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Progressive Multifocal Leukoencephalopathy and Newer Biological Agents

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Abstract

Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease of the brain due to a polyoma virus, JC virus. Despite the ubiquity of this virus, PML is rare and almost always seen in association with an underlying immunosuppressive condition. In the last 30 years, AIDS has been the most common predisposing factor. The observation of PML attending the use of certain monoclonal antibody therapies and other pharmacological agents has raised concerns about the safety profile of these agents, but has also provided a window into the pathogenesis of PML. Certain agents, such as the monoclonal antibodies natalizumab, an $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrin inhibitor, and efalizumab, an antibody directed against CD11a, appear to uniquely predispose to PML. Prior to their introduction for multiple sclerosis and Crohn's disease with respect to natalizumab, and psoriasis with respect to efalizumab, PML had never been observed with these disorders. PML occurring with other agents that currently carry US FDA-mandated 'black-box' warnings, such as rituximab, an antibody directed to CD20, or mycophenolate mofetil, a drug that inhibits T- and B-cell proliferation, typically occur in the background of underlying disorders that have already been identified as risks for PML. This review will focus on the available data regarding the risk for PML with monoclonal antibodies and other drugs. A biologically plausible explanation for the increased risk of PML will be proposed, as well as potential strategies for mitigating disease risk.

Identifying the risks of a drug during phase III studies or in the postmarketing period is often rendered difficult by low incidence rates and relatively high background prevalences of the complication, such as suicidal risk with antidepressant drugs[1] or congestive heart failure with thiazolidinediones (glitazones) in treating diabetes mellitus.[2] However, the rarity of progressive multifocal leukoencephalopathy (PML) clearly facilitated the recognition of a link between its occurrence and the use of several therapeutic agents. The observation of PML in two patients with multiple sclerosis (MS)[3,4] and one patient with Crohn's disease[5] in 2005 was truly unique as neither illness, MS or Crohn's disease, had been previously associated with PML. This observation is rather astonishing in light of the large number of patients with these disorders who had been treated with immunosuppressive regimens. Similarly, the observation of confirmed PML in three patients and suspected PML in a fourth who had been treated with efalizumab for psoriasis was remarkable for the same reasons.[6-8] PML had not been previously reported with psoriasis; therefore, the recognition of a link between these agents and PML was obvious but not necessarily predictable, and has provided a window into the pathogenesis of the disease. On the other hand, a large number of other drugs have been purported to increase the risk of PML. Two of these, rituximab and mycophenolate mofetil, carry US FDA-mandated 'black-box' warnings but, unlike natalizumab and efalizumab, both have been used to treat disorders that are commonly recognized as underlying risks for PML, and determining the risk for the development of PML with these agents is more difficult. There are other immunosuppressive and immunomodulatory agents that also increase the risk for PML. For instance, the introduction of high-dose therapy with the purine analogue, fludarabine, for chronic lymphocytic leukaemia (CLL) appears to have increased the risk of PML with this condition 6-fold or more.[9,10]

This review addresses the issue of PML with newer therapeutic agents, in particular the monoclonal antibodies and mycophenolate mofetil. Issues addressed include the biology of JC virus (JCV), plausible mechanisms underlying the increased risk for PML with these agents, risk mitigation strategies and future directions for research.

1. JC Virus Biology and Progressive Multifocal Leukoencephalopathy (PML)

In their seminal description of PML in 1958, Astrom et al.[11] stated "We do not know the cause of this condition … most frequently a complication of chronic lymphatic leukemia or Hodgkin's … Whether there is some factor shared in common between these diseases and sarcoidosis and tuberculosis … remains unknown." Seven years later, ZuRhein and Chou[12] suggested that a papovavirus was the cause of PML after observing intracellular paracrystalline inclusions on electron microscopy. This hypothesis was proven correct when Padgett et al.[13] cultured a polyoma virus, subsequently referred to as JCV, in human fetal glial cells.

Although seroprevalence rates for antibody to JCV has varied among series, almost all show seroconversion rates in the adult populations ≥50%. The seroprevalence rate is fairly constant worldwide, with the exception of rare isolated populations in whom antibody studies suggest that the rate of exposure to JCV is negligible.[14] In some urban areas, the rate of JCV seroconversion may exceed 90%.[15] Several methods have been employed to determine seroprevalence rates. Early studies relied on the ability of the virus to haemagglutinate type O erythrocytes. Subsequent antibody studies have typically employed haemagglutination inhibition and the more sensitive enzyme immunoassay.[16] Cross reactivity with other polyoma viruses, such as BK virus, is a potential concern, but is probably not significant.[16] Between the ages of 1 and 5 years, approximately 10% of children demonstrate antibody to JCV, and by age 10 years, it can be observed in 40–60% of the population. The acquisition of JCV during childhood appears to occur slowly[17] and primary infection has yet to be correlated with an identifiable clinical disorder. By early adulthood, as many as 70–80% of the population has been infected.[15,17] However, continued exposure throughout adulthood is suggested by a study demonstrating seroprevalence rates of 50% in the 20- to

