab treatment can induce a shift toward a regulatory-like cytokine profile. Early during B cell reconstitution after rituximab treatment, the predominant response seems to be Breg-like, while Be1- and Be2-like responses only appear once memory B cells emerge.

The effect of rituximab on B and T cell responses in the CNS is well documented because of the beneficial effects of this drug in MS (123, 124). In particular, rituximab has been shown to deplete B cells in CSF (123, 125–127). In addition, necropsy studies of patients who died of rituximab-induced PML have shown that rituximab also depletes B cells in cerebral perivascular spaces (127). Rituximab could promote the onset of PML by successive effects on B cell homeostasis. First, it eliminates Be1 cells, thereby inhibiting the activation of effector T cells (Figure 1). Then, as shown in Figure 1, repopulation by Breg-like cells such as IL-10-producing B cells and plasma cells, initially in the periphery and then in the CNS, promotes a Treg-like response and inhibits inflammatory responses (81, 128, 129). In vitro experiments suggest that Bregs could influence T cell responses in brain via IL-10, by inhibiting microglia activation following viral antigen stimulation and promoting Treg proliferation (128). It remains to be determined whether B cell-depleting antibodies other than anti-CD20 have the same potential to induce PML. In the EAE model, a single injection of monoclonal anti-CD19 inhibited leukocyte infiltration into the spinal cord and disrupted disease development (130). In contrast to anti-CD20, anti-CD19 depletes not only mature B cells but also short- and long-lived CD138hi plasma cells (130). However, CD19hi CD5+ regulatory B cells showed some resistance to anti-CD19-mediated depletion, which was not related to decreased CD19 expression (130). Together, these observations suggest that while anti-CD9 may reduce the B cell-related immune response, it may also spare some regulatory mechanisms (Figure 1). This may have a positive effect on autoimmune diseases but might favor the onset of opportunistic infections.

**Conclusion**

The role of B cells in JCV infection and PML is likely more complex than initially thought. Indeed, on the one hand, B cells represent a potential reservoir for JCV and may disseminate the virus to the CNS while, on the other hand, they likely play a regulatory role in the immune response that controls JCV infection. The role of the humoral response in the control of JCV remains to be clarified but is probably less important than the T cell response. The association between rituximab and PML suggests that B cells may help to control JCV infection through functions other than antibody production. B cells secreting Th1-type cytokines such as IFN-γ probably enhance the Th1 response and thereby help to establish effective CD8 T cell activity against JCV. In addition, Treg responses are enhanced in B cell-depleted human and mouse models. These Treg responses could be induced by post-rituximab repopulating B cells, which could be predominantly IL-10-producing cells. A better understanding of the complex relations between JCV and B cells may have significant implications for the prevention and treatment of PML.

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**References**

1. Carson KR, Focosi D, Major EO, Petrini M, Richey EA, West DP, et al. Monoclonal antibody-associated progressive multifocal leucoencephalopathy in patients treated with rituximab, natalizumab, and efalizumab: a review from the research on adverse drug events and reports (RADAR) project. *Lancet Oncol* (2009) 10:816–24. doi:10.1016/S1474-4422(10)70260-8
2. Tan CS, Koralnik IJ. Progressive multifocal leucoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. *Lancet Neurol* (2010) 9:425–37. doi:10.1016/S1474-4422(10)70040-5
3. Gasnault J, Costagliola D, Hendel-Chavez H, Dulouest A, Pakianather S, Mazer AA, et al. Improved survival of HIV-1-infected patients with progressive multifocal leucoencephalopathy receiving early 5-drug combination antiretroviral therapy. *PLoS One* (2011) 6:e20967. doi:10.1371/journal.pone.0020967
4. Gasnault J, Kahraman M, de Goer de Herve MG, Durali D, Delfraisey JF, Taoufik Y. Critical role of JC virus-specific CD4 T-cell responses in preventing progressive multifocal leucoencephalopathy. *AIDS* (2003) 17:1443–9. doi:10.1097/00001883-200307040-00004
5. Engsig FN, Hansen AB, Omland LH, Kronborg G, Gerstoft J, Laursen AL, et al. Incidence, clinical presentation, and outcome of progressive multifocal leucoencephalopathy in HIV-infected patients during the highly active antiretroviral therapy era: a nationwide cohort study. *J Infect Dis* (2009) 199:77–83. doi:10.1086/595299
6. Carson KR, Evens AM, Richey EA, Habermann TM, Focosi D, Seymour JF, et al. Progressive multifocal leucoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the research on adverse drug events and reports project. *Blood* (2009) 113:4834–40. doi:10.1182/blood-2008-10-186999
7. Clifford DB, Ances B, Costello C, Rosen-Schmidt S, Andersson M, Parks D, et al. Rituximab-associated progressive multifocal leucoencephalopathy in rheumatoid arthritis. *Arch Neurol* (2011) 68:1156–64. doi:10.1001/archneurol.2011.103
8. White MK, Khalili K. Pathogenesis of progressive multifocal leucoencephalopathy – revisited. *J Infect Dis* (2011) 203:578–86. doi:10.1093/infdis/jiq997
9. Wei G, Liu CK, Atwood WJ. JC virus binds to primary human glial cells, tonsillar stromal cells, and B-lymphocytes, but not to T lymphocytes. *J Neurovirol* (2000) 6:127–36. doi:10.3109/13550280090131516
10. Major EO, Amemiya K, Elder G, Houff SA. Glial cells of the human developing brain and B cells of the immune system share a common DNA binding factor...
for recognition of the regulatory sequences of the human polyomavirus, JCV. J Neurosci Res (1990) 27:641–71. doi:10.1002/jnr.490270405

11. Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev (1992) 5:49–73.

12. Chapagain ML, Nerurkar VR. Human polyomavirus JC (JCV) infection of human B lymphocytes: a possible mechanism for JCV transmigration across the blood-brain barrier. J Infect Dis (2010) 202:184–91. doi:10.1086/653823

13. Monaco MC, Shin J, Major EO. JC virus infection in cells from lymphoid tissue. Dev Biol Stand (1998) 94:115–22.

14. Houff SA, Major EO, Katz DA, Kufta CV, Sever JI, Pittaluga S, et al. Involvement of JC virus-infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. N Engl J Med (1988) 318:301–5. doi:10.1056/NEJM198802043180507

15. Monaco MC, Atwood WJ, Gravell M, Monceau-Oliver HD, Latorre A, Major EO. Detection of JC virus DNA and proteins in the bone marrow of HIV-positive immunosuppressed patients. Ann Neurol (2001) 50:397–406. doi:10.1002/ana.10573

16. Weaver AL, Miller S, Berlin E, Rybicki EB, Major EO. JC virus infection in bone marrow stromal cells: implications for viral latency. J Virol (1996) 70:704–12.

17. Major EO. Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. Annu Rev Med (2010) 61:35–47. doi:10.1146/annurev.med.080708.082655

18. Tan CS, Dezube BJ, Bhargava P, Auville J, Wuthrich C, Miller C, et al. Detection of JC virus DNA and proteins in the bone marrow of HIV-positive and HIV-negative patients: implications for viral latency and neurotropic transformation. J Infect Dis (2009) 199:861–8. doi:10.1086/597117

19. Brew BJ, Davies NW, Cinque P, Clifford DB, Nath A. Progressive multifocal leukoencephalopathy and other forms of JC virus disease. Nat Rev Neurol (2010) 6:667–79. doi:10.1038/nrneurol.2010.164

20. Yogo Y, Kitamura T, Sugimoto C, Ueki T, Aoyama H, Kato K, et al. Isolation of a possible archetypical JC virus DNA sequence from nonimmunocompromised individuals. J Virol (1990) 64:3139–43.

21. Pietropaolo V, Videtta M, Fioriti D, Mischitelli M, Arancio A, Orsi N, et al. Rearrangement patterns of JC virus noncoding control region from different biological samples. J Virol (2003) 76:903–10. doi:10.1128/JVI.76.9.903-911.2002

22. Han GP, Miura K, Ide Y, Tsurusaki Y. Genetic analysis of JC virus and BK virus from a patient with progressive multifocal leukoencephalopathy with hyper IgM syndrome. J Med Virol (2005) 76:398–405. doi:10.1002/jmv.20377

23. Marzocchetti A, Wuthrich C, Tan CS, Tompkins T, Bernal-Canfo F, Bhargava P, et al. Rearrangement of the JC virus regulatory region sequence in the bone marrow of a patient with rheumatoid arthritis and progressive multifocal leukoencephalopathy. J Neurovirol (2008) 14:455–8. doi:10.1080/13550280802356837

24. Reid CE, Li H, Sur G, Carmillo P, Bushnell S, Tizard R, et al. Sequencing and analysis of JC virus DNA from natalizumab-treated PML patients. J Infect Dis (2011) 204:237–44. doi:10.1093/infdis/jir256

25. Major EO, Lymphocyte gene expression and JC virus noncoding control region sequences are linked with the risk of progressive multifocal leukoencephalopathy. J Virol (2014) 88:5177–83. doi:10.1128/JVI.03221-13

