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Aging and lymphocyte changes by immunomodulatory therapies impact PML risk in multiple sclerosis patients

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Abstract

New potent immunomodulatory therapies for multiple sclerosis (MS) are associated with increased risk for progressive multifocal leukoencephalopathy (PML). It is unclear why a subset of treated patients develops PML, but patient age has emerged as an important risk factor. PML is caused by the JC virus and aging is associated with immune senescence, which increases susceptibility to infection. With the goal of improving PML risk stratification, we here describe the lymphocyte changes that occur with drugs associated with high or moderate risk toward PML in MS patients, how these changes compare to immune aging, and which measures best correlate with risk. We reviewed studies examining how these therapies alter patient immune profiles, which revealed the induction of changes to lymphocyte number and/or function that resemble immunosenescence. Therefore, the immunosuppressive activity of these drugs may be enhanced in the context of an immune system that is already exhibiting features of senescence.

Keywords

PML; immunosenescence; disease modifying therapies; multiple sclerosis; risk assessment

Introduction

Progressive multifocal leukoencephalopathy (PML) is an opportunistic infection caused by the JC virus (JCV), and currently represents a significant concern for multiple sclerosis (MS) patients taking potent immunomodulatory disease modifying therapies (DMTs). The JCV antibody index is the current standard for assessing risk, with antibody titers greater than 1.5 associated with elevated risk[1, 2], but its reliability is compromised by false negatives[3]. While it is not yet possible to accurately predict which patients will develop PML, age has emerged as a significant risk factor[4]. Indeed, JCV seroprevalence has been found to increase with age in the MS population[5–7]. This trend of increasing seroprevalence with age is hypothesized to

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Conflict of interest

EAM has no conflict of interest.

stem from transmission of the reactivated virus in adults[8], and suggests that age increases the risk for both viral infection and reactivation. This elevated risk is believed to be the result of decreased immune capacity due to immunosenescence[9]. Many of the lymphocyte changes induced by the DMTs also reduce immune capacity and could potentially accelerate immunosenescence. However, the risk for PML is not uniform across all DMTs[10] (see Table 1), and an analysis of how these therapies modify immune function in relation to their risk profile for PML could help identify the most significant contributing factors.

Aging and Immunosenescence

Older MS patients tend to have lower lymphocyte counts than younger patients, placing them at higher risk for DMT induced lymphopenia[11]. Low lymphocyte counts, particularly in the central nervous system (CNS), increase PML risk, but the extent to which a therapy lowers cell counts is insufficient to explain the disparity in PML incidence. Instead, it is the anti-viral function of the remaining immune cells that is likely to be the most significant driver of PML risk. Therefore, DMTs impacting both immune cell number and function would be most detrimental to the already compromised pathogen fighting capacity of the aged immune system.

Aging of the immune system involves a decreased production of naive T cells coupled with an increase in terminally differentiated late effector memory T cells (T_{EMRA}), leading to a narrowing of the T cell repertoire[12]. Circulating $CD4^+$ naïve T cells have a deficit in IL-2 production which impairs effector cell differentiation[13], and have a higher threshold for productive T cell receptor (TCR) signaling, leading to less activation[14]. The decline of $CD4^+$ T cell helper activity then results in the production of non-functional $CD8^+$ memory T cells, thereby hampering viral clearance[15]. Therefore, DMTs which further impair $CD4^+$ T cell responses may cause aged patients to become particularly vulnerable to chronic infections.

The highly differentiated memory T cells critical for long-term pathogen specific immune protection lose expression of the costimulatory molecule CD28[16], and often take on a more regulatory phenotype. $CD8^+CD28^-$ T cells arise in response to persistent antigenic stimulation, and in combination with an inverted CD4/CD8 ratio and decreased naïve T cells are part of the ‘immune risk profile’ associated with higher susceptibility to infection and mortality in aged individuals[17]. The loss of CD28 is also a prominent feature of a variety of autoimmune diseases[18], suggesting that these patients experience aspects of premature immune aging. Consequently, it is immunosenescence rather than age itself that is the relevant risk factor, since the aging of the immune system does not necessarily correspond to chronological age.