29-year-old age group, but 68% in those aged 68–100 years.[18]

The mechanism by which JCV, one of five currently identified human polyomaviruses,[19] is acquired remains unknown. The absence of a recognized clinical illness accompanying the initial infection with JCV has undoubtedly hampered the ability to identify the means of transmission. Asymptomatic JC viral shedding in urine has been demonstrated in 19%[18] to >70% of immunologically normal individuals,[20] averaging about 30% in most series.[21] While some investigators have suggested that the rate of JCV isolation from the urine increases with age,[22] this has not been universally demonstrated.[20] In light of its frequent occurrence in urine, perhaps it is not surprising that JCV has also been detected worldwide in virtually every sample of sewage that has been examined.[23] Contaminated food and water are potential sources of infection.[23] Conversely, the virus is not detectable in the saliva or oropharyngeal washings of young healthy adults, though it may rarely be detected in these fluids of immunosuppressed individuals.[20] Infection is likely to be by an oropharyngeal or respiratory route, with replication of the virus in parapharyngeal lymph nodes and tonsils. Support for this hypothesis includes the finding of JCV DNA in about 40% of harvested tonsils.[24] Following replication at these sites, the virus is proposed to seed sites of viral latency elsewhere in the body with periodic re-expression during periods of immunosuppression.[25]

Prior to the inception of the AIDS pandemic in 1981, PML was a rare disorder. From 1958, when PML was initially described, through 1984, Brooks and Walker[26] were able to identify only 230 cases that had been published in the English language or from their own experience. Ninety-five percent of the patients in this series had a recognized underlying condition that predisposed them to PML. Only five cases of AIDS-associated PML had been reported at that time.[27-29] Lymphoproliferative disorders, predominantly B-cell disorders, accounted for approximately 66% of the underlying causes. In a single-site study from Canada, the incidence of PML with all haematological malignancies was 0.07%.[30] The incidence of PML with CLL has ranged from 0.52%[31] to >3%,[9,10] with the higher incidence rates only noted after the introduction of treatment with high-dose purine analogues.[9] Other underlying disorders recognized to predispose to PML were other haematological and solid malignancies, immunodeficiency states, autoimmune disorders and granulomatous diseases, such as tuberculosis and sarcoidosis. The incidence rate of PML changed dramatically by the late 1980s during the AIDS pandemic. Five percent of HIV-infected persons developed PML prior to the introduction of effective antiretroviral therapy,[32,33] resulting in a 4-fold increase in the frequency of PML between 1979 and 1989 in the US.[34] By 1993, AIDS was the underlying predisposing disorder for 87% of PML cases in the US.[35] AIDS-associated PML has been most frequently observed in severely immunosuppressed individuals (CD4 counts <200 cells/mm^3). Although controversial,[36] the incidence appears to have declined following the introduction of highly active antiretroviral therapeutic (HAART) regimen.[37]

Development of PML proceeds as a stochastic sequence of events. Based on our current incomplete understanding, a plausible scenario is

Table I. The proposed stages of development of progressive multifocal leukoencephalopathy (adapted from Berger et al.,[38] with permission)

1. Initial infection JCV
2. Establishment of JCV latency, most importantly in CD34 and other B-cell lineages
3. Release of B cells (immature and pre-B cells) from bone marrow
a. Re-activation of JCV within these immature B cells due to viral transactivation by transcriptional factors that are released during B-cell maturation
b. Mutation of JCV to a neurotropic form within the B cells that have the unique genetic engineering to permit this to occur, namely, mechanisms for gene rearrangement, the addition or deletion of nucleotides to the genome, and somatic hypermutation
4. Actively replicating neurotropic JCV in the circulation
5. Brain entry of neurotropic JCV
6. Establishment of productive infection of oligodendrocytes
7. Impairment of CNS immunosurveillance
a. Inability of JCV-specific cytotoxic T cells to enter the brain
b. Inability to process JCV antigen locally in the brain due to depletion of perivascular dendritic cells

JCV = JC virus.

outlined in table I. Following initial infection with JCV, the virus disseminates to various sites within the body. Sites of viral latency include kidney, tonsils, bone marrow, spleen, lymph node and lung.[39,40] Latency within the brain remains controversial; some investigators have detected JCV DNA but not protein expression,[41] and others have found the T antigen in rare instances.[42] In light of the pervasive presence in urine of the archetype JCV, it is likely, though not proven, that the initial infection occurs with this virus, which is incapable of effectively replicating in glial tissues. It would then require genetic modification, in particular the insertion of a 98 base pair tandem repeat, in its non-coding control region (NCCR) to become neurotropic. B cells have the capacity to harbour JCV with diverse regulatory regions, including neurotropic JCV.[40] The gene rearrangement in the NCCR of the neurotropic JCV permits binding to the NF-1X binding protein, a protein that glial cells share with B cells.[43-46] Therefore, B cells are the logical, though unproven, site of the mutation as they have a unique genetic machinery that facilitates gene rearrangements, the addition and deletion of nucleotides, and somatic hypermutation.

