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The Role of Thymic Reconstitution in the Outcome of AIDS-Related PML

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Abstract

The implications of thymopoiesis in acquired immunodeficiency syndrome (AIDS) - related opportunistic infections (OI) remain unexplored. We used progressive multifocal leukoencephalopathy (PML), caused by JC virus, as an OI model and we simultaneously investigated thymic output and T-cell responses against JCV in 22 PML patients treated with combined antiretroviral therapy. Thymic output was significantly associated with JCV-specific CD4⁺ and CD8⁺ T-cell responses and improved survival. Our data suggest that patients with AIDS-related PML and impaired thymopoiesis are less likely to develop a robust JCV-specific cellular immune response and consequently are at an increased risk for a poor clinical outcome.

Keys words

Human Immunodeficiency Virus; thymus; JC virus; progressive multifocal leukoencephalopathy

INTRODUCTION

Impaired thymic T-cell reconstitution is one of the mechanisms for suboptimal T-cell recovery after effectively inhibiting Human Immunodeficiency Virus (HIV) replication with combined antiretroviral therapy (cART) in patients with acquired immunodeficiency syndrome (AIDS)^{1–6}. The implications of impaired thymopoiesis in AIDS-related

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opportunistic infections have not yet been elucidated. We hypothesized that thymic contribution to the naïve T-cell pool enhances T-cell responses against opportunistic viruses. We used progressive multifocal leukoencephalopathy (PML), caused by polyomavirus JC (JCV), as an AIDS-defining opportunistic infection model and we investigated the impact of thymic reconstitution in the development of T-cell responses against JCV and in survival from PML.

In the past, radiographic imaging of the thymus and T-cell surface markers have been used for assessing thymic T-cell reconstitution^{4,7-9}. However, given considerable limitations of volumetric imaging studies and the lack of specific cell markers for recent thymic emigrant cells, none of these methods have proven to be a reliable assessment of thymic function¹⁰. A more accurate assessment of thymic output may be ascertained by quantification of T-cell receptor rearrangement excision circles (TREC) via polymerase chain reaction (PCR)¹¹⁻¹³. TREC are episomal deoxyribonucleic acid (DNA) circles which are stably retained during cell division and generated during the intrathymic rearrangement of T-cell receptor genes. After initiating cART in AIDS patients, quantification of TREC in blood allows monitoring of the thymic contribution to the peripheral pool of naïve T-cells^{14,15}. TREC levels can be influenced by T-cell activation and proliferation in the periphery. Given the profound CD4⁺ T-cell depletion in patients with AIDS, TREC levels reflect the thymic contribution to the T-cell pool especially when measured early in the course of immune reconstitution^{5,11,14,15}. In the present study, we measured TREC levels in blood in order to assess thymic output in patients with AIDS-related PML. We simultaneously monitored the development of JCV-specific CD4⁺ and CD8⁺ T-cell responses using intracellular cytokine staining (ICS) and recorded the clinical outcomes. We paired thymic output with ICS and clinical data in order to investigate the relationship between thymic reconstitution and the development of T-cell responses against JCV, the opportunistic pathogen.

METHODS

This study was approved by the Institutional Review Board at Beth Israel Deaconess Medical Center. Twenty two patients with AIDS-related PML and five control subjects with AIDS were treated with cART and followed longitudinally at the HIV/Neurology Clinic within our Center. Interferon- γ production from JCV-specific T-cells was detected using ICS, as previously described, after isolating peripheral blood mononuclear cells (PBMC) from whole blood via Ficoll centrifugation¹⁶. T-cell activation was assessed using anti-CD25 monoclonal antibodies¹⁷. Quantification of TREC in blood was performed as previously described by Douek et al¹⁸. We extracted deoxyribonucleic acid (DNA) from the same PBMC obtained for ICS and performed quantitative PCR for the signal-joint (sj)TREC. Results are expressed in sjTREC copies per microgram of PBMC DNA, hereafter abbreviated as TREC cps/ μ g PBMC DNA^{13,19}. We compared the survival experience in those with high (>2000 copies/ μ g PBMC DNA) versus low (<2000 copies/ μ g PBMC DNA) TREC levels using Kaplan-Meier survival analysis based on baseline levels and we used the log-rank test to compare survival experiences. The cut-off TREC level of 2000 copies/ μ g PBMC DNA has been previously used in thymopoiesis studies¹⁹. We used Cox proportional hazards regression models to estimate hazard ratios of death, comparing those with high versus low TREC levels at baseline, and we repeated the analysis accounting for all follow-

up TREC measurements. We used Prism 6.0 and STATA 13 software to complete the analyses. Two-sided p-values < 0.05 were considered statistically significant in order to perform logistic regression analysis, Cox proportional hazards regression, and Kaplan-Meier survival analysis.

