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Frequent expression of activation induced cytidine deaminase in diffuse large B-cell lymphoma tissues from persons living with HIV

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Abstract

Objective: The increased risk for persons living with HIV to develop diffuse large B-cell lymphoma (DLBCL) even in the post-antiretroviral therapy eras suggests a role beyond immunosuppression in lymphoma development. However, the mechanisms leading to lymphoma in the HIV setting are not fully understood. HIV is known to induce activation induced cytidine deaminase (AID) levels in non-neoplastic B-cells in vitro and chronic AID expression may play an important role in lymphomagenesis. While AID expression is observed in B-cell lymphoma, studies in HIV-associated DLBCL are limited.

Design: In this study, we conducted a retrospective review of DLBCL tissues from patients with and without HIV infection to compare expression of AID and B-cell receptors potentially involved in HIV and B-cell interaction.

Methods: We evaluated DLBCL formalin-fixed paraffin-embedded tissues from 72 HIV seropositive and 58 HIV seronegative patients for AID, DC-SIGN, and CD40 protein expression. BCL2 and MYC, two well-established prognostically significant oncoproteins in DLBCL, were also assessed at the protein and mRNA levels. Subset analysis was performed according to DLBCL subtype and EBV status.

Results: Of note, AID expression was more frequent in HIV-associated DLBCL compared to non-HIV-associated DLBCL regardless of cell-of-origin subtype, and also displayed significantly less BCL2 expression. Despite no direct correlation with AID expression, the HIV-DLBCL tissues also exhibited high levels of the DC-SIGN receptor.

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Conclusions: Collectively, these findings support a potential role for AID in the pathogenesis of HIV-associated lymphomas and suggest the need of further investigations into the involvement of the DC-SIGN receptor-signaling pathway.

Keywords

HIV; diffuse large B-cell lymphoma; activation induced cytidine deaminase; non-Hodgkin's lymphoma; BCL2

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma (NHL) in persons living with HIV. The decrease in incidence with the advent of combined anti-retroviral therapy (cART) indicates a compromised immune system is one of the key factors for HIV-infected patients' increased risk of lymphoma [1,2]. However, even in HIV seropositive [HIV(+)] patients treated with cART, there still remains an increased risk for DLBCL compared to the general population [1–4]. In addition, frequent chemotherapy resistance leads to fewer potential cure options, resulting in a poor overall patient outcome, [1–6]. Favorable responses were observed in one study treating HIV-non-Hodgkin's lymphoma patients, including those with DLBCL, with a rituximab-chemotherapy regimen (R-EPOCH) [7]. Although, a recent randomized trial in HIV(−) DLBCL demonstrated no survival benefit with R-dose adjusted-EPOCH [8]. Other than the role of immunocompetency, the mechanisms of HIV-related lymphomagenesis are not well defined. The oncogenic role of viruses, such as Epstein Barr Virus (EBV) and Kaposi's Sarcoma Herpes Virus, in HIV associated cancers is clearly established and remains an area of active research exploring the underlying mechanisms of viral induced neoplastic transformation [6]. Whether HIV itself has a direct influence on critical oncogenic events in B cell lymphomagenesis, as well as cellular mechanisms behind the HIV viral and B-lymphocyte interaction is not clearly understood.

Activation induced cytidine deaminase (AID) is a critical enzyme for adaptive immunity in physiologic conditions that also has a recognized mutagenic effect and possible role in lymphomagenesis. Following antigen-dependent B-cell activation, AID orchestrates the secondary antibody diversification in the germinal centers of lymph nodes. AID functions by deaminating cytosine to uracil, thus creating a point mutation, which if left unrepaired, allows for the incorporation of an altered nucleotide into the gene and results in somatic hypermutation and ultimately antibody class switching [9]. More recently, it was also shown that AID is active at earlier, pre-germinal center stages of B-cell maturation [10]. However, in pathological contexts such as in Burkitt's lymphoma, some DLBCL, and other mature B cell lymphomas, AID facilitates mutation and rearrangements in non-immunogloblin genes including *C-MYC, PAX5* and *DDX6*, which are likely key lymphomagenic events [11–13]. In the context of HIV-infection in vitro, AID expression in primary germinal center B cells is elevated following exposure to HIV and this may occur through a CD40/CD40L process [14,15]. However, another study demonstrates HIV can also stimulate primary B cells in a CD40L independent manner via an encoded protein, gp120 [16]. In further support of a CD40 independent mechanism, Rappocciolo, *et al* reported that the dendritic

cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) receptor, normally present on a small subset of activated B-cells, mediates a distinct pathway of HIV-1 infection of T-cells [17]. Although, there may be crosstalk between the two pathways as recent work demonstrates an enhanced expression of DC-SIGN by B-cells in response to CD40L stimulation [18].