The decline in pathogen clearance capacity with age cannot be attributed solely to intrinsic differences within T cells or a shift in the distribution of T cell subsets. Rather, it is the decline in T cell priming and activation by antigen presenting cells (APCs) that appears to be the most relevant[19]. Aged animals have impaired dendritic cell (DC) maturation, resulting in the production of DCs with decreased antigen uptake and presentation capacity[20, 21]. T cell activation in the aged immune system is then further impaired by the loss of costimulatory molecule engagement with APCs, such as decreased expression of CD40L by aged $CD4^+$ T

cells[22]. The CD40-CD40L interaction promotes the activation and antigen presenting capacity of APCs, such as DCs and B cells, and is critical for promoting the antigen specific $CD8^+$ T cell response to infection[23]. Therefore, the manner in which DMTs alter the function of APCs is likely to have critical implications for immunosurveillance, irrespective of whether they alter absolute levels of T cells.

DMTs can induce changes to the immune system that mimic immunosenescence (see Table 2), and potentially shift a patient's immune age well after drug cessation, further compounding risk. Cell depleting agents such as ocrelizumab and alemtuzumab are not readily reversible and even the effects of therapies primarily targeting cell migration such as natalizumab and fingolimod could be long lasting. Therefore, it would be useful to test immune capacity and calculate an adjusted 'immune system age' before and after starting DMTs in MS patients.

Natalizumab

Natalizumab is a humanized monoclonal antibody targeting α-4 integrin (CD49d), which at 4.2 per 1000 patients[24], has the highest global incidence for PML of all approved RRMS DMTs[10]. Though it does not alter peripheral lymphocyte counts, natalizumab significantly lower rates of $CD4^+$, $CD8^+$, and $CD19^+$ cells in the CSF[25]. Natalizumab is associated with increasing anti-JCV antibody indices over time (0.091 units/year or 13%), and high annual seroconversion rates of 8.5–10%[26, 27]. In combination with the age induced narrowing of the T cell repertoire[28], its further restriction of TCR repertoire and impairment of antigen specific T cells[29] may account for why the initiation of natalizumab treatment at older age is associated with earlier onset of PML and worse outcomes[30]. The percentage of CD49d expressing T cells also decreases with age[31], which may stem from a rise in $CD49d^{lo}$ virtual memory $CD8^+$ T cells during immunosenescence[32]. Downregulation of CD49d by natalizumab blocks lymphocytes that depend on the VLA-4/VCAM-1 interaction from entering the CNS. Decreased expression of CD49d may then account for lower CNS lymphocyte infiltrates and reduced clinical efficacy of natalizumab in older MS patients[33].

Mechanistic insight into the importance of CD49d for CNS T cell activation comes from a histology based report of a fatal case of natalizumab-associated PML[34]. The study found a decrease in MHC class II expression on APCs within cerebral perivascular spaces driven largely by decreased numbers of $CD209^+$ DCs, leading to a complete lack of $CD4^+$ T cells within the cerebral perivascular spaces. CD49d is upregulated as monocyte derived DCs mature, and facilitates their transendothelial migration[35], but in its absence, these mature DCs are unable to localize to the perivascular space and present antigens to peripherally activated $CD4^+$ T cells in order to initiate a CNS immune response[36]. Natalizumab also decreases $CD4^+$ expression of the microRNA miR-17, which is implicated in T-cell activation and proliferation[37]. The specific loss of $CD4^+$ T cell function is thought to be particularly important, since low $CD4^+$ T cell count is a risk factor for HIV-associated PML[38] and idiopathic PML[39]. The secretion of IFN-γ by $CD4^+$ T cells has also been shown to be necessary for mobilizing antiviral antibodies into the CNS[40, 41]. This suggests that a high peripheral titer of anti-JCV antibodies is a risk factor for natalizumab-associated PML[2]

because it is indicative of an active infection, and without $CD4^+$ T cell help, these antibodies cannot reach the CNS.