26. Bellizzi A, Nardis C, Anzivino E, Rodio D, Fioriti D, Mischitelli M, et al. Human polyomavirus JC reactivation and pathogenetic mechanisms of progressive multifocal leukoencephalopathy and cancer in the era of monoclonal antibody therapies. J Neurovirol (2012) 18:1–11. doi:10.1007/s13365-012-0080-7

27. Marshall LJ, Major EO. Molecular regulation of JC virus tropism: insights into potential therapeutic targets for progressive multifocal leukoencephalopathy. J Neuroimmune Pharmacol (2010) 5:404–17. doi:10.1100/jnp.2010.0192-03

28. Atwood WJ, Amemiya K, Traub R, Harms J, Major EO. Interaction of the human polyomavirus, JCV, with human B-lymphocytes. Virology (1992) 190:716–23. doi:10.1006/2042-6822(92)90099-9

29. Reickmann P, Michel U, Kehrl JH. Regulation of JC virus expression in B lymphocytes. J Virol (1994) 68:217–22.

30. Miyamura T, Furuno A, Yoshike K. DNA rearrangement in the control region for early transcription in a human polyomavirus JC host range mutant capable of growing in human embryonic kidney cells. J Virol (1985) 54:750–6.
50. Vandvik B, Nilsen RE, Vartdal F, Norby B. Mumps meningitis: specific and non-specific antibody responses in the central nervous system. Acta Neurol Scand (1982) 65:468–87. doi:10.1111/j.1600-0404.1982.tb03104.x

51. Luxton RW, Thompson EF. Affinity distributions of antigen-specific IgG in patients with multiple sclerosis and in patients with viral encephalitis. J Immunol Methods (1990) 131:277–82. doi:10.1016/0022-1759(90)90199-6

52. Felgenhauer K, Reiber H. The diagnostic significance of antibody specificity indices in multiple sclerosis and herpes virus induced diseases of the nervous system. Clin Investig (1992) 79:28–37. doi:10.1007/BF00422934

53. Sindic CJ, Monjevey P, Lakerre EC. The intrathecal synthesis of virus-specific oligoclonal IgG in multiple sclerosis. J Neuroimmunol (1994) 54:75–80. doi:10.1016/0165-5728(94)90223-X

54. Luxton RW, Zeman A, Holzel H, Harvey P, Wilson J, Kocen R, et al. Affinity of antigen-specific IgG distinguishes multiple sclerosis from encephalitis. J Neurol Sci (1995) 132:11–9. doi:10.1016/S0022-510X(95)00115-I

55. Khanna N, Wolbers M, Mueller NJ, Garzoni C, Du Pasquier RA, Fux CA, et al. JC virus-specific immune responses in human immunodeficiency virus type 1 patients with progressive multifocal leukoencephalopathy. J Viral (2009) 83:4404–11. doi:10.1128/JVI.02657-08

56. Weber F, Goldmann C, Kramer M, Kaup FJ, Pickhardt M, Young P, et al. Cellular and humoral immune response in progressive multifocal leukoencephalopathy. Ann Neurol (2001) 49:636–42. doi:10.1002/ana.10043. abs

57. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. J Neuromunol (2006) 180:63–70. doi:10.1016/j.jneuroim.2006.06.029

58. Reis EA, Athanazio DA, Lima I, Oliveira e Silva N, Andrade JC, Jesus RN, et al. NK and NK cell dynamics after rituximab therapy for systemic lupus erythematosus and rheumatoid arthritis. Rheumatol Int (2009) 29:469–75. doi:10.1007/s00296-008-0719-0

59. Tamimoto Y, Horuchi T, Tsukamoto H, Ottsuka J, Mitoma H, Kimoto Y, et al. A dose-escalation study of rituximab for treatment of systemic lupus erythematosus and Evans’ syndrome: immunological analysis of B cells, T cells and cytokines. Rheumatology (Oxford) (2008) 47:821–7. doi:10.1093/rheumatology/ken071

60. Stasi R. Rituximab in autoimmune hematologic diseases: not just a matter of B cells. Semin Hematol (2010) 47:170–9. doi:10.1053/j.seminhematol.2010. 01.010

61. Melet J, Mulleran D, Goupille P, Riboutart B, Watier H, Thibault G. Rituximab-induced T cell depletion in patients with rheumatoid arthritis: association with clinical response. Arthritis Rheum (2013) 65:2783–90. doi:10.1002/art.38107

62. Lykken JM, DiLillo DJ, Weimer ET, Roser-Page S, Heise MT, Grayson JM, et al. Acute and chronic B cell depletion disrupts CD4+ and CD8+ T cell homeostasis and expansion during acute viral infection in mice. J Immunol (2014) 193:746–56. doi:10.4049/jimmunol.1302848