Here, we sought to determine whether AID, CD40, and DC-SIGN expression are elevated in HIV-associated malignant B-cells as a possible mechanism by which HIV stimulates B-cells and consequently leads to malignant phenotypes. To further investigate the impact of HIV infection in DLBCL pathogenesis we also examined the frequency of expression of well-known prognostic proteins, BCL2 and MYC. Previous immunophenotypic analyses of HIV/AIDS-related DLBCL demonstrated BCL2 was expressed in almost half of the tumors, and HIV(+) DLBCL displayed higher BCL2/MYC dual and MYC only protein expression with similar levels of BCL2 only compared to HIV(−) DLBCL [19, 20]. Concurrent Epstein Barr Virus (EBV) infection with HIV is also a recognized event in HIV-related lymphoma and previous studies show EBV co-infection tended to occur in the non-germinal center B-cell like (non-GCB) DLBCL cell of origin (COO) tumors [19], but no associations were found with BCL2 and MYC expression [20]. Recognizing the relevance of DLBCL COO and EBV positivity, we also performed our analyses according to COO and EBV status.

Methods:

Patients

We used HIV(+) (n=72) and de novo HIV(−) (n=58) DLBCL formalin-fixed paraffin-embedded tissues (FFPET) provided by the AIDS and Cancer Specimen Resource (ACSR; https://acsr.ucsf.edu) and the Arizona Lymphoma Tissue Bio-repository at the Banner University Medical Center Tucson (Tucson, AZ). These samples are expanded cohorts from a previous study we conducted with an overlap of 17 HIV(+) and 13 HIV(−) cases [21]. The FFPET were available either as cores arrayed on two separate tissue microarrays or as whole tissue sections. As criteria for this study, the cases had known HIV sero-status, EBV- encoded small RNA (EBER) in situ hybridization (ISH) staining, and a minimum 70% tumor content. In 28 of the 72 HIV(+) cases provided by ACSR, HIV RNA copy number was known from previous quantitative PCR. This retrospective study of HIV(+) samples from the centralized, anonymized ACSR biobank is limited by the unknown status of whether patients received cART and as such, none of the analyses were performed according to cART. However, of the 72 HIV(+) tissues, only 7 are most likely of the pre-cART era and 65 of the post-cART based on year the sample was collected using 1996 as the cut-off for when this antiretroviral therapy became standard of care [21,22]. The use of human tissues and clinical data for this study was approved from the University of Arizona Institutional Review Board in accordance with the Declaration of Helsinki (#500000226R005).

Immunohistochemistry and scoring

We performed immunohistochemistry (IHC) using rabbit polyclonal antibodies for AID (ab59361, Abcam), DC-SIGN (ab5716, Abcam), and CD40 (ab13545, Abcam), and rabbit monoclonal antibodies for BCL2 (clone SP66, Spring Biosciences) and MYC (clone EP121,

Epitomics), on the Ventana BenchMark® XT as described [23]. Two or three independent observers manually scored AID (V.S, S.A., and S.K.), DC-SIGN and CD40 (V.S. and C.R), and BCL2 and MYC (L.M.R. and S.K.) expression for the number of positive malignant cells in 5% increments. Benign reactive tonsil FFPET served as an external positive control tissue. A negative control (ie. absence of primary antibody) was run in parallel for each stain. For all stains, membranous or cytoplasmic staining was considered positive, and no nuclear staining was observed. Similar staining patterns and protein identification were previously reported using these same antibodies [23–30]. AID expression was evaluated as both a range and at ≥5%, ≥25%, and ≥75% distributions as previously described [31]. This approach to scoring was reproducible (85% agreement; 93/109) between the observers. We then applied this same evaluation system to DC-SIGN and CD40 staining. Positivity for BCL2 and MYC was assessed at well-established cutoffs of ≥50% and ≥40% positive tumor cells, respectively [23,24,32]. Discrepancies in scoring were resolved either by achieving consensus together or by a third observer (L.M.R. for AID).