Supporting this hypothesis is the detection of JCV DNA in bone marrow samples of 13% of HIV-negative patients and 47% of HIV-positive patients in a recent study.[47] Genetic sequencing of these isolates revealed that the regulatory region of the virus isolated from bone marrow was consistent with neurotropic JCV, suggesting that the bone marrow is an important reservoir in the pathogenesis of PML.[47] The detection of JCV in plasma or peripheral blood mononuclear cells has been reported in 0–29% of healthy controls and in 5–38% of HIV-positive individuals.[20,48-54] The frequency with which JCV is detected in the blood increases with the increasing immunosuppression, as has been demonstrated in patients with AIDS,[49,55] and its appearance may presage the development of PML, as noted in the number of instances.[5,56] These observations imply that the virus is periodically re-expressed, ultimately trafficking to the brain and establishing productive infection of oligodendrocytes.[25] Alternatively, it is possible that the virus is harboured latently within the brain and its expression suppressed by an effective immune response. Conceivably, both possibilities may be correct, with some PML patients recently seeding the brain with the virus and others harbouring it latently within glial tissue.

The cellular immune response is most important in preventing and controlling JCV.[57,58] There is no evidence that the presence of antibody to JCV offers any protection. IgG directed against JCV protein 1 (VP1) is almost always seen in PML, and an intrathecal humoral response to JCV is seen in 76% of PML patients compared with <3.2% of healthy controls.[59] On the other hand, the importance of cell-mediated immunity is indicated by the correlation between an impaired T_h1-type T helper cell function, the appearance of PML[58] and the importance of JCV-cytotoxic T lymphocytes (CTLs) in controlling the disease once it is established.[60,61]

In light of the large number of people infected with JCV, the rarity of PML indicates that substantial barriers to its development must exist in immunologically healthy individuals. How these barriers are possibly broached to facilitate the development of PML is addressed with each agent.

2. Monoclonal Antibodies and PML

Certain monoclonal antibody products have been demonstrated to predispose to the development of PML (table II). Among the monoclonal antibodies that increase the risk of PML are natalizumab (Tysabri®), an $\alpha4\beta1$ and $\alpha4\beta7$ integrin antagonist; efalizumab (Raptiva®), an anti-CD11a antibody; and rituximab (Rituxan®), an anti-CD20 antibody. Until the precise mechanisms that lead to PML are fully understood, the risk posed by other monoclonal antibodies targeting specific arms of the immune system will remain uncertain. The complexity of this issue is highlighted by the observation that the monoclonal antibody, alemtuzumab (Campath®), an anti-CD52 antibody that depletes both T and B cells, recapitulating many of the immune abnormalities of HIV infection, has not yet been demonstrated to increase the risk of PML. Perhaps not unexpectedly, $\alpha4\beta7$ integrin inhibitors, used in the treatment of inflammatory bowel disorders, have also not been

Table II. Summary of agents predisposing to progressive multifocal leukoencephalopathy (PML)

Agent	Mechanism of action	Possible explanation for increased risk of PML	Estimated risk of PML	Unique predisposition for PML[a]
Natalizumab	α4β1 and α4β7 integrin antibodies	↓ JCV-specific CTL trafficking into CNS; ↓ CNS perivascular dendritic cells for antigen processing; ?↑ neurotrophic JCV expression	1 : 1000 after 2 y of treatment	Yes
Efalizumab	Anti-CD11a antibody	Blockade of co-stimulatory molecules on T cells; ↓ JCV-specific CTLs trafficking into CNS	Unknown	Yes
Rituximab	Anti-CD20 antibody	↑ JCV expression with recovery of B-cell population; ↓ B-cell antigen presentation	Unknown	No
Mycophenolate mofetil	Selective, non-competitive and reversible inhibitor of inosine-5′-monophosphate dehydrogenase	↓ JCV-specific CTLs trafficking into CNS; ↑ JCV expression on recovery of B-cell population	Unknown	No

a Unique predisposition for PML refers to the development of PML with the drug in the absence of underlying disorders that increase its risk.

CTLs = cytotoxic T lymphocytes; **JCV** = JC virus; ↓ indicates decreasing; ↑ indicates increasing; **?**↑ indicates possibly increasing.

associated with an increased risk of PML. Piecing these observations together will undoubtedly be critical in our understanding of the pathogenesis of PML.

