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Decreased JC Virus-Specific Antibody-Dependent Cellular Cytotoxicity in HIV-Seropositive PML Survivors

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Abstract

Background: Progressive multifocal leukoencephalopathy (PML) is an often fatal disease caused by JC virus (JCV) in severely immunocompromised patients, including HIV patients. Development of therapeutics to prevent or treat PML is an urgent medical need. While JCV-specific T cells are crucial to control JCV and recover from PML, the role played by antibodies remains unclear. Anti-JCV antibodies, including potent neutralizing antibodies, can be detected in most infected adults, yet in PML patients, JCV seems to escape from neutralization. Whether antibodies can contribute to JCV control by eliciting Fc-mediated effector functions activity has not been evaluated.

Methods: We measured the capacity of plasma anti-JCV VP1 antibodies to recruit Fc receptor (FcR)-bearing effector cell functions in 28 HIV patients, comparing subjects without PML with PML survivors (PML S) who were alive 1 year after disease onset or PML progressors (PML P) who succumbed within the first year. Antibody titers against JCV VP1 and HIV gp140 trimer were determined by endpoint titer dilution ELISA. FcR-mediated natural killer cell degranulation and IFN- γ production were measured as surrogate for in vitro antibody-dependent cellular cytotoxicity (ADCC).

Results: PML S had higher JCV antibody titers than PML P and patients without PML. However, anti-JCV antibodies had a higher ability to functionally engage FcR in PML P than PML S. Antibody titers and ADCC activity did not vary over time in PML S. Anti-HIV antibody titers and ADCC activity were similar among groups.

Conclusions: The ability of anti-JCV antibodies to stimulate FcR-bearing effector cell activity might contribute to the outcome of PML. Further studies are warranted to define Fc-mediated functions of anti-JCV antibodies and evaluate whether ADCC can contain JCV replication.

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Keywords

JC virus; PML; Fc receptor-mediated response

INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is a demyelinating neurological infection caused by polyomavirus JC (JCV). Primary infection most likely occurs in childhood, with antibody responses to JCV being detectable in up to 86% of the general adult population.¹ Persistent JCV infection in the renal uroepithelium has little consequences in healthy individuals, but uncontrolled viral replication in the oligodendrocytes in persons living with HIV (PLWH) and in patients with severe T-cell deficiency leads to PML, which is often fatal as there is currently no cure against this disease. PML occurs mostly in patients with poor cellular immune responses,^{2,3} including those with HIV³⁻⁵ and those treated with natalizumab—a monoclonal antibody used to decrease inflammation of the brain in multiple sclerosis.

It has long been assumed that antibody-mediated protection is not sufficient to contain JCV as most infected adults have detectable antibodies.¹ Notably, a mixture of antibodies against different capsid mutations was detected in the cerebrospinal fluid (CSF) and in serum of PML patients, indicating potential viral immune escape.⁶ To date, studies have demonstrated that a portion of antibodies against JCV contain potent neutralization function.^{6,7} Neutralizing antibodies can bind extracellular virus and block infection, yet non-neutralizing antibodies can coat virus-infected cells and, through their Fc region, promote lysis of target cells by Fc receptor (FcR)-bearing cells such as natural killer (NK) cells, neutrophils, and monocytes. Accumulating evidence suggest that such antibody-dependent cellular cytotoxicity (ADCC) responses contribute to immune protection against several viruses,^{8,9} including HIV,¹⁰⁻¹⁴ EBV,^{15,16} and influenza viruses.¹⁷ Fc-mediated functions of anti-JCV antibody have been suggested to play a role in the recovery from PML-immune reconstitution inflammatory syndrome (IRIS) in 2 natalizumab-treated patients, based on the predominance of IgG1 and IgG3 in their CSF.¹⁸ However, whether antibodies against JCV can activate FcR-bearing effector cells in PML patients has not been evaluated in detail and could inform the development of urgently needed novel immune-based therapies to treat PML.

Here, we hypothesized that Fc-mediated effector functions elicited by antibodies against the JCV major capsid protein, VP1, contribute to controlling JCV replication. To start addressing this question, we compared anti-JCV and anti-HIV in vitro ADCC responses between PLWH with no PML (PML Neg) and PLWH who developed PML and either survived for over a year (PML S) or progressed to death within a year of diagnosis (PML P).