Out of the 72 HIV(+) cases, 54 were successfully stained for AID, 67 for MYC, 54 for BCL2, 66 for CD40, and 64 for DC-SIGN. In the HIV(−) cohort (n=58), 55 were successfully stained for AID, 46 for MYC, 54 for BCL2, 32 for CD40, and 31 for DC-SIGN. Photomicrographs were obtained using an Olympus BX53 microscope and Olympus DP 22 camera.

Cell of origin and gene expression determination

In DLBCL patient samples with at least 360 ng RNA, the Lymph2Cx assay was performed to determine the DLBCL COO [33]; otherwise the Hans IHC algorithm was used [34]. *BCL2* and *MYC* gene expression were assessed using the PanCancer Pathways Panel (NanoString Technologies) with an RNA input of 300 ng as per manufacturer guidelines previously described [21]. RNA was extracted from 10 μM scrolls or unstained slides of the FFPET using the AllPrep RNA/DNA FFPE isolation kit (Qiagen) and evaluated for quality and quantity by using the Agilent BioAnalyzer and a NanoDrop. Those samples that met the minimum criteria (HIV(−), n=40 and HIV(+), n=34) were then hybridized to the pool of capture/reporter probe pairs for each RNA target. The Lymph2Cx and PanCancer pathways panel were run on the NanoString nCounter system with one of the HIV(−) samples loaded onto each cartridge serving as an inter-cartridge control. Batch effect was also internally corrected for by the inclusion of negative and positive control probes provided by NanoString, as well as by applying the Robust Multichip Averaging, quantile normalization method to all data [35]. The mRNA levels are shown as log2 transformed normalized values.

Statistical analysis

Prism 6 software was used to determine statistical significance at $P<0.05$ by applying the Fisher's exact test or Student's t-test for two-tailed, two-group comparisons with Sidak's multiple comparison test adjusted (adj.) *P*-values for multiple comparisons. One-way ANOVA was used for more than two-group comparisons. The Spearman correlation coefficient was used to evaluate correlations between nonparametric data. For mRNA expression, differences were assessed by a one-way ANOVA adjusted with the Benjamini

and Hochberg false discovery rate (FDR) for multiple-test correction using the Partek Genomics Suite software [36].

Results:

DLBCL characteristics of HIV-associated DLBCL

We first set out to determine the status of factors known to correlate with DLBCL biology in HIV(−) patients within the ACSR HIV(+) DLBCL cohort. These factors included the expression of two prognostic oncogenes, *BCL2* and *MYC*, subtype of DLBCL according to COO, and EBV co-infection. The Lymph2Cx molecular COO assay was performed on 23 of the 72 HIV(+) DLBCL samples to classify 14 as GCB, 4 as activated B-cell like (ABC), and 5 as unclassifiable. As well, 49 of the 58 HIV(−) DLBCL tissues were classified as 23 GCB, 21 ABC, and 5 unclassifiable using the Lymph2Cx. Since not all of the tissues were available for Lymph2Cx COO assignment, we grouped the ABC and unclassifiable into the “non-GCB” category. GCB and non-GCB status were determined for the remaining cases using the Hans IHC algorithm [34]. The total HIV(+) DLBCL cohort determined by molecular or IHC assays consisted of 33/72 (46%) GCB and 39/72 (54%) non-GCB; while, the total HIV(−) DLBCL demonstrated a similar COO distribution: 24/53 (45%) GCB and 29/53 (55%) non-GCB ($P = 1.00$; Supplemental Table 1). Due to limited tissue quantity in some samples, there were 5 DLBCL tissues with unknown subtype in the HIV(−) cohorts, which were excluded from COO specific analyses.

EBER ISH is routinely performed at time of diagnosis and serves as the standard for determining EBV status in lymphoma biopsy tissues [37,38]. Images of EBER staining of 72 HIV(+) DLBCL on the TMA provided by the ACSR were reviewed and scored as positive or negative with 1 failure. Of the 71 HIV(+) DLBCL, 34% (24/71) were EBER positive (Supplemental Table 1), which is consistent with previous findings of EBV co-infection rates [19]. The HIV(+)/EBV(+) cases were predominantly of the non-GCB DLBCL COO compared to the GCB subtype (79% (19/24) vs. 21% (5/24)) relative to the HIV(+)/EBV(−) cases ($P = 0.003$, Fig. 1a).