A longitudinal analysis on a patient with natalizumab-associated PML provides potential peripheral correlates for the loss of CNS immunosurveillance[42]. In contrast to the majority of natalizumab treated MS patients, his peripheral $CD4^+/CD8^+$ ratio decreased within the first 12 months, while *in vitro* assays indicated dramatically decreased expression of CD49d and migratory capacity of T cells. Therefore, in addition to measures of T cell activation, monitoring the peripheral $CD4^+/CD8^+$ ratio as well as CD49d expression and *in vitro* migration of JCV specific T cells could serve as relevant metrics for determining PML risk, though they require further verification. Another longitudinal case report of natalizumab-associated PML revealed a decrease in levels of newly produced circulating $CD31^+$ naïve T cells coupled with increased numbers of T_{EMRA} cells, indicative of an aged immune system, despite the patient's relatively young age[43], emphasizing the need for comprehensive immune assessments prior to therapy initiation and continued monitoring for the duration of treatment.

The risk for PML is not eliminated when MS patients stop taking natalizumab, as several cases have occurred after switching to a different DMT, as the lower rates of $CD4^+$, $CD8^+$ and $CD19^+$ cells in the CSF persisted for 6 months after stopping natalizumab and normal levels are not regained until 14 months after cessation[44]. Furthermore, many of the other DMTs effective at reducing relapses have also been linked to cases of PML. Some of these DMTs induce lymphocyte function changes that are common with natalizumab and/or immunosenescence, which may further compound risk.

Dimethyl fumarate

DMF is a fumaric acid with antioxidant and immunomodulatory properties approved for the treatment of RRMS that has been linked to PML in MS patients over 50 years old[4, 45]. Lymphopenia is a common side effect of DMF, and chronic grade 3 lymphopenia, which has been established as a risk factor for DMF-associated PML, is more common in older patients[46]. However, DMF-associated PML has also occurred in patients with absolute lymphocyte counts above the guideline threshold[47, 48], suggesting that changes in specific subsets might be more important. The ability of DMF to negatively impact the survival and activation of pro-inflammatory T cell subsets possibly play a role in its clinical efficacy[49] and drive PML risk. In addition to the higher tendency for older MS patients to become lymphopenic, the decreased $CD69^+$ activation status for T_{EMRA} cells following DMF treatment[50] may also contribute to the age-associated PML risk.

Similar to natalizumab, DMF impacts the activation of $CD4^+$ T cells by DCs. Instead of impeding the migration of mature DCs, DMF prevents Th1 and Th17 $CD4^+$ T cell differentiation through the inhibition of DC maturation[51], leading to decreased peripheral blood levels of Th1 and Th17 cells[50]. DMF also acts directly on activated T cells, inhibiting their survival[52]. The peripheral loss of $CD8^+$ T cells is generally more pronounced than the loss of $CD4^+$ T cells[50], but the CSF immune profile has not been studied in DMF treated patients. The $CD4^+/CD8^+$ ratio may differ between peripheral and central compartments

since DMF has been found to decrease transendothelial migration *in vitro* by decreasing expression of adhesive molecules[53]. The downregulation of CD49d has also been shown in animal models[54]. Therefore, similar to natalizumab, assays designed for testing migratory capacity, CD49d expression, and JCV-specific cytotoxic responses may also be useful for assessing PML risk in DMF treated patients.