2.1 Natalizumab

Natalizumab is a humanized monoclonal antibody to α4β1 and α4β7 integrin that has demonstrated significant efficacy in relapsing-remitting MS. The proposed mechanism of action in MS is the ability of natalizumab to prevent activated lymphocytes from entering the brain. Following the observation that three patients had developed PML while taking natalizumab (two in the Sentinel study for MS who were also being treated with intramuscular interferon α1b [Avonex®][3,4] and a third in a clinical trial of Crohn's disease treatment),[5] it was withdrawn from the market on 28 February 2005 and reintroduced in July 2006 as a monotherapy for relapsing-remitting MS and moderately to severely active Crohn's disease. It was initially suggested that the risk for developing PML was a consequence of the combined use of interferon-β1a with natalizumab in MS. However, subsequent experience revealed that PML can occur with natalizumab monotherapy. As interferon-β1a is a potent inhibitor of the JCV infection and early antigen expression,[62] it is unlikely to have contributed to the appearance of PML. Based on the initial observation of three cases of PML in the premarketing era, it was estimated that approximately 1 in 1000 persons would develop PML after 17.8 months of treatment.[63] As of April 2010, there have been a total of 42 MS patients who have developed PML[64] while under treatment with natalizumab, resulting in nine deaths. Twenty-four (57%) of the cases have occurred in Europe, with most cases in Germany and 15 (36%) in the US, although more people have been treated with natalizumab in the US than Europe. A disproportionate number of patients (54%) developing PML had received prior immunosuppressive therapy with mitoxantrone, azathioprine and methotrexate versus those enrolled in the TYGRIS (TYSABRI Global Observational Program in Safety) and TOUCH (TYSABRI Outreach: Unified Commitment to Health) programmes (13%). TYGRIS is simply an observational programme to determine what risks may exist with natalizumab administration. TOUCH is a mandatory programme designed to decrease the risk of PML. It was introduced in the US when natalizumab was returned to market. With respect to the use of natalizumab, it is recommended that all immunomodulatory agents be discontinued at least 1 month prior to the initiation of natalizumab; however, the apparent significantly increased risk of PML with natalizumab following the use of immunosuppressants may suggest a more stringent regimen be employed in patients who have previously received these

agents. In rare instances, symptoms attributable to PML have occurred during the first 12 months of therapy,[65] but no cases of PML have actually been diagnosed during this timeframe. The rate appears to increase gradually thereafter with the incidence per 1000 being 1.35 (95% CI 1.04, 1.73) [1 in 741] after 12 infusions. This rate increases to 1.76 after 24 infusions (95% CI 1.30, 2.32) [1 in 568].[66] Wide confidence intervals make these estimates questionable and there are too few patients treated continuously beyond 30 months to comment meaningfully. Whether longer durations of therapy will result in further increases in the incidence rate or not remains to be determined. It is conceivable, though probably not likely, that the at-risk population declines after several years of exposure. Only continued vigilance for PML will permit an answer to this question.

Although the precise explanation for the increased risk of PML with natalizumab remains unknown, the drug has a number of mechanisms of action that might increase the risk by affecting the immunosurveillance for the virus and potentially by increasing the expression of neurotropic JCV. With respect to lowering the immunological barriers for the development of PML, the same mechanism that accounts for the efficacy of natalizumab in treating relapsing-remitting MS, namely blocking activated T cells from entering the brain, may also be responsible for increasing the risk of PML. As JCV-CTLs are commonly found in normal individuals[67] and have been demonstrated to correlate very strongly with PML survival,[60,61] preventing their entry into the CNS may predispose to the development of PML.[68] Another immunological insult that may increase the risk of PML is the significant reduction in the number of CD209+ dendritic cells in cerebral perivascular spaces and the elimination of CD4+ T cells in the brain tissue following natalizumab administration.[69] Dendritic cells are essential for the expansion of the JCV-CTL response[70] and may be important for locally processing JCV antigen and assisting in viral clearance. Other potential mechanisms contributing to the development of PML are related to the release of CD19+ B cells, particularly CD19+ CD10+ pre-B cells[71] following natalizumab administration. These cells can be latently infected with JCV. As circulating immature B cells mature, JCV transactivation may be elicited by the cellular transcriptional factors that are upregulated during their maturation.[72,73] This potential increased expression of actively replicating virus occurs in cells that are uniquely equipped to rearrange the virus' transcriptional control region permitting the evolution of neurotropic JCV. In a small study of patients treated with natalizumab for MS, no JCV DNA in plasma or peripheral blood mononuclear cells was detected before treatment, but after 18 months of continuous therapy 20% of plasma samples and 60% of peripheral blood mononuclear cells had detectable virus.[74] Genetic sequencing of the regulatory region was consistent with neurotropic JCV.[74] Larger studies that were designed differently have not replicated this observation.[75,76] One study intriguingly demonstrated an increase in cellular immune response to JCV when pretreatment values were compared with those during natalizumab treatment.[76] Nonetheless, it is likely that the risk for PML is increased with natalizumab by multiple mechanisms.