RESULTS

Patient characteristics are shown in table 1. Fifteen patients survived for more than one year after PML onset (PML-survivors) and 7 patients died within one year after disease onset (PML-progressors). There was no significant difference in age among the patient groups and control subjects. Patients in both PML groups had low CD4⁺ T-cell counts (median below 60 cells/mL) at the time of PML onset and all patients were started on cART within 35 days. PML-survivors had a higher median CD4⁺ count compared to PML progressors (123 vs 86, p=0.05) at the time of first TREC measurement as well as 6 months after starting cART (301 versus 144, p=0.02). Since clinical factors such as CD4⁺ count nadir, hepatitis C virus infection or other opportunistic infections have been associated with a poor immunologic response after starting cART, we included these factors in our analysis but did not find significant differences between the PML-survivors and PML-progressors groups²⁰. HIV viral load in blood decreased below 50 copies/mL within 6 months of cART initiation in 12/15 (80%) PML-survivors and 5/7 (71.4%) PML-progressors. Three PML-survivors and two PML-progressors had detectable HIV viremia after six months of treatment; however these patients experienced at least a 2-log drop in HIV viral load within 6 months of starting cART. The cART central nervous system penetration effectiveness (CPE) score was seven for both PML groups indicating that all cART regimens had similar penetration into the brain²¹. HIV-infected control subjects had similar age, baseline CD4⁺ counts and time elapsed between cART initiation and TREC PCR compared to PML patients.

Initial TREC levels in blood were obtained within 45 days (range 38–57) from starting cART for all patients. Repeat TREC levels were measured 180 days (range 167–203) from starting cART for 12 PML-survivors and 7 PML-progressors. All PML-survivors underwent additional testing after one year of starting cART (12 to 58 months). PML-survivors displayed significantly higher TREC levels (median = 3001, range = 931–12201 cps/μg PBMC DNA), compared to PML-progressors (median = undetectable, range = undetectable–1507) upon initial testing (p=0.0007, Figure 1A). T-cell activation at the time of the first TREC PCR was modest and comparable between PML-survivors and PML-progressors (CD4⁺CD25⁺ T-cell frequencies ranged from 0.13% to 7.08%, median = 3.68% for PML-survivors and 0.17% to 8.63%, median = 4.11% for PML-progressors, p=0.2). The difference in TREC levels remained significant during repeat testing (p=0.0004 Figure 1B) between PML-survivors (median = 3039, range = 1895–13815) and PML-progressors (median = 152, range = undetectable–1836). There was no significant difference in TREC levels between PML-survivors and control subjects (p=0.3, figure 1C). At the time of the first TREC PCR for each study subject, we performed ICS and we detected a JCV-specific CD4⁺ T-cell mediated response in 13/15 (86.7%) of PML-survivors and in 3/7 (42.8%) of PML-progressors. In addition, we detected a JCV-specific CD8⁺ T-cell mediated response in 14/15 (93.3%) PML-survivors and in 2/7 (28.5%) of PML-progressors. Previously, we reported that a cytotoxic T-cell response against JCV is more frequently present in PML-

survivors compared to PML-progressors²². We further investigated the relationship between thymic output and the development of JCV-specific T-cell response using logistical regression analysis. Interestingly, we found that initial TREC levels correlated with a detectable CD4⁺ and CD8⁺ T-cell response against JCV ($p=0.023$ and $p=0.045$ respectively).

We then performed survival analysis in order to investigate whether initial TREC levels correlate with survival from PML. We found that thymic output above 2000 TREC copies/ μ g PBMC DNA is strongly associated with survival from PML ($p=0.009$, hazard ratio = 0.041, 95% confidence interval = 0.01–0.531, Figure 2A). We repeated the survival analysis but allowed TREC levels to vary over time so as to take into account all TREC data measured during repeat testing. Similarly, PML patients who maintained a thymic output above 2000 TREC copies/ μ g PBMC DNA had improved survival ($p=0.013$, hazard ratio = 0.071, 95% confidence interval = 0.008–0.579, Figure 2B). The association between TREC levels and survival remained statistically significant and was not materially altered when we adjusted for the number of days elapsed between initiation of cART and TREC PCR.

DISCUSSION

In the present study, thymic reconstitution after initiating cART correlates with the development of a cellular immune response against JCV and favors survival from AIDS-related PML. In particular, PML-survivors displayed higher thymic output and robust JCV-specific T-cell responses in comparison to PML-progressors. We estimated thymic output early in the course of immune reconstitution and simultaneously found evidence of modest T-cell activation for all patients. We therefore believe that TREC measurement provides an assessment of thymic output in our cohort and that thymic reconstitution explains, at least in part, the superior CD4⁺ T-cell recovery in the PML-survivors group. Our findings are in line with previously published data suggesting a central role of the thymus in naïve T-cell recovery in the setting of cART^{4,5,8,11,23}. In animal studies, recent thymic emigrant T-cells have been shown to participate in immune responses against viral pathogens and this is likely due to the reconstitution of a T-cell receptor repertoire with broad antigenic specificity^{3,11,20,24–26}. It is therefore likely that a well-replenished pool of naïve T-cells allows for *de novo* priming against viruses, such as JCV, and for the development of a virus-specific cellular immune response. Alternatively, JCV-specific T-cells could originate from memory T-cell expansion after starting cART. Nonetheless, memory T-cell depletion is thought to be the cause of AIDS-defining opportunistic infections²⁷. Our findings expand upon the previous observation that subsets of HIV-infected individuals treated with cART exhibit reduced thymic output and immunological failure for reasons that are poorly understood^{3,4,6,28}. In particular, our data suggest that patients with impaired thymopoiesis are likely unable to mount an adequate response against opportunistic viruses such as JCV, which leads to poor clinical outcomes.