Based on the known associations of BCL2 protein and MYC mRNA/protein expression with a negative prognosis in HIV(−) patients [23,32,39–41], we next examined the DLBCL tissues for these markers and summarized the findings in Supplemental Table 1. We analyzed gene expression using the Nanostring PanCancer Pathways Panel. On average, HIV(+) DLBCL had 3-fold less *BCL2* mRNA compared to HIV(−) DLBCL ($P = 0.0005$, $FDR = 0.006$, Fig. 1b). To determine if the differential expression in *BCL2* mRNA also occurred at the protein level, we performed immunohistochemistry (IHC) for BCL2. BCL2 protein expression was also significantly lower in HIV(+) DLBCL tissues compared to HIV(−) DLBCL (53% vs. 78%, adj. $P = 0.0004$, Fig. 1c). However when MYC mRNA and protein were evaluated, there was no difference in *MYC* mRNA expression relative to HIV(−) DLBCL ($P = 0.08$, $FDR = 0.20$, Fig. 1d), which paralleled the observations at the protein level (42% vs. 46%, adj. $P = 0.75$, Fig. 1e). We then compared the frequency of BCL2 and MYC double expression in HIV(+) cohort and HIV(−) counterparts, but found no difference (22/52 (42%) vs. 22/46 (48%), $P = 0.68$). Since correlations between EBV infection of B-cells and BCL2 expression are known [42], we repeated the BCL2 analyses according to EBV status. There

was no difference in percentages of BCL2 positive DLBCL cells in the absence or presence of EBV co-infection (52% vs. 60%, $P = 0.54$, Fig. 1f).

AID expression is more frequent in HIV(+) DLBCL compared to HIV(−) DLBCL

Previous literature demonstrated that HIV induces AID in primary B-lymphocytes [14–16], here, we aimed to determine whether neoplastic B-cells of HIV(+) DLBCL also show increased levels of AID. Our benign tonsil control tissue displayed cytoplasmic staining in GC B cells (Fig. 2a), and was used as a reference for assessing lymphoma cell staining in both HIV(−) and HIV(+) DLBCL tissues (Fig. 2b,c). When cases were evaluated for AID positivity, the HIV(+) DLBCL displayed a higher mean percentage of AID positive cells compared to HIV(−) cases (80% vs. 64%, $P = 0.04$, Fig. 2d; Supplemental Table 1). Of note, assessing AID expression at different cutoffs demonstrated HIV(+) DLBCL more frequently express AID in at least 25% of tumor cells than HIV(−) DLBCL (89%, 48/54 vs. 62%, 34/55, $P = 0.002$; Fig. 2e; Supplemental Table 1). Although the pre-cART status is unknown in the HIV(+) cases, 7 samples were collected from 1989 to 1995 and are presumably from pre-cART patients. The expression of AID in these tumors ranged from 20% to 100% (n=3 at 100%, n=1 at 90%, n=1 at 80%, n=1 at 50%, and n=1 at 20%). Given the importance of COO in the pathology and molecular profile of DLBCL, we also analyzed AID expression according to DLBCL COO subtype. There was no difference in the percent of AID positive DLBCL cells among the subtypes within the HIV(+) and HIV(−) cohorts [HIV(−): GCB, 58% vs. nonGCB, 64% adj. $P = 0.96$; HIV(+): GCB, 82%; nonGCB, 77%, adj. $P = 0.99$, Fig. 2f)]. However, there was a slight tendency for HIV(+) GCB tumors to exhibit more frequent positive AID staining on average compared to HIV(−) GCB tumors (82% vs. 58%, adj. $P = 0.12$, Fig. 2f).