2.2 Efalizumab

Efalizumab is an anti-CD11a IgG1 antibody with demonstrated efficacy in moderate to severe plaque psoriasis[77,78] and sustained improvement observed during 36 months of continuous therapy.[79] By binding to the I domain of the α chain of CD11a, it triggers a conformational change in lymphocyte function-associated antigen-1 (LFA), the site that binds to intercellular adhesion molecule (ICAM) and can affect apoptosis, cytotoxicity, cell proliferation, cytokine production, antigen presentation and gene activation.[80] This set of events affects psoriasis pathogenesis at multiple levels, perhaps most importantly by inhibiting the initial T-cell activation in lymph nodes, preventing binding of T cells to endothelial cells and blocking trafficking of T cells from the circulation into the psoriatic skin preventing their reactivation in the dermal and epidermal layer.[81] Blockade of costimulatory molecules on T cells, particularly CD11a, as occurs with efalizumab, also results in a sustained unresponsiveness to

viral and other pathogens in animal models.[82-84] It has been demonstrated to reduce T-cell activation produced by polyclonal stimuli; this T-cell hyporesponsiveness is fully reversible following efalizumab washout.[85] Similarly, during active therapy but not following its elimination, it has been demonstrated to reduce the cellular immune response to intracutaneous recall antigens.[86] While efalizumab-like natalizumab results in an increase in peripheral blood leukocytes during treatment, these cells are predominantly circulating CD3+ cells with the largest increase in memory CD8 T cells.[87] Eflalizumab reduces cutaneous dendritic cells,[88] but its affect on cerebral perivascular dendritic cells is unknown.

More than 6000 patients had been treated with efalizumab before its removal from the European and US markets in the spring of 2009. Of these, only 166 patients had received more than 3 years of therapy. In a review of the effects of efalizumab on infection rates in 2335 patients receiving 12 weeks of therapy, 1115 receiving 24 weeks of therapy and 170 receiving 108 weeks (27 months) of continuous therapy, there appeared to be no increased risk of infection.[89] However, this follow-up period was clearly of inadequate length as four patients ranging in age from 47 to 73 years who were treated with efalizumab for more than 3 years for psoriasis were subsequently observed to have developed PML (three confirmed and one suspected). PML was confirmed in three cases and suspected in one. As with MS and Crohn's disease, PML had not previously been observed complicating psoriasis. The T-cell hyporesponsiveness to viral antigens may be the explanation for the increased risk for PML with efalizumab, but this explanation remains unsatisfactory and does not explain the failure to observe other viral illnesses at substantially higher incidence during treatment. Blockade of the entry of JCV-CTLs into the brain is also a likely contributor.

2.3 Rituximab

Rituximab is a monoclonal antibody directed against CD20. It results in profound B-cell depletion by depleting pre-B and B cells that express CD20, but it does not affect stem cells or plasma cells.[90] It has demonstrated efficacy in treating lymphoproliferative diseases and a wide variety of autoimmune diseases,[91] especially rheumatoid arthritis (RA), systemic lupus erythematosus (SLE)[92] and MS.[93]

The Research on Adverse Drug Event and Reports project identified 57 patients with PML who had been treated with rituximab.[92] In this study, 52 patients had lymphoproliferative disorders (generally B-cell malignancies), two had SLE, one had RA, one had autoimmune pancytopenia and one had autoimmune thrombocytopenia, and all had been treated with other immunosuppressive regimens, including haematopoietic stem cell transplantation in seven.[92] As of November 2009, there have been three reported cases of rituximab associated-PML in RA,[94] and others have since been reported. Unlike natalizumab, where the case fatality rate for PML is <50%, the case fatality rate with rituximab associated-PML is 90% and 100% among those diagnosed with PML within 3 months of their last dose of rituximab.[92] In part, this may reflect the nature of the underlying disease. Alternatively, it may also reflect an inability to reverse the immune abnormalities consequent to rituximab administration, in contrast to HIV and natalizumab-associated PML.