Our findings have specific clinical implications. First, TREC levels bear prognostic value since they correlate with survival from AIDS-related PML early during the disease course. This is especially important given the lack of prognostic biomarkers for PML, a devastating brain infection with no specific treatment. TREC are readily quantifiable via PCR, and

therefore, constitute a potentially scalable blood test. Second, assessment of thymic output has therapeutic ramifications since it allows for identification of HIV-infected PML patients who could benefit from immunotherapies that stimulate thymic function. One such immunotherapy is recombinant human interleukin-7 (IL-7) which is critical for intrathymic T-cell selection, differentiation, and proliferation¹⁰. Investigational use of recombinant IL-7 has been successful in HIV-infected individuals and in PML patients with idiopathic lymphocytopenias^{29–33}. Lastly, since PML is also known to occur in patients with congenital thymic aplasia and in thymectomized individuals, our findings provide insight into the mechanisms of disease in individuals with known thymic dysfunction^{34,35}.

Our study has certain limitations in that our cohort is small and we did not control for multiple covariates. Nonetheless, PML is a rare disease with poorly understood immunopathogenesis and our findings shed light to the mechanisms of T-cell repopulation in AIDS patients who suffer from opportunistic infections such as PML.

In conclusion, in the setting of cART, thymus-dependent T-cell recovery enhances the development of CD4⁺ and CD8⁺ T-cell responses against JCV and leads to improved survival from AIDS-related PML. This is the first study to address the implications of thymopoiesis in AIDS-related PML, and the application of our results to other opportunistic infections merits further investigation. Estimating thymic T-cell output early in the course of immune reconstitution has prognostic value in AIDS-related PML and will help screen for patients who might benefit the most from immunotherapies such as recombinant IL-7.

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Figure 1.

TREC levels in blood of PML-survivors (PML-S), PML-progressors (PML-P) and control subjects (CTRL). TREC levels in blood of PML-survivors, compared to PML-progressors, were significantly higher within 45 days after starting cART (Fig A, $p=0.0007$) and 180 days after starting cART (Fig B, $p=0.0004$). TREC levels were overall higher in PML-survivors than PML-progressors (Fig C, 1–58 months of starting cART, $p=0.0001$) and TREC levels were not significantly different between PML-survivors and control subjects ($p=0.3$).

Figure 2.

Kaplan-Meier curves based on TREC levels in blood of patients with AIDS-related PML. Probability of survival was significantly higher in PML patients with TREC > 2000 copies/μg PBMC DNA 45 days after starting cART, compared to PML patients with TREC < 2000 copies/μg PBMC DNA ($p=0.009$, Fig A). Probability of survival remained significantly higher in patients who maintained TREC >2000 copies/μg PBMC DNA up to 58 months of starting cART ($p=0.013$, Fig B).

Table 1

HIV-infected patient characteristics.

	PML-survivors (n=15)	PML-progressors (n=7)	P value	Controls (n=5)
Age (median, range)	43 (21–53)	44 (41–52)	p=0.1	46 (30–53)
Gender (male : female)	14:1	6:1	-	4:1
Hepatitis C co-infection	3/15 (20%)	2/7 (28.6%)	p=1.0	1/5 (20%)
Other opportunistic infection	1/15 (6.67%)	0/7 (0%)	p=1.0	
CD4 ⁺ counts nadir (median, range)	31 (11–131)	27 (4–88)	p=0.4	33 (8–67)
CD4 ⁺ counts at PML onset	47 (14–192)	53 (4–96)	p=0.35	-
CD4 ⁺ counts at time of first TREC PCR	123 (69–301)	86 (54–251)	p=0.05	101 (79–347)
CD4 ⁺ counts after 6 months of cART	301 (179–455)	144 (86–299)	p=0.02	325 (350–1024)
HIV viral load <50 cps/mL within 6 month of cART	12/15 (80%)	5/7 (71.4%)	p=1.0	4/5 (80%)

CD4 counts are expressed in cells/mL, HIV viral loads in copies/mL.

Abbreviations: PML, progressive multifocal leukoencephalopathy; cART combined antiretroviral therapy.