METHODS

Study Subjects

Study was approved by Beth Israel Deaconess Medical Center Institutional Review Board. PLWH with and without PML were prospectively recruited from outpatient clinics and inpatient units as they were diagnosed with PML. Definitive diagnosis of PML was established by clinical and radiographic criteria in accordance to AAN criteria¹⁹ and confirmed by detection of JCV by PCR in the CSF obtained at enrollment. PML P were defined as patients who succumbed to PML within 12 months of disease onset. PML S were defined as patients who were still alive 12 months after disease onset. We also included a group of PLWH who did not have PML (PML Neg). PML Neg subjects were selected based on absence of central neurological symptoms consistent with PML and no detectable JCV in CSF and were seen in the HIV Neurology Clinic mostly for peripheral neuropathy. Demographics, clinical data and blood samples were collected at enrollment and at 12-month follow-up. Plasma samples collected at enrollment were used to compare PML P and PML S. Follow-up samples were only available for a subset of PML S and allowed us to assess changes between enrollment (PML S T1) and 12 months after disease onset (PML S T2).

Serological Assays

HIV gp140, clade C (C97ZA.012), trimers were obtained from Dr. Dan Barouch.^{20,21} JCV capsid protein VP1 was obtained from Abcam (AB74569). Endpoint titer dilution ELISA was performed as previously published.²⁰ Briefly, polystyrene-type ELISA plates were coated overnight with 1 ug/well of either gp140 or the JCV VP1 protein. Plasma were then added in serial dilutions and incubated for 1 hour at room temperature. The plates were washed 3 times with PBS containing 0.05% Tween 20 and incubated for 1 hour with a dilution of a 1/1000 horseradish peroxidase (HRP)-conjugated goat anti-human secondary antibody (ImmunoResearch Laboratories, West Grove, PA), developed with TMB Peroxidase Substrate (SeraCare, Milford, MA), stopped by addition of stopping solution (SeraCare), and analyzed at 450/550 nm with Spectramax Plus ELISA plate reader using Softmax Pro 4.7.1 software. ELISA endpoint titers were defined as the highest reciprocal plasma dilution that yielded an absorbance > 2-fold over background values.

ADCC

FcR-mediated NK cell degranulation and IFN- γ production were evaluated as surrogate for ADCC using an ELISA-based assay previously shown to be associated with Fc γ RIIIa-binding capacity of antigen-specific antibodies.²²⁻²⁴ The 96-well plates were coated overnight at 4°C with 300 ng of HIV gp140 or JCV VP1 recombinant protein per well.

Five percent BSA-blocked plates were used as antigen controls. The next day, plates were washed 3 times with PBS, blocked with 5% BSA, and washed again before adding 100 μ L of undiluted plasma to each well and incubating at 37°C for 2 hours. PBS alone as well as HIV-negative plasma pools and Rhesus macaque plasma pools were used as negative controls for HIV and JCV, respectively. Pooled HIV-positive or JCV-positive plasma was used as positive controls. After removing plasma and washing with PBS, 5×10^4 NK cells

enriched through negative selection from a single healthy blood donor were added to each well in the presence of Brefeldin A, Golgi stop, and anti-CD107a BV786 and incubated for 5 hours at 37°C and 5% CO₂.

After incubation, cells were stained with CD3 (A700), CD16 (APC-Cy7), and CD56 (BV605) antibodies, fixed (perm A), permeabilized (perm B), and stained intracellularly with anti-IFN- γ (FITC). The cells were then fixed with 2% paraformaldehyde and analyzed by flow cytometry.

Statistical Analysis

The distributions of characteristics of each group were compared by Kruskal–Wallis, Fischer exact, or Mann–Whitney tests. The distributions of anti-JCV VP1 antibodies and anti-HIV gp140 antibodies as well as the NK cell characteristics were compared by the nonparametric Mann–Whitney method using GraphPad Prism Version 7.

RESULTS

Characteristics of Study Population

We analyzed samples from 22 PLWH, of whom 7 were PML P and 15 were PML S (Table 1). Among PML S, we had pairs of samples from 9 subjects where the first time point was obtained at enrollment, as close to disease onset as possible, and the second time point after an average of 367.8 days (range 280–479, SD 64.83).

We also included a group of 6 PML Neg PLWH. The age was similar among the 3 groups. There was an overall male predominance in the study subjects. At PML diagnosis, there were no differences in HIV viral load among all groups. However, PML P had significantly lower CD4⁺ T-cell counts. For the 2 time point comparisons, PML S had significantly lower HIV viral loads about a year after PML onset, but similar CD4⁺ T-cell counts overtime. At enrollment, 4 of 6 PML Neg, 4 of 7 PML P, and 10 of 15 PML S were on antiretroviral therapy (ART). All 9 PML S were on ART at the 12-month follow-up time point.