Distilling the precise risk that rituximab poses for the development of PML is difficult as the underlying disorders, in particular B-cell malignancies, independently carry a risk for PML. The risk of PML with CLL for instance may exceed 3% in the absence of rituximab.[9] Garcia-Suarez and colleagues[9] have even argued that the use of rituximab after high-dose therapy and haematopoietic stem cell transplantation delays the onset of PML. However, it is likely that the number of cases of PML reported with rituximab is an underestimate of the true incidence. PML has also been reported with SLE and RA in the absence of rituximab. There are at least 26 cases of PML that have been reported with SLE, and >40% of cases have occurred with minimal immunosuppression, suggesting that SLE itself predisposes to PML.[95] PML has also been reported with RA in the absence of rituximab therapy.[26,96,97] The incidence rate of PML from the Nationwide Inpatient Sample Database in SLE was 4/100 000 hospital

discharges, for RA it was 0.4/100 000 and for other connective tissue diseases it was 2/100 000,[98] indicating that there is an increased risk of PML with all these disorders, particularly SLE, even in the absence of treatment with rituximab.

PML occurs a median of 5.5 months after the last rituximab dose.[92] The rate of B-cell reconstitution following rituximab administration varies from 6 months to 24 months depending on additional antilymphocyte treatments, especially stem cell transplantation.[99,100] With repopulation of the peripheral B cells, immature B cells predominate and certain cells such as memory cells are significantly delayed in their reappearance.[101] Therefore, the time course for the development of PML following rituximab therapy is consistent with the time course for the reconstitution of the B-cell population. Conceivably, the reappearance of these immature B cells with JCV may contribute to the appearance of PML. Additionally, rituximab also reduces CD3+ T cells in the cerebrospinal fluid (CSF).[102] The temporal profile and functional consequences of a reduction in T cells in patients treated with rituximab is unknown, but rituximab may affect antigen presentation and T-cell regulation;[103] the former may affect the immune response to JCV and contribute to the development of the disease. Many of the mechanisms that predispose to PML that are operant with natalizumab are, therefore, also present with rituximab, such as the migration of pre-B cells into the peripheral circulation in response to B-cell depletion. However, the incidence rate of PML appears to be lower with rituximab than natalizumab.

3. Mycophenolate Mofetil

Mycophenolate mofetil is a selective, noncompetitive and reversible inhibitor of inosine-5′-monophosphate dehydrogenase. This enzyme is the first of two enzymes responsible for the conversion of inosine monophosphate to guanosine monophosphate, and by depleting guanosine and deoxyguanosine nucleotides inhibits proliferation of T and B lymphocytes.[104] It is approved for allograft rejection after renal, cardiac and liver transplant, and has also demonstrated efficacy in the treatment of some autoimmune diseases, including SLE, autoimmune glomerular disease and myasthenia gravis,[105] although its effectiveness in the latter disorder remains controversial.[106]

In February 2008, following the observation of isolated cases of PML with mycophenolate mofetil in kidney, heart and lung transplant recipients and in patients with long-standing SLE, the drug's manufacturer, Hoffmann-LaRoche, and regulatory agencies advised healthcare workers of the risk. The transplant patients were all men between the ages of 33 and 62 years who were taking concomitant immunosuppressive medications, including tracrolimus, basiliximab, corticosteroids and ciclosporin. The SLE patients were all women ranging in age from 40 to 53 years who were also taking concomitant immunosuppressive medications, including ciclosporin, cyclophosphamide and corticosteroids. As recognized by the manufacturer, it is difficult to know the exact contribution of mycophenolate mofetil to the pathogenesis of PML in these patients. All had underlying diseases that have been previously associated with PML and all were on other immunosuppressive regimens. While mycophenolate mofetil has been used extensively with myasthenia gravis, a disease not linked to PML, no cases have yet been reported. In a retrospective cohort study of nearly 33 000 renal transplant recipients in the US, the incidence of PML among mycophenolate mofetil users was 14.4 cases/100 000 versus 0 in non-mycophenolate mofetil users, but this did not meet statistical significance.[107] Stabilization of PML and prolonged survival following the drug's discontinuation in some patients[108] in the absence of other therapeutic interventions argues in favour of its role in the development of PML.

The mechanism by which mycophenolate mofetil increases the risk of PML may parallel that of natalizumab. By depleting T cells, it may lower the immunological barrier for the disease by reducing or eliminating JCV-CTLs. Additionally, as the depleted pools of B cells begin to recover, there may be a potential for the expression of immature B cells containing JCV with an upregulation of viral replication and the potential for mutation to a neurotropic strain.