Fingolimod

Fingolimod traps subsets of lymphocytes that depend on S1P for egress from lymph nodes by acting as a functional antagonist for the S1P receptors[55, 56]. Since $CCR7^{-}$ effector memory T cells are not subject to this mechanism of lymphocyte trapping[57], it was expected that fingolimod would not interfere with pathogen elimination. However, it is actually the shift in balance toward decreased $CD4^{+}$ central memory and more effector memory T cells that is associated with PML[58], and several cases of PML have been attributed to fingolimod, mostly in patients over 50[4, 59]. Notably, fingolimod treatment induces similar immune system changes as occur during aging, including a shift in the balance toward less naive T cells and more T_{EMRA}[60], suggesting that patients showing evidence of immunosenescence prior to starting fingolimod may be at greater risk for opportunistic infections. Indeed, fingolimod has been shown to also increase the risk for cryptococcal infection in patients over 50 through induction of immunosenescent changes in T cell subsets[61]. While effector memory T cells may be spared from trapping, they are still subject to fingolimod's inhibitory effects on T cell activation mediated through TCF-1[62]. Specifically, fingolimod impairs the ability of activated $CD4^{+}$ T cells to produce IFN-γ, which may then also limit the ability of anti-JCV antibodies to reach the CNS. S1P plays an important role in DC maturation, thus fingolimod can also interfere with antigen presentation and T cell activation[63]. Similar to DMF, fingolimod treated DCs skew $CD4^{+}$ T cell differentiation away from Th1 and towards anti-inflammatory subsets[64, 65]. Fingolimod treated RRMS patients have decreased $CD4^{+}/CD8^{+}$ ratios in both the periphery and CNS[66], thus in addition to functional measures, peripheral and especially CNS $CD4^{+}$ lymphocytopenia may be a relevant risk metric.

Ocrelizumab

Ocrelizumab is a humanized anti-CD20 monoclonal antibody approved for RRMS and PPMS in 2017, while rituximab was the first generation anti-CD20 therapy and used off-label for RRMS. Anti-CD20 therapies primarily target B cells since CD20 is expressed on the majority of B cells but is also found on 5% of T cells[67]. However, the therapeutic action is thought to result from a decrease in T cell activation and the stimulation of autoreactive T cells by B cells. In MS patients with prior exposure to natalizumab, there have been 3 cases of PML with rituximab and 2 cases with ocrelizumab[68], but none solely attributed to anti-CD20 use. Meanwhile, the risk of rituximab-associated PML in rheumatoid arthritis (RA) is about 1:25,000[69]. This discrepancy is likely due to the combinatorial use of rituximab with other immunosuppressants for RA, such that T cell function is more likely to be compromised in RA patients. Indeed, depletion of $CD4^{+}$ T cells has also been found in to occur in response to rituximab treatment, and differences in $CD4^{+}$ T cell loss may partly account for the differential risk[70]. $CD4^{+}$ T cell loss up to 37% was found to occur in nearly

all RA patients treated with rituximab plus methylprednisone[70], whereas less than half of MS rituximab treated MS patients' experienced significant loss of $CD4^+$ T cells[71]. It is currently unclear whether the MS patients with lower $CD4^+$ counts are at increased risk for infection, but may be worth monitoring. Since anti-CD20 therapies do not block lymphocyte migration, assays of peripheral JCV specific T cell function would likely be the most informative.

Teriflunomide

Teriflunomide is a pyrimidine synthesis inhibitor approved for RRMS, which decreases proliferation of activated B and T cells by reversibly inhibiting the mitochondrial enzyme dihydro-orotate dehydrogenase[72]. PML has been reported in a patient after switching from natalizumab to teriflunomide[73], but no cases due to teriflunomide alone. One small study found that teriflunomide significantly reduced absolute levels of $CD19^+$ B cells, but only led to a minor reduction in T cells[74], though a prior report showed a significant reduction in $CD4^+$ and $CD8^+$ T cells[75]. Within the $CD4^+$ T cell population, there was a selective decrease in Th1 cells, however, the *ex vivo* proliferation and cytokine expression of the T cells was maintained[75]. The preservation of appropriate T cell function may underlie the low risk of PML associated with teriflunomide in MS patients, but more studies are needed.

Alemtuzumab

Alemtuzumab is a humanized anti-CD52 monoclonal antibody used for refractory cases of RRMS which effectively depletes circulating B and T cells[76]. Similar to teriflunomide, PML has only occurred in one alemtuzumab treated patient that could be attributed to prior natalizumab exposure[77], despite the loss of $CD19^+$ B cells, $CD8^+$ and $CD4^+$ T cells which can last for approximately 3–8 months, 20 months, and 3 years, respectively[78, 79]. The maintenance of pathogen clearance in these patients is thought to stem from the sparing of immunocompetent cells in lymphoid organs[80], and critically, the preservation of functional anti-viral responses in the remaining B and T cells[81, 82].