JCV VP1 and HIV gp140 Antibody Titers

We performed endpoint dilution ELISA against the HIV gp140 clade c trimer (Figs. 1A, B). All subjects had antibodies that bound to the trimer. The endpoint titers did not differ between the PML Neg and the combined PML-positive groups; likewise, it was also not significantly different between the 2 PML-positive groups (PML P and PML S). Furthermore, there was also no significant difference in anti-gp140 antibody titers in the PML S group over time. We then applied the same endpoint dilution protocol to compare relative quantities of antibodies against JCV VP1 between the 3 groups.

The combined PML positive group showed significantly higher titer than the PML Neg group ($P = 0.0037$), and the PML S group had higher antibody titers at the time of PML diagnosis than the PML P group ($P = 0.026$). The titers did not change over time in the PML S group ($P = 0.98$).

Anti-JCV and anti-HIV ADCC Activity

As higher ADCC activity in a subset of patients could be partly explained by overall higher antibody titers, for each subject we normalized anti-HIV and anti-JCV ADCC activity, as measured by proportions of IFN- γ and CD107a+ NK cells, with relative titers of corresponding antibodies. After normalization, anti-HIV gp140 ADCC activity (measured by either IFN- γ production or degranulation by NK cells) did not differ between the PML-positive and the PML Neg groups, although patients with no PML tended to have higher ADCC responses against HIV gp140. Similarly, there were no significant differences between the PML P and PML S groups, nor within the PML S group over time (Figs. 1C, D). On the contrary, anti-JCV VP1 ADCC responses were significantly lower in patients with PML than in those without PML ($P=0.0016$ for %IFN- γ + NK cells; $P=0.0064$ for %CD107a+ NK cells) (Figs. 1E, F). Interestingly, among the 2 PML groups, survival was associated with lower anti-JCV ADCC activity compared with progression ($P=0.021$ for %IFN- γ + NK cells; $P=0.015$ for %CD107a+ NK cells). There were no significant differences in ADCC activity between the 2 time points in the PML S group. Similar trends in ADCC activity could be observed before normalization with antibody titers (See Figure, Supplemental Digital Content 1, <http://links.lww.com/QAI/B342>).

DISCUSSION

In this study, we compared Fc-mediated functions of antibodies against JCV VP1 between PLWH with distinct PML outcomes, using an in vitro assay. We found that as expected, PLWH with PML displayed higher titers of antibodies against JCV than PLWH without PML and showed that the highest levels were associated with survival over a year after PML diagnosis. Despite higher titers of anti-JCV antibodies, the ability of anti-JCV antibodies to activate FcR-bearing effector cells in PLWH with PML was decreased compared to that in PLWH without PML, and among PML patients, a better outcome was linked to decreased Fc-mediated functionality of anti-JCV antibodies.

While our in vitro ADCC assay may not reflect actual in vivo FcR-mediated immune responses, where the JCV agnoprotein is so far the only known JCV protein that can be detected at the surface of JCV-infected cells,²⁶ nonetheless, our data demonstrate functional differences in antibodies against JCV in PML patient with distinct clinical outcome, while their anti-HIV antibodies showed similar Fc-mediated functions in the same assay. Among our cohorts, PML S displayed the highest titers of JCV antibodies, yet the lowest ability to functionally engage FcR. Previous studies have shown that levels of antibodies that can mediate ADCC do not necessarily reflect titers of neutralizing antibodies, and that ADCC activity can even be detected in the absence of the latter.²⁴⁻²⁸ Although it would be crucial to compare the neutralization potential of those antibodies between PML S and P, our findings do not exclude a role for Fc-mediated antibody functions in protection against JCV. For instance, ADCC activity elicited by antibodies against other JCV antigens than the major capsid protein VP1, such as the JCV agnoprotein, was not examined in this study and might significantly contribute to JCV control. In particular, further investigations to assess ADCC responses against JCV-infected cell lines are warranted.

Likewise, to assess anti-HIV ADCC responses, we only evaluated antibodies against the clade C gp140 trimer. Of note, PLWH included in this study were from North America and likely infected with HIV clade B. However, previous studies showed equivalent IgG binding to gp140 trimers from different clades,²⁹ and accordingly, all subjects had antibodies binding to the clade C trimer.

Alternatively, potent Fc-mediated antibody functions elicited by specific domains of VP1 might be detrimental to PML outcome. Exacerbated tissue damage associated with ADCC activity has been suggested in mouse models of infection.^{30,31} Increased titers of JCV antibodies that activate FcR-bearing effector cells could then potentially have deleterious effects, in line with previous observations. Indeed, high titers of JCV antibody have been associated with a significant increased risk of PML in patients treated with natalizumab.³²⁻³⁴ In PLWH, JCV antibody levels, measured years before onset of PML, are also associated with a trend toward increased risk of developing PML,³⁵ indicating potential antibody disruption of immune control of JCV.