4. Risk Mitigation Strategies

A number of strategies have been proposed to mitigate the risk of PML in patients being treated with drugs that increase the risk of the disorder. A first step may be determining whether the patient has been infected with JCV or not. In almost all, if not all instances, PML appears to arise as a recrudescence of a latent infection. Evidence for the latter include the presence of IgG antibody to JCV but not IgM,[59] rarity of PML in children,[109] demonstration of JCV isolates with the same NCCRs from plasma and peripheral blood mononuclear cells 8 months before its isolation from the PML brain tissue,[56] and six instances in which JCV isolated from lymphoid tissue, spleen or bone marrow 0.5–4.1 years before the development of PML showed the same NCCR genetic profile as that isolated from the brain (Major EO, personal communication). Coupled with the observation that seroepidemiological studies indicate that the preponderance of JCV exposure occurs prior to adulthood, determining whether the prospective patient is JCV seropositive is a reasonable approach to assess risk. Enzyme immunoassays IgG and IgM directed against JCV VP1 appear to be sufficiently specific to avoid cross reactivity with another common polyoma virus, BK virus.[18] However, before this approach is widely adopted, it should be determined to be highly sensitive. Until the latter is comfortably demonstrated, PCR of blood and urine for JCV and perhaps even JCV-specific cellular immune response measures[110] should be determined concomitantly to ensure the test is sensitive. If a patient is seronegative for JCV antibody, repeat determinations would be warranted at regular intervals (perhaps 6 months) as the increase in antibody response with age suggests that the risk for primary infection continues into late adulthood.[18]

While a JCV seronegative patient can be treated with these agents with relative impunity, those who have been previously exposed to the virus are at risk for PML. In these patients, periodic PCR for JCV in blood has been proposed. JCV has been detected in the blood prior to the clinical appearance of PML in several instances in which blood has been available.[5] Conversely, the presence of JCV in the blood is not predictive of PML and, therefore, the value of this strategy remains open to question. An alternative or complementary strategy might be to determine those at greatest risk for opportunistic infection among treated patients. Khoury and colleagues[111] have performed a pilot trial of the Cylex ImmuKnow Assay in MS patients. This test measures adenosine triphosphate production in CD4+ T cells activated by phytohaemagglutinin, and has been used to monitor the risk of organ transplant rejection and infection in patients receiving immunosuppressive therapies. Conceivably, this test may help predict patients who are at greatest risk for developing PML and other opportunistic infections. However, it is unclear whether this strategy would be effective with immunomodulatory therapies that alter cellular trafficking as opposed to immunosuppressive regimens.

In JCV seropositive individuals in whom treatment is necessary, interval serial magnetic resonance imaging (MRI) of the brain might prove valuable in detecting the disorder early, thus permitting the elimination of the offending drug and institution of therapies that might be beneficial. To reduce the inconvenience and expense of this study, one might consider performing only fluid attenuated inversion recovery scans at 6-month intervals as this MR sequence is very sensitive to the presence of PML. Radiographically evident PML prior to the development of clinical symptoms has been well described.[4,112] This form of monitoring is rendered more difficult in the MS population receiving an $\alpha 4\beta 1$ integrin inhibitor or an anti-CD20 antibody as the lesions of MS may be indistinguishable from the demyelinating lesions of PML. Suggested MRI features to distinguish between MS and PML include the location of the lesions, borders, mode of extension, mass effect, appearance on various MRI sequences, enhancement and atrophy.[63] However, none of these distinctions is absolute. For instance, posterior fossa lesions are not that uncommon with MS. Contrast enhancement was observed in 12 of 28 (43%) natalizumab PML cases at the time of diagnosis.[113] Similarly, confluent lesions, mass effect and focal atrophy, albeit relatively rare, can all be seen with PML.[65] In a comparative study

of PML and relapsing-remitting MS,[114] certain clinical and MRI features helped differentiate the two disorders. Optic neuritis and transverse myelitis were not seen with PML, whereas clinically monosymptomatic hemiparesis and altered mental status were far more common with PML.[114] Distinguishing MRI characteristics included large confluent granular T2-weighted lesions, deep grey matter involvement and crescentic cerebellar lesions for PML,[114] and gadolinium enhancement and periventricular and transcallosal lesions for MS, although 23% of the PML lesions were enhancing, 9% were periventricular and 9% were transcallosal. The features that suggest either MS or PML described previously are unlikely to be very helpful in an effort to distinguish between the two disorders with small early lesions as noted in the experience of Linda and colleagues.[112] Therefore, serial imaging studies to detect early PML are unlikely to be of substantial benefit.

Some individuals have adopted the use of serotonin receptor antagonism with agents such as mirtazapine as a prophylaxis for the development of PML in patients being treated with natalizumab. This strategy is based on the observation that JCV uses a 5-HT_{2A} serotonin receptor to attach to the cell[115] and that infection *in vitro* can be blocked by this and related drugs, such as ketanserin and ritanserin.[116] Serotonin receptor antagonism has been suggested as therapy for PML[117] and there is a case report suggesting that mirtazapine was useful in treating PML.[118] However, this strategy presupposes that the serotonin receptor is the only mechanism by which the virus accesses the cell and that the doses employed are sufficient to effectively block these receptors. Neither is likely to be the case[119] and until evidence of this is available in a clinical trial, their use is discouraged.