63. Hunziker L, Klenerman P, Zinkernagel RM, Ehl S. Excision of cytokot T cells during adoptive immunotherapy of virus carrier mice can be prevented by B cells or CD4+ T cells. Eur J Immunol (2002) 32:574–82. doi:10.1002/ ejim.200202322-374. AID-IMMU374=3.0.CO;2-9

64. Brundler MA, Aichele P, Rachmann M, Kitamura D, Rajewsky K, Zinkernagel RM, et al. Immunity to viruses in B cell-deficient mice: influence of antibodies on virus persistence and on T cell memory. Eur J Immunol (1996) 26:2257–62. doi:10.1002/eji.1830260943

65. Asano MS, Ahmed R. CD8 T cell memory in B cell-deficient mice. J Exp Med (1996) 183:2165–74. doi:10.1084/jem.183.5.2165

66. Topham DJ, Tripp RA, Hamilton-Easton AM, Sarawar SR, Doherty PC. Quantitative analysis of the influenza virus-specific CD4+ T cell memory in the absence of B cells and Ig. J Immunol (1996) 157:2947–52.

67. Thomsen AR, Johnson I, Johannson I, Marko O, Christensen JP. Exhaustion of CTL memory and recrudescence of viremia in lymphocytic choriomeningitis virus-infected MHC class II-deficient mice and B cell-deficient mice. J Immunol (1996) 157:3074–80.

68. Whitmire JK, Asano MS, Kaech SM, Sarkar S, Hannum LG, Shlomchik MJ, et al. Requirement of B cells for generating CD4+ T cell memory. J Immunol (2009) 182:1868–76. doi:10.4049/jimmunol.0802501

69. Shen H, Whitmire JK, Fan X, Shedlock DJ, Kaech SM, Ahmed R. A specific role for B cells in the generation of CD8+ T cell memory by recombinant Listeria monocytogenes. J Immunol (2003) 170:1443–51. doi:10.4049/jimmunol.170.3.1443

70. Rocha R, Tanchot C. Towards a cellular definition of CD8+ T-cell memory: the role of CD4+ T-cell help in CD8+ T-cell responses. Curr Opin Immunol (2004) 16:259–63. doi:10.1016/j.coi.2004.03.004

71. Sun JC, Williams MA, Bevan MJ. CD4+ T cells are required for the mainte- nance, not programming, of memory CD8+ T cells after acute infection. Nat Immunol (2004) 5:927–33. doi:10.1038/ni105

72. Williams MA, Bevan MJ. Effector and memory CTL differentiation. Annu Rev Immunol (2007) 25:171–92. doi:10.1146/annurev.immunol.25.021206.141548

73. de Goer de Herge MG, Cariou A, Simonetta F, Taoufiq Y. Heterospecific CD4 help to rescue CD8 T cell killers. J Immunol (2008) 181:5974–80. doi:10.4049/jimmunol.181.9.5974

74. de Goer de Herge MG, Dembele B, Vallee M, Herr F, Cariou A, Taoufiq Y. Direct CD4 help provision following interaction of memory CD4 and CD8 T cells with distinct antigen-presenting dendritic cells. J Immunol (2010) 185:1028–36. doi:10.4049/jimmunol.0904209

75. de Goer de Herge MG, Iaafoura S, Vallee M, Taoufiq Y. FoxP3+ regulatory CD4 T cells control the generation of functional CD8 memory. Nat Commun (2012) 3:986. doi:10.1038/ncomms1992

76. Homann D, Tishon A, Berger DP, Weigle WO, von Herrath MG, Oldstone MB. Evidence for an underlying CD4 helper and CD8 T-cell defect in B-cell-deficient mice: failure to clear persistent virus infection after adoptive immunotherapy with virus-specific memory cells from murM/muMT mice. J Viral (1998) 72:9208–16.