Circulating antibodies levels and FcR-mediated responses promoted by JCV-specific antibodies are likely to be impacted in the context of uncontrolled inflammatory responses such as PML-IRIS. However, our cohorts of PML subjects did not have radiographic brain images nor clinical notes consistent with PML-IRIS, based on previously established definitions of PML-IRIS.³⁶ As the prognostic value of PML-IRIS is still under debate, further investigations in patients with PML-IRIS or diagnosed with different stages of PML disease are warranted.

There is no specific treatment for PML, yet ART can restore immune responses against JCV in PLWH.³⁷ However, due to irreversible neurological destruction, only 50% of ART-treated PLWH survive PML. Similar percentages of PML P and PML S were on ART at study entry, which is as close to PML diagnosis as possible, and all PML S were on treatment at the follow-up time point. Thus, while ART significantly improves survival of PLWH with PML, therapy may not have been started early enough to prevent death from PML in our cohorts. ART also restores NK cell function, which deteriorates with HIV disease progression.³⁸ This is relevant as the potential impact of Fc-mediated antibody functions on the outcome of PML would depend on the ability of NK cells and other FcR-bearing cells to mediate FcR-dependent responses to autologous antibodies. Unfortunately, we did not have access to peripheral blood mononuclear cell samples from these PML cohorts to assess NK cell functionality in PML Neg, PML P and PML S. Finally, we and other have showed the critical favorable impact of the T-cell-mediated response against JCV in PML outcome.²⁻⁴ It is therefore possible that a strong T-cell response to JCV obviates the need for antibody-mediated cytotoxic functions in PML S, while absent or weak anti-JCV T-cell response triggers a higher, yet unsuccessful, antibody response in PML P. Future studies are warranted to better understand the role played by antibodies and NK cells in the setting of PML.

The AIDS pandemic and, more recently, the growing use of immunosuppressive drugs have been associated with increased incidence rates of PML over the past decades.³⁹ Here, we provide the first report of distinct Fc-mediated anti-JCV antibody function in PLWH with

differing PML out-comes. Our results highlight the need to define FcR-mediated functions of JCV antibodies in detail. Such studies may help to guide the development of novel immunotherapeutics to prevent JCV reactivation or treat PML, especially in PLWH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

PML S display the highest titers of anti-JCV antibodies and the lowest anti-JCV ADCC activity. Antibody levels against HIV gp140 trimer clade C (A) and JCV VP1 (B) were determined by ELISA endpoint titers. Values are represented in log scale. Antibodies against HIV gp140 trimer (C, D) and JCV VP1 (E, F) from study groups were evaluated for the induction of ADCC by measuring Ab-dependent degranulation (CD107a) and production of IFN- γ by NK cells using flow cytometry. NK cells were isolated by negative selection from one single healthy donor. Each dot represents the mean of 2 replicates normalized by dividing the corresponding antibody titer and multiplied by 1000. PML NEG, PLWH without PML; PML P, PLWH deceased within a year after PML diagnosis; PML S, PLWH alive 1 year after PML diagnosis; PML P + S, all PLWH with PML; T1, first sample at PML diagnosis; T2, closest time point to 1 year after PML diagnosis. Bars represent median. * $P < 0.05$; ** $P < 0.01$.

TABLE 1.

Clinical characteristics of the study groups.

Variable	PML Neg (n = 6)	PML P (n = 7)	PML S (n = 15)	PML S T1 (n = 9)	PML S T2 (n = 9)	P
Age (yrs, median, IQR)	44.5 (35–60.8)	39.5 (30.5–45)	45 (35–57)			0.55 [*]
Gender (M)	5	5	12			0.99 [†]
HIV viral load (copies/mL, median, IQR)	2e3 (51–3.45e4)	73 (50–6.4e4)	50 (50–345)	98 (50–670)	50 (33–52.5)	0.62 [*] 0.047 [‡]
CD4 count (Cells/ μ L, median, IQR)	356 (206–486)	51.5 (20.75–139.8)	283 (143.5–423)	314 (176–423.5)	509 (27.5–691.5)	0.038 [*] 0.44 [‡]

HIV viral load of 50 is the limit of the assay and considered undetectable.

^{*} Comparing PML Neg, PML P, and PML S groups using Kruskal–Wallis.

[†] Comparing PML Neg, PML P, and PML S groups using Fisher Exact.

[‡] Comparing PML S T1 and PML S T2 using Mann–Whitney.

PML Neg, PLWH with no PML diagnosis; PML P, PML progressor; PML S, PML survivor; PML S T1 and T2, subset of 9 subjects of PML S who had samples available at 2 time points approximately a year apart; T1, first time point at study enrollment as close to PML diagnosis as possible; T2, second time point closest to one year after PML diagnosis; IQR, interquartile range.