Conclusion

While DMT associated $CD4^+$ T cell lymphocytopenia, particularly within the CNS, appears be an important component of PML risk, the loss of lymphocyte functional capacity is associated with the highest risk, and age induced immunosenescence can magnify these effects. Therefore, accelerated immunosenescence is a concern for MS patients, and reliance on chronological age rather than functional immune capacity can skew risk assessments. Going forward, assessing the function of JC virus specific T cells may provide a better metric to determine PML susceptibility amongst MS patients treated with immunomodulatory therapies.

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Table 1

Incidence of PML in MS DMTs

DMT	Cases of PML	PML Patient Age
Natalizumab	763 in >181,300 patients[a]	15–73
Fingolimod	19 in >225,000 patients[b*]	34–71
Dimethyl Fumarate	5 in >270,000 patients[a*]	54–66
Ocrelizumab	0 in >40,000 patients[c*]	
Teriflunomide	0 in >71,000 patients[d*]	
Alemtuzumab	0 in >18,400 patients[d*]	

[*] Cases of PML not attributable to prior natalizumab exposure

[a] Biogen internal data file,

[b] Novartis internal data file,

[c] Genentech internal data file,

[d] Sanofi Genzyme internal data file.

Table 2

Changes to lymphocyte populations in response to immunotherapy in MS patients.

		Aging	Natalizumab	Fingolimod	DMF	Anti-CD20	Teriflunomide	Alemtuzumab
PERIPHERY	**$CD19^+$ B cells**	▼	▲	▼▼	▼▼	▼▼▼	▼▼	▼▼
	B memory	▽	△	▽	▽	▽	▭	▽
	B naïve	△	▽	△	△	△	▭	△
	B regulatory	▽	△	△	△	△	▭	△
	$CD3^+$ T cells	▼	▲	▼▼	▼▼	▼	▼	▼▼▼
	T naïve	▽	▽	▽	△	▭	▭	▽
	T memory	△	△	△	▽	▭	▭	△
	T regulatory	△	▽	△	▭	▭	▭	△
	$CD4^+$ T cells	▼	▬	▼▼	▼	▬/▼	▼	▼▼▼
	$T_{Central\ Memory}$	▭/△	▭/△	▽	▽			▽
	$T_{Effector\ Memory}$	△	△	△	▽			△
	$T_{Helper\ 1}$	▽	△	▽	▽			▽
	$T_{Helper\ 17}$	▽	△	▽	▽			▽
	$T_{Helper\ 2}$	△		▽	△			△
	$CD8^+$ T cells	▼	▬/▲	▼	▼▼	▬	▼	▼▼▼
	$T_{Central\ Memory}$	△	▭	▽	▭			▽
	$T_{Effector\ Memory}$	△	△	▭	▽			△
	T_{EMRA}	△	△	△	▭			△
	$CD4^+/CD8^+$	▼	▬/▼	▼	▲	▬/▼	▲	▼
CNS	**$CD19^+$ B cells**		▼▼	▼		▼▼▼		
	B memory		▽	▽				
	B naïve			▽				
	B regulatory			△				
	$CD3^+$ T cells		▼▼	▼▼		▼▼		
	$CD4^+$ T cells		▼▼	▼▼				

		Aging	Natalizumab	Fingolimod	DMF	Anti-CD20	Teriflunomide	Alemtuzumab
	$CD8^+$ T cells		▼	▼				
	T regulatory							
	$CD4^+/CD8^+$		▼	▼				

Bold font and closed symbols indicates changes in absolute cell numbers.
Regular font and open symbols indicates relative changes.

▲ Increase, ▼ Decrease, ▬ No change, use of / indicates different effects in different subsets of patients. Magnitude of absolute cell count changes noted by number of symbols. Empty boxes indicate no reported data.