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Immunovirology Report

Immune response in progressive multifocal leukoencephalopathy: An overview

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Progressive multifocal leukoencephalopathy (PML) is a disease usually ocurring in immunosuppressed patients. By far the most common underlying immunosuppressive illness is the acquired immune deficiency snydrome, accounting for about 85% of PML cases currently seen in clinical practice. PML may occur in patients with deficits in the humoral and/or cellular immune response such as lymphoproliferative diseases, myeloproliferative diseases, carcinomatous diseases and acquired immunodeficiency due to autoimmune diseases and immunosuppressive therapy. The humoral immune response in PML is indicative of a persistent, reactivated infection with a prominent immunoglobulin (lgG) G synthesis to virus protein 1 (VP1). An lgM synthesis in serum is rarely found. In about 76% of PML cases, an intrathecal humoral immune response to recombinant VP1 can be found as compared to only 3.2% in healthy controls. The detection of intrathecally synthesized lgG antibodies to VP1 can be used as an additional diagnostic test for the diagnosis of PML. The magnitude of the intrathecal humoral immune response appears to rise over time and may be associated with a decrease of viral load in cerebrospinal fluid (CSF) and possibly the central nervous system (CNS). Compared to healthy controls, proliferation of peripheral blood mononuclear cells (PBMC) is reduced in PML patients. Immunological studies suggest a general impairment of the Th1-type T-helper cell function of cell-mediated immunity. Furthermore, the appearance of JCV-specific cytotoxic T-lymphocytes appears to be associated with a favorable clinical outcome. *Journal of NeuroVirology* (2001) **7,** 311–317.

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Until the advent of the polymerase chain reaction (PCR), diagnosis of progressive multifocal leukoencephalopathy (PML) *intra vitam* was based on brain biopsy (Walker, 1985; Major *et al*, 1992) or relayed for confirmation at autopsy (Åström *et al*, 1958; Richardson, 1961; Budka and Shah, 1983). A sporadic report suggested diagnosis of PML by the detection of viral antigen in cerebrospinal fluid (CSF) (Peters *et al*, 1980) but these findings could not be reproduced. Antibody detection by hemagglutination inhibition test (HAI) failed to detect JCV capsid-specific antibody in the CSF of 9 autopsy-proven cases of PML (Padgett and Walker, 1983). With the introduction of highly active anti-retroviral therapy (HAART), PML cases with prolonged survival are reported with increased frequency (Baldeweg and Catalan, 1997; Baqi *et al*, 1997; Domingo *et al*, 1997; Elliot *et al*, 1997; Power *et al*, 1997; Albrecht *et al*, 1998; Miralles *et al*, 1998; Teofilo *et al*, 1998; Dworkin *et al*, 1999; Gasnault *et al*, 1999; Inui *et al*, 1999; Tantisiriwat *et al*, 1999; Yiannoutsos *et al*, 1999; Giudici *et al*, 2000). Current evidence suggests that JC viral load in CSF as measured by semiquantitative or quantitative PCR is a predictor of survival time (De Luca *et al*, 1999; Yiannoutsos *et al*, 1999; Taoufik *et al*, 2000). Survival time has also been linked to the CD4 count at the time of presentation with symptoms suggestive of PML (Berger *et al*, 1998; Dworkin *et al*, 1999). Patients with a CD4 count below 50 cells/mm^3

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have a median survival time ranging from 1 month to, at most, 5 months (Dworkin *et al*, 1999).

Humoral Immunity

JCV agglutinates human type 0 erythrocytes (Padgett and Walker, 1973). Antibodies to JCV can be determined by a hemagglutination-inhibition (HAI) test using human type 0 erythrocytes from donors lacking antibodies against JCV. Padgett and her colleagues used JCV grown in cultures of primary human fetal glial (PHFG) cells and assayed sera of 406 persons. In their series, seroprevalence rose from 10% for those ages 0–4 years to 60% for those ages 20–29 years. The highest rate of conversion to seropositivity, defined as an HAI titer of 32 or greater, occurred during the first 14 years. In addition, sera with HAI titers of 32 or greater contained detectable levels of neutralizing antibodies. No sex difference in the incidence of antibodies against JCV was seen (Padgett and Walker, 1973). Serological assays either by HAI, complement fixation, neutralizing antibodies, or various immunoassays are not diagnostic for the diagnosis of PML (Padgett and Walker, 1976; Knowles *et al*, 1995; Weber *et al*, 1997).