HIV(+) DLBCL express high levels of DC-SIGN that tend to coincide with AID expression

After observing a high prevalence of AID expression in HIV(+) DLBCL tissues relative to HIV(−), we next investigated whether these HIV(+) DLBCL tumors express the B-cell surface receptors, DC-SIGN and CD40, previously implicated in HIV and B-cell interaction. These published studies suggest signaling through DC-SIGN and CD40 may potentially lead to an increase in AID expression [14–17]. We observed that in benign tonsil tissue, most GC cells were negative for DC-SIGN expression with rare scattered cells displaying an intense rimming of the cytoplasm (Fig. 3a). These positive cells were used as the intensity reference for scoring malignant B-cells positive in both HIV(−) and HIV(+) DLBCL tissues (Fig. 3b,c). When percent DC-SIGN positive DLBCL cells per tumor were plotted, tumors from HIV(+) patients exhibited a higher average percent of DC-SIGN positive cells compared to HIV(−) DLBCL (93% vs. 80%, $P = 0.02$, Fig. 3d; Supplemental Table 1). In particular, HIV(+) DLBCL tissues displayed frequent DC-SIGN expression at or above 75% tumor cells staining positive (91%, 58/64 vs. 61%, 19/31, $P = 0.001$; Fig. 3e; Supplemental Table 1). Similar to AID expression, there was no correlation between DC-SIGN positivity and COO in either HIV(−) DLBCL or HIV(+) DLBCL (GCB, 81% vs. non-GCB, 73%, adj. $P = 0.20$; GCB, 98% vs. non-GCB, 88%, adj. $P = 0.20$, Fig. 3f).

Qualitatively IHC for CD40, the control tonsil tissue displayed mostly cytoplasmic staining in GC and some inter-follicular cells (Fig. 4a), while in contrast malignant B-cells in both

HIV(−) and HIV(+) DLBCL tissues showed both cytoplasmic and surface staining (Fig. 4b,c). However, quantitatively, there was no difference in percent DLBCL cells positive for CD40 expression between the HIV(+) and HIV(−) cohorts (60% vs. 74%, $P = 0.14$, Fig. 4d; Supplemental Table 1). Likewise, there were no differences in CD40 positive cases at any of the predetermined three cutoffs (Fig. 4e; Supplemental Table 1). CD40 positive tumors were not enriched in a particular COO classification for either the HIV(+) or the HIV(−) DLBCL tissues (GCB, 53% vs. non-GCB, 65%, adj. $P = 0.73$; and GCB, 85% vs. non-GCB, 61%, adj. $P = 0.51$, Fig. 4f). Although, there was a slight trend towards HIV(−) GCB tumors to stain at a higher percent positivity than HIV(+) GCB (85% vs. 53%, adj. $P = 0.14$, Fig. 4f).

We then examined whether the presence of either receptor or co-infection of EBV correlated with the elevated AID expression in the HIV(+) DLBCL tissues. In the 51 HIV(+) DLBCL tissues with successful staining of both DC-SIGN and AID, there was a slight trend for DC-SIGN and AID expression to increase concurrently (r = 0.21, $P = 0.14$, Fig. 5a). In contrast, CD40 expression did not correlate with AID expression (n = 54, r = 0.03, $P = 0.85$, Fig. 5b). EBV co-infection also did not appear to correlate with an increased AID expression, as there was no difference in the percentage of AID positive DLBCL cells according to EBV status (EBER negative, 83% vs. EBER positive, 73%, $P = 0.28$). In a subset of the HIV(+) DLBCL, 28 samples were subjected to quantitative PCR for HIV RNA copy number and 10 cases had detectable HIV RNA with a range of 1,355 to 238,170 copies while the other 18 cases were negative. We compared expression of the markers evaluated in this study (BCL2, MYC, AID, DC-SIGN, and CD40) between the HIV RNA positive cases and the HIV RNA negative cases. There was no significant difference in the average percent DLBCL cells positive for any of the proteins (Supplemental Figure 1).

Discussion

While the advent of potent cART substantially reduced the incidence of DLBCL in HIV-infected individuals, DLBCL is still elevated as compared to the general population [1,2]. This increased risk suggests that, in addition to a diminished immune response, alternative molecular pathways also contribute to the development of DLBCL in these individuals. Several lines of evidence have implicated AID in the development of B-cell lymphomas particularly related to chromosomal translocations. In particular, in vitro-based data demonstrate HIV induces AID expression in non-neoplastic B-cells, indicating that HIV may facilitate lymphomagenesis through upregulation of AID [14–16,43,44]. In further support, we show, here, for the first time that AID expression is frequent and elevated in HIV(+) DLBCL tissues that occurred over a continuous range as compared to HIV(−) counterparts. This expression pattern is suggestive of a more active, sustained induction of AID in the presence of HIV infection compared to HIV(−) DLBCL where expression was more of an “off - on” pattern consistent with normal AID activity in GC B-cells [45]. The mainly cytoplasmic staining pattern with virtually no nuclear localization is also in line with previous observations [25, 45–48]. The AID expression did not correlate with a specific DLBCL COO subtype, despite the expectation that non-GCB DLBCL may exhibit an increase in AID since previous studies show higher *AID* mRNA levels in ABC DLBCL compared to GCB DLBCL [49, 50]. However, quite often mRNA and protein levels do not

correlate due to post-transcription or -translation regulation and a lack of dynamic range for protein levels detected by IHC.