77. Bouaziz JD, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses. Curr Op Immunol (2003) 20:332–8. doi:10.1016/j.coi.2003.03.003

78. Land F. Cytokine-producing B lymphocytes-ke regulators of immunity. Curr Op Immunol (2008) 20:332–8. doi:10.1016/j.coi.2008.03.003

79. Land F, Randall TD. Effector and regulatory B cells: modulators of CD4+ T cell immunity. Nat Rev Immunol (2010) 10:236–47. doi:10.1038/ nri2729

80. Millo SR, Zajac A1, Harrington LE. Temporal requirements for B cells in the establishment of CD4 T cell memory, J Immunol (2013) 191:6052–9. doi:10.4049/jimmunol.1302033

81. Misumi I, Whitmire JK. B cell depletion curtails CD4+ T cell memory and reduces protection against disseminating virus infection. J Immunol (2014) 192:1597–608. doi:10.4049/jimmunol.1302661

82. Vandvik B, et al. Interferon-alpha triggers B cell effector 1 (Be1) commitment. PLoS One (2011) 6:e19366. doi:10.1371/journal.pone.0019366

83. Scandella E, Fink K, Jun T, Senn BM, Lattmann E, Förster R, et al. Dendritic cell-independent B cell activation during acute virus infection: a role for early
CCR7-driven B-T helper cell collaboration. *J Immunol* (2007) 178:1468–76. doi:10.4049/jimmunol.178.3.1468

92. Wu J, Qin D, Burton GF, Szalak AK, Tew JG. Follicular dendritic cell-derived antigen and accessory activity in initiation of memory IgG responses in vitro. *J Immunol* (1996) 157:3404–11.

93. Tew JG, Wu J, Qin D, Helm S, Burton GF, Szalak AK. Follicular dendritic cells and presentation of antigen and costimulatory signals to B cells. *Immunity* (1997) 156:39–52. doi:10.1011/jem.1997.00957.x

94. Dustin ML, Dustin LB. The immunological relay race: B cells take antigen by synapse. *Nat Immunol* (2001) 2:480–2. doi:10.1038/sj.ni.4401287

95. Weber T, Weber F, Petry H, Luke W. Immune response in progressive multifocal leuкоencephalopathy: an overview. *J Neurovirol* (2001) 7:311–7. doi:10.1080/13552801552537166

96. Perez D, Baranda L, Abud-Mendoza C, et al. Clinical and immunological with lupus nephritis. *Arthritis Rheum* (2015) 70:387–96. doi:10.1002/art.39155

97. Duddy M, Niino M, Adatia F, Hebert S, Freedman M, Atkins H, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J Immunol* (2007) 178:6092–9. doi:10.4049/jimmunol.178.10.6092

98. Leandro MJ, Cambrige G, Ehrenstein MR, Edwards JC. Restoration of peripheral blood B cells after deletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum* (2006) 54:2377–86. doi:10.1002/art.20199

99. Tedder TF, Leonard WJ. Autoimmunity: regulatory B cells – IL-35 and IL-67.

100. Dustin ML, Dustin LB. The immunological relay race: B cells take antigen by synapse. *Nat Immunol* (2001) 2:480–2. doi:10.1038/sj.ni.4401287

101. Perez-D, Baranda-L, Abud-Mendoza-C, et al. Clinical and immunological with lupus nephritis. *Arthritis Rheum* (2015) 70:387–96. doi:10.1002/art.39155

102. Leandro MJ, Cambrige G, Ehrenstein MR, Edwards JC. Restoration of peripheral blood B cells after deletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum* (2006) 54:2377–86. doi:10.1002/art.20199

103. Tedder TF, Leonard WJ. Autoimmunity: regulatory B cells – IL-35 and IL-67.

104. Dustin ML, Dustin LB. The immunological relay race: B cells take antigen by synapse. *Nat Immunol* (2001) 2:480–2. doi:10.1038/sj.ni.4401287

105. Tedder TF, Leonard WJ. Autoimmunity: regulatory B cells – IL-35 and IL-67.

106. Dustin ML, Dustin LB. The immunological relay race: B cells take antigen by synapse. *Nat Immunol* (2001) 2:480–2. doi:10.1038/sj.ni.4401287

107. Tedder TF, Leonard WJ. Autoimmunity: regulatory B cells – IL-35 and IL-67.

108. Dustin ML, Dustin LB. The immunological relay race: B cells take antigen by synapse. *Nat Immunol* (2001) 2:480–2. doi:10.1038/sj.ni.4401287

109. Tedder TF, Leonard WJ. Autoimmunity: regulatory B cells – IL-35 and IL-67.
129. Ren X, Akiyoshi K, Dziennis S, Vandenbark AA, Herson PS, Hurn PD, et al. Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. J Neurosci (2011) 31:8556–63. doi:10.1523/JNEUROSCI.1623-11.2011

130. Chen D, Blazek M, Ireland S, Ortega S, Kong X, Meeuwissen A, et al. Single dose of glycoengineered anti-CD19 antibody (MEDI551) disrupts experimental autoimmune encephalomyelitis by inhibiting pathogenic adaptive immune responses in the bone marrow and spinal cord while preserving peripheral regulatory mechanisms. J Immunol (2014) 193:4823–32. doi:10.4049/jimmunol.1401478

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