Almost all patients except those few cases with hypo- or agammaglobulinemia with PML have detectable IgG antibodies in their serum, thus further substantiating JCV as causative agent of PML. In 1995, Knowles and her colleagues used a HAI and a JC M-antibody capture radioimmunoassay (MACRIA) to analyze the humoral immune response to JCV in 28 PML patients and 71 controls (Knowles *et al*, 1995). JCV HAI antibodies were detected in the serum of all but one patient with PML (96%) compared to 68% of control patients. As immunoassays based on recombinant VP1 have several advantages over tissue culture–derived virus, Weber and colleagues developed a recombinant antigen-based ELISA (Weber *et al*, 1997). First, sufficient amounts of JCV are difficult to grow. Second, the amount of viral protein may be insufficient for an optimal antigen concentration in an immunoassay. Third, purified JCV is not stable. Fourth, HAI titer decreases with time (Major *et al*, 1992; Weber *et al*, 1997). A quantitative ELISA using recombinant JCV VP1 revealed an overall seroprevalence of VP1-specific IgG antibodies of 84.5% in 155 patients (Weber *et al*, 1997) (Figure 1). Of these, 86% of healthy controls (43/50), 88% of patients with an impaired blood–brain barrier (29/33), 76% of patients with multiple sclerosis (28/37), and 89% of HIV seropositive patients (31/35) had detectable antibodies in their sera.

The CSF in PML has been reported as normal with a regular protein content (≤50 mg/dL), a normal cell count (≤4 cells/μL) and absent intrathecal antibody synthesis as demonstrated by the lack of oligoclonal bands (Walker, 1985). More recently, however, Berger

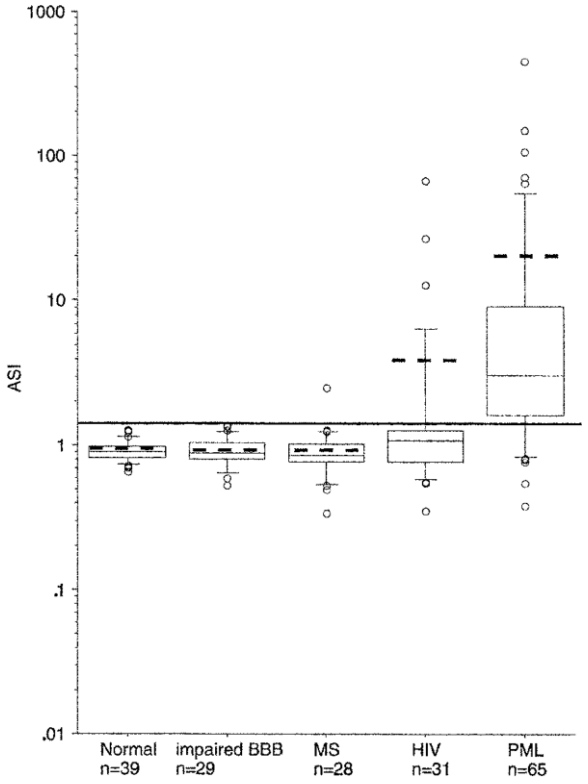

Figure 1 The plot shows 10th, 25th, 50th, 75th, and 90th percentiles and outliers. The solid line indicates the cutoff at 1.5, above which an intra blood–brain barrier (BBB) synthesis of immunoglobulin G antibodies to VP1 is present. The mean values are shown as solid lines for each group. In the normal group, the mean ASI_{VP1} was 0.92 (±0.159; range 0.65–1.26), in the patients with impaired BBB (IBBB) it was 0.92 (±0.216; range 0.52–1.36), in the multiple sclerosis (MS) group it was 0.908 (±0.382; range 0.34–2.49), in the HIV-positive patients (HIV-pos.) it was 4.355 (±12.71; range 0.35–67.2), and in the PML group it was 21.836 (±61.906; range 0.38–451). By the Kruskal–Wallis test, there is a significant difference between the 5 groups ($P < 0.001$). By the Mann–Whitney *U*-test, there is highly a significant difference between PML and normal, IBBB, MS, and HIV-pos ($P < 0.001$), whereas the difference between MS vs HIV-pos. was marginally significant at $P = 0.0305$. The differences between normal vs MS ($P = 0.2972$), normal vs IBBB ($P = 0.9604$), MS vs IBBB ($P = 0.4388$), IBBB vs HIV-pos. ($P = 0.1276$) and normal vs HIV-pos. ($P = 0.0562$) were not significant.