As the risk of PML following treatment with natalizumab appears to increase over time, particularly after 2 years of continuous therapy, some MS authorities have proposed discontinuing the therapy after this period of time and perhaps reinstituting it after a hiatus of 6 or more months. If the mechanisms proposed are responsible for the increased risk of PML with natalizumab, the reinstitution of the drug would likely result in the patient returning to the risk curve for disease that existed its termination. Furthermore, presuming therapy was effective for their MS, it would expose them to the risk of significantly higher disease activity.

5. Treatment of PML Occurring with Newer Biological Agents

The fundamental first step in treating confirmed or suspected PML in the setting of an offending agent is discontinuing the treatment and reversing the effects of the drug. Natalizumab has been best studied. It has a long biological half-life after administration. One month after administration, 80% of binding sites remain occupied,[120] activity with respect to suppression of gadolinium-enhancing lesions in MS is still evident 3 months after discontinuation following two intravenous infusions separated by 4 weeks,[121] and 6 months after cessation, an altered CD4/CD8 T-cell ratio in the CSF remains evident.[122] Therefore, simply discontinuing the drug is insufficient; it is important to eliminate the antibody from the body as rapidly as possible. Plasma exchange as three 1.5 volume exchanges over 5–8 days not only decreased the concentration of natalizumab in the blood but also desaturated α4 integrin.[123] Professor Ralf Gold at the University of Bochum, who has been responsible for treating most of the natalizumab-associated PML patients in Germany, has employed protein immunoadsorption with a tryptophan column (TR-350-L),[124] with the typical plasma volume of 2000 mL (Gold R, personal communication). Although survival in patients treated with immunoadsorption appears to be better (eight US deaths vs one EU death), the small numbers of patients treated with either modality preclude meaningful comment regarding efficacy.

To date, there is no demonstrated effective treatment for PML. The use of a serotonin receptor is unlikely to be particularly helpful, as noted in the previous discussion. Scientists at Biogen, while screening 2000 approved drugs for activity against neurotropic strains of JCV, found that mefloquine inhibited viral replication in cells after viral entry.[125] A small study of the effect of

mefloquine on CSF viral titres has been undertaken. Many investigators have been using this antimalarial compound off-label in treating PML with seemingly rare instances of success (unpublished observation of the author). However, one needs to recall the prior experience with cytosine arabinoside. *In vivo*, this drug is highly efficient in preventing JCV replication,[126] and there were many anecdotal case reports and series that suggested efficacy. However, when studied in a large placebo-controlled trial, no effect was demonstrated in HIV-associated PML.[127]

The return of immune function is often associated with an entity referred to as immune reconstitution inflammatory syndrome (IRIS). IRIS is a paradoxical worsening of the clinical condition attributed to recovery of the immune system. Although first described in patients with HIV infection with PML and other opportunistic infections who had experienced a relatively rapid recovery of immune function following the introduction of an effective HAART,[128] it occurs with other conditions associated with rapid immune recovery, such as following natalizumab withdrawal. PML-IRIS has been observed in 18% of HIV-associated PML cases[129] but appears to be almost invariable based on new or worsening symptoms or MRI findings with natalizumab-associated PML.[113] Pathological examination of affected tissue reveals an intense perivascular inflammation with CD8+ T cells.[130] PML-IRIS can be associated with significant morbidity and mortality. While no controlled trial regarding treatment has been conducted, preliminary data from both the experience with HIV-associated[129] and natalizumab-associated PML-IRIS[131] suggests that treatment with high-dose intravenous corticosteroids ameliorates the condition. The duration of therapy remains to be determined, but PML-IRIS may last from weeks to months.

6. Future Directions and Conclusions

The proposed steps in the pathogenesis of PML remain theoretical and remain to be determined precisely. Many of the as yet unanswered questions are fundamental to developing effective methods to reduce the risk of PML. Is the initial infection with the archetype virus? Does PML always result from reactivated JCV infection? What are the specific sites of viral latency? Is the brain included in these latently infected sites? Does JCV presence in the blood always occur prior to the development of PML? Do the incidence rates of PML necessarily increase with longer durations of therapy? Are there subpopulations that can be treated with these agents without fear of PML developing?

The use of biological agents remains in its infancy and our understanding of the targets for disease control as well as the complications arising from perturbing these targets has not been fully explored. That an autoimmune disease can respond to therapies targeting very different arms of the immune system is illustrated by the treatment of psoriasis. Biological agents that have been successfully employed in treating psoriasis include those that target T cells, alefacept and efalizumab, tumour necrosis factor-α (TNFα) inhibition with etanercept, infliximab and adalimumab, and new biological agents targeting anti-interleukin (IL)-12 and IL-23 (ustekinumab and ABT-874).[132] The risk for PML with each of these agents is very different. While PML occurs with efalizumab, it has not been observed with TNFα antagonism. Almost certainly, PML is but one of many risks that will be observed with these newer biological agents.

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