With the recent discovery that extracellular HIV virions accumulate in B cell follicle GCs and that these can serve as a large reservoir site [51] along with the possible mediation of HIV – B-cell interaction through DC-SIGN and CD40 [14–18], we investigated the expression for these two receptors in HIV(+) DLBCL. We found DC-SIGN is highly expressed in HIV(+) DLBCL compared to HIV(−), which indicates an altered phenotype that B-cells may acquire when undergoing neoplastic transformation in the presence of HIV. This phenomenon was observed to a greater extent in the HIV+/EBV- cases suggesting similar to the elevated AID, the increased DC-SIGN expression is not dependent on co-infection with EBV (HIV+/EBV- DC-SIGN, 99% ± 1.2% vs. HIV+/EBV+, 81% ± 7.9%, $P = 0.04$). While we were unable to evaluate HIV antigens including Env and gp120 in the scope of this study, our findings in DLBCL tissues support the potential for Env-mediated B-cell regulation of HIV observed in the *in vitro* studies from a pathological standpoint [16]. But, whether the enhanced AID and DC-SIGN expression is a direct effect mediated by HIV antigens or is a result of a chronic B-cell activation feedback loop remains unclear. In contrast, presence of CD40 did not differ between HIV(+) DLBCL and HIV(−) DLBCL, which is not entirely unexpected since CD40 is a normal B-cell surface marker.

We also explored correlation of HIV status to two well-known negative prognostic biomarkers in DLBCL, BCL2 and MYC [32,39]. Neither concurrent expression of BCL2/MYC nor MYC alone differed in the HIV(+) DLBCL cohort compared to the HIV(−) tumors. Of note, HIV(+) DLBCL were more often BCL2 negative, which is in agreement with our previous findings in a smaller subset of cases [21]. However, the lower BCL2 expression in HIV(+) DLBCL differs from previous findings by Chao *et al.*, which did not find a difference in BCL2 expression between HIV(+) and HIV(−) DLBCL. This discrepancy may be explained by the lower positivity cutoff that was selected by Chao's group [20]. As well, we utilized a different, newer rabbit clone, SP66, which is less susceptible to false negative staining [23]. The less frequent BCL2 expression could associate with a better patient outcome in the HIV-DLBCL cohort; however, AID expression was recently shown to predict inferior survival in high-risk DLBCL patients to first-line therapy as well as in relapse/refractory patients to salvage therapy [52]. Additional studies are needed to clarify the significance of DLBCL prognostic factors in the context of HIV infection.

Overall, our study contributes to the accumulating data that HIV(+) DLBCL likely represents a distinct biologic entity within a broad B-cell lymphoma category and supports a direct involvement in the development of lymphoma possibly through the misappropriated activity of AID. How this important enzyme is dysregulated in HIV(+) DLBCL remains unclear, but the elevated DC-SIGN expression may provide a means for which HIV-associated protein stimulate B-cells to upregulate AID expression. The high level of AID expression in the HIV(+) DLBCL presents the question as to whether the high level of AID expression would differ from non-lymphoma HIV(+) tissues. The presence of HIV infection and associated antigens may provide a chronic stimulation of B-cells regardless of lymphoma development. Understanding the molecular characteristics of HIV(+) DLBCL, and the fine interplay between the patient's immune response and the lymphomagenic

viral effects, including a potential role for AID, should offer additional insights into the mechanisms behind the increased risk of lymphoma during HIV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

V.S. wrote the manuscript. M.J. performed the NanoString Lymph2Cx and PanCancer Pathways Panel assays. L.M.R. provided the patient tissues, and along with V.S., C.Y.R, and S.A. reviewed the immunohistochemistry. L.M.R. also edited the manuscript. A.M. assisted in the data analysis interpretation and edited the manuscript. S.K. designed the research, performed the data analyses, and edited the manuscript. All authors approved the manuscript.

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