and colleagues reported a slight increase in CSF protein (range 52 to 72 mg/dL) in about one quarter, an elevated IgG or IgG-albumin index in about one fifth, and a slight pleocytosis in about one eighth of the patients (Berger *et al*, 1987). Others have seen a pleocytosis in 14%, an increased CSF protein in 54% (median 54, mean 52, range 33 to 130 mg/dL), and an intrathecal IgG synthesis in 29% of cases (Weber *et al*, 1996). A more specific indicator of blood–brain barrier (BBB) function is the albumin quotient Q(Alb) (Reiber and Felgenhauer, 1987; Reiber and Lange, 1991), which is calculated according to the formula:

$$Q(Alb) = \frac{[Alb_{CSF}] * 10^3}{[Alb_{Serum}]}$$

The normal value is age-dependent; values exceeding 6.5 indicate an impaired BBB in patients ages 15–40, in those ages 40–60, the upper limit of normal is 8.0 (Reiber and Felgenhauer, 1987). In a series of 62 PML patients, Q(Alb) was normal for 74% (6.5 ± 4; range 1.9–22.03) (Weber *et al*, 1997). An even higher percentage of normal findings in 87% of PML patients has been reported in another study (Monno *et al*, 1999). Thus, in between 13%–26% of patients with PML, a moderate BBB impairment may be found. In HIV-positive patients in particular, these changes may indicate a concomitant opportunistic infection such as cerebral toxoplasmosis, cryptococcal meningitis, or CMV ventriculitis/encephalitis. Oligoclonal bands were seen in 42% of PML patients as compared to 41% in asymptomatic HIV-positive patients (Weber *et al*, 1997). These data suggest that these oligoclonal bands are nonspecific CSF findings due to the underlying HIV infection. Until 1995, it was widely believed that CSF does not contain antibodies to JCV (Padgett *et al*, 1976). CSF analysis for JCV-specific antibodies was thus considered nondiagnostic (Major *et al*, 1992; Major and Ault, 1995).

To determine an intrathecal humoral immune response, Knowles and colleagues (Knowles *et al*, 1995) calculated an antibody index (AI) according to the formula:

$$\text{AI} = \frac{\text{CSF JCV antibody titer}}{\text{Serum JCV antibody titer}} \div \frac{\text{CSF albumin}}{\text{Serum albumin}}$$

Using this approach, a JCV-specific intrathecal immune response was defined by an AI of 2 or greater. In 12 of 18 PML patients (67%), an intrathecal immune response by JCV HI antibodies was found, but was not found in any of the controls. As further proof of an intrathecal humoral immune response, JCV-specific IgG bands were detected in 7 PML patients. In a comparable approach, the systemic and intrathecal humoral immune response was analyzed in 62 PML patients and 155 controls (Weber *et al*, 1997). Recombinant JCV VP1 expressed as virus-like particles (VP1-VLP) in the baculovirus system served as antigen instead of tissue culture–derived JCV (Goldmann *et al*, 1999). The recombinant VP1-VLP exhibits the same structural and functional properties as natural JCV (Goldmann *et al*, 1999). The recombinant VP1 was used in an ELISA assay for the detection of VP1-specific IgG. Antibody titers in CSF and serum were measured in arbitrary units (E). The VP1-specific CSF/serum ratio was calculated according to the formula:

$$\text{Q(IgG)spec} = \frac{\text{E}_{\text{CSF}} * 10^3}{\text{E}_{\text{Serum}}}$$

For the detection of locally synthezised VP1 specific antibodies, the Q(IgG)spec was related to the ratio of CSF to serum IgG (Q(IgG)tot), according to the formula:

$$\text{Q(IgG)tot} = \frac{[\text{IgG}_{\text{CSF}}] * 10^3}{[\text{IgG}_{\text{Serum}}]}$$

The ratio of Q(IgG)spec to Q(IgG)tot is defined as the antibody specificity index (ASI) (Reiber and Lange, 1991; Weber *et al*, 1991; Weber *et al*, 1997). To compensate for a potential blood–brain barrier leak, Q(IgG)spec has to be related to the individual albumin ratio (Reiber and Felgenhauer, 1987). This is achieved by using the upper limit of the normal range of IgG in relation to the BBB function Q(IgG)lim, which is calculated according to the formula:

$$\text{Q(IgG)lim} = 0.8 * \sqrt{(\text{QAlb})^2 + 1.5 * 10^{-6}} - 1.8 * 10^{-3}$$

In cases with a normal IgG ratio, i.e., without intrathecal synthesis of polyspecific IgG antibodies [Q(IgG)tot < Q(IgG)lim] (Reiber and Lange, 1991), the ASI is calculated as follows:

$$\text{ASI} = \frac{\text{Q(IgG)spec}}{\text{Q(IgG)tot}}$$

In those cases with a polyspecific intrathecal IgG synthesis, i.e., Q(IgG) tot > Q(IgG)lim, the ASI is calculated according to the formula:

$$\text{ASI} = \frac{\text{Q(IgG)spec}}{\text{Q(IgG)lim}}$$

Using this approach, an intrathecal humoral immune response to JCV-specific VP1 ($\text{ASI}_{\text{VP1}} \geq 1.5$) was found in 78% of PML patients (47/62) but only in 3.2% (5/155) of controls (Figure 2; Weber *et al*, 1997). By Western blot analysis of paired serum/CSF samples using identical IgG concentrations for each pair, these findings could be supported by the demonstration of more intense bands in those CSF samples from patients with PML as compared to the respective serum samples (Weber *et al*, 1997). Knowles and colleagues could detect JCV-specific oligoclonal bands in 7 PML patients tested (Knowles *et al*, 1995). By antigen-driven immunoblotting (ADI) (Sindic *et al*, 1994), JCV-VP1-specific oligoclonal bands were detected in 55% of PML cases (10/18) and in 6% (2/31) of controls (Sindic *et al*, 1997).

Of these patients, one with multiple sclerosis also had an ASI of 2.5, whereas the second patient with neuroborreliosis had an ASI_{VP1} of 0.64. No oligoclonal bands were detected in 4 PML patients with an $\text{ASI}_{\text{VP1}} \leq 1.5$ and in 4 cases with a moderately elevated ASI_{VP1} ranging from 1.78 to 3.04 (Sindic *et al*, 1997). In a case of PML without any underlying immunodeficiency, comparative analysis of the intrathecal humoral immune response ADI and VP1 ELISA of serial CSF/serum samples revealed clearly detectable VP1-specific oligoclonal bands with an $\text{ASI}_{\text{VP1}} \geq 3.0$ (Guillaume *et al*, 2000). Taken together, these data suggest that analysis of the intrathecal

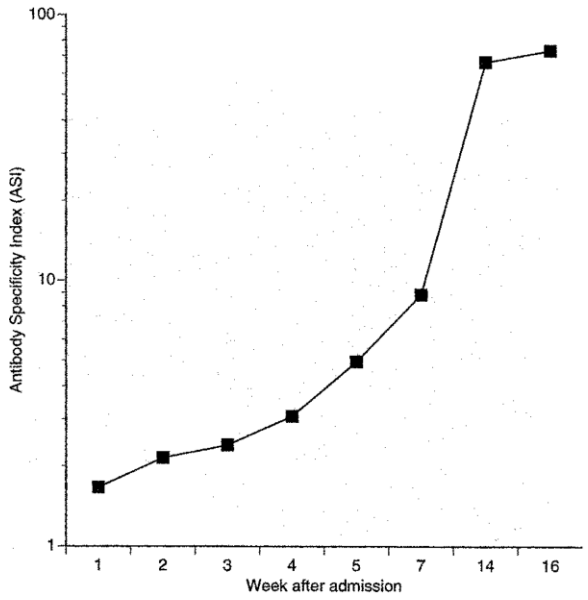

Figure 2 Time course of intrathecal VP1-specific antibody synthesis as determined by an ELISA. This 65-year-old patient had no obvious underlying immunodeficiency and was treated from week 2 to week 7 by 3 cycles of intravenous cytarabine 2 mg/kg/day for 5 days and by weekly intrathecal administration of 50 mg/m^2 cytarabine. PCR for JCV DNA in CSF was positive in weeks 1 to 7 and was negative in weeks 14 and 16 (Guillaume *et al*, 2000).

humoral immune response by ELISA is more sensitive than by ADI. It appears that those cases with an $ASI_{VP1} \geq 3$ are also positive by ADI (Sindic *et al*, 1997; Guillaume *et al*, 2000). In AIDS patients with PML receiving highly active anti-retroviral therapy (HAART), an intrathecal immune response to recombinant VP1 evolved during therapy in 3 of 4 patients (Giudici *et al*, 2000). It appears that an intrathecal immune response evolves over time (Figure 2) in the majority of PML patients and may be positive in cases in which the JC virus load in CSF is below the detection limit of PCR (Berner *et al*, 1999; Giudici *et al*, 2000).

Neuropathological analysis in 11 PML patients showed an excellent correlation ($r = .985$) between the plasma cell count in brain tissue and the humoral immune response to VP1 (Weber *et al*, 1997). The comparable rates of 67% and 76%, respectively, found for an intrathecal humoral immune response are in close agreement with neuropathological findings reporting perivascular infiltrates in about 70% of cases of PML in AIDS patients (Kuchelmeister *et al*, 1993). Further studies are necessary to evaluate the clinical relevance of the detection of an intrathecal immune response to VP1 for the diagnosis of PML. This assay could be used to diagnose PML in those cases with clinical and/or neuroradiological findings suggestive of PMNL but without detectable JCV DNA in CSF (Eggers *et al*, 1999; Koralnik *et al*, 1999; Yiannoutsos *et al*, 1999; Giudici *et al*, 2000). In addition, these data clearly indicate the plasma cells as source of the intrathecally produced IgG and suggest a role for B cells in the development of PML. They further suggest that PML should be considered as an inflammatory disease of the CNS and thus may be better designated as progressive multifocal leukoencephalitis.

Cellular Immune Response

EP Richardson was the first to suggest an altered immune response as underlying cause leading to the development of PML (Richardson, 1961). In 1969, Ellison reported the first study on the immunological status of a patient with PML (Ellison, 1969). This patient showed no evidence at all of a delayed-type hypersensitivity (DHT) reaction to tetanus toxoid (TT), diphtheria toxoid, mumps vaccine, or 2,4-dinitrofluorobenzene (DNFB). Furthermore, immunoglobulin A (IgA) and M (IgM) levels were below the lower limit of normal values. IgG was within the normal range. In 1972, Knight and colleagues reported on a 22-year-old patient who developed PML in the setting of a malabsorption syndrome with longstanding hypogammaglobulinemia of IgG and IgA (Knight *et al*, 1972). This patient also had no DHT reaction and showed a decreased proliferation in one-way mixed leukocyte cultures. In addition, incorporation of H^3-thymidine after stimulation with phytohemagglutinin (PHA) was significantly reduced.

Rockwell and colleagues described in 1975 a 45-year-old female patient with a biopsy-proven PML without evidence for deficits in either cellular or humoral immunity (Rockwell *et al*, 1976). Skin tests for tuberculin and Candida antigens as well as dinitrochlorobenzene were normal. The patient's lymphocytes transformed normally in response to pokeweed and PHA as well as purified protein derivative tuberculin antigen. The number and function of T lymphocytes, as determined by sheep erythrocyte rosette test, were also normal. In contrast to these findings are those by Willoughby and colleagues, who tested 7 patients with PML (Willoughby *et al*, 1980). Lymphocyte proliferation in response to the mitogens PHA, concanavalin A (ConA), and pokeweed mitogen (PWM) was significantly reduced for the 7 PML patients as compared to seven healthy controls (Willoughby *et al*, 1980). In addition, production of the pleitrophic cytokine leukocyte migration inhibitory factor or leukemia inhibitory factor (LIF) in response to stimulation with JCV antigen was absent. Katz and colleagues described a 15-year-old boy with Wiskott–Aldrich syndrome with a CD4 count of 0.12×10^9 L and depressed T-cell function as measured by mitogen-induced proliferation to ConA and PHA (Katz *et al*, 1994).

In 1995, Owen and colleagues described a 43-year-old man with chronic myeloid leukemia who received allogeneic bone marrow transplantation after immunosuppression. He underwent CMV seroconversion and developed PML. By reactive expansion of CD3+ large granular lymphocytes, humoral and

cellular immunity were reduced, and the CD4 count ranged between 0.3 to 0.4 $\times$ 10^9 L (Owen *et al*, 1995). Frye *et al* studied the cellular immune response of a PML patient and compared it to the proliferative response of healthy donors (Frye *et al*, 1997). Using purified JCV particles as antigen a stimulation index (SI) of 8 and 9 was found for the healthy donors, respectively, and a SI of 4 for the PML patient. After stimulation with PHA, healthy donors showed SI values of 15 and 25, whereas no response was seen with the PML patients' peripheral blood mononuclear cells (PBMCs).

Using recombinant VP1 as target antigen, a reduced proliferation of PBMCs was seen in PML patients as compared to healthy donors (Weber *et al*, 2001). We studied 7 healthy donors (HD), 6 HIV-infected patients without PML (HIV), 8 HIV-infected patients with PML (HIV/PML), and 4 HIVC-negative patients with PML (PML). We used TT, PHA, and recombinant VP1-virus-like particles (VP1-VLP) as antigens. After antigen stimulation, the production of IFN-γ was reduced in PML, in HIV/PML, and in HIV patients. The production of interleukin-10 (IL-10) was elevated in HIV/PML patients (Weber *et al*, 2001). A cytotoxic T-cell assay showed specific killing of autologous targets expressing recombinant JCV VP1 in PBMCS of 3/3 HIV-positive PML patients with longtime survival in 1/3 HIV-positive patients without PML but not in 2/0 HIV-negative PML patients or in 0/2 healthy donors, indicating a role of CD8+ effector cells in the control of the progression of PML (Koralnik *et al*, 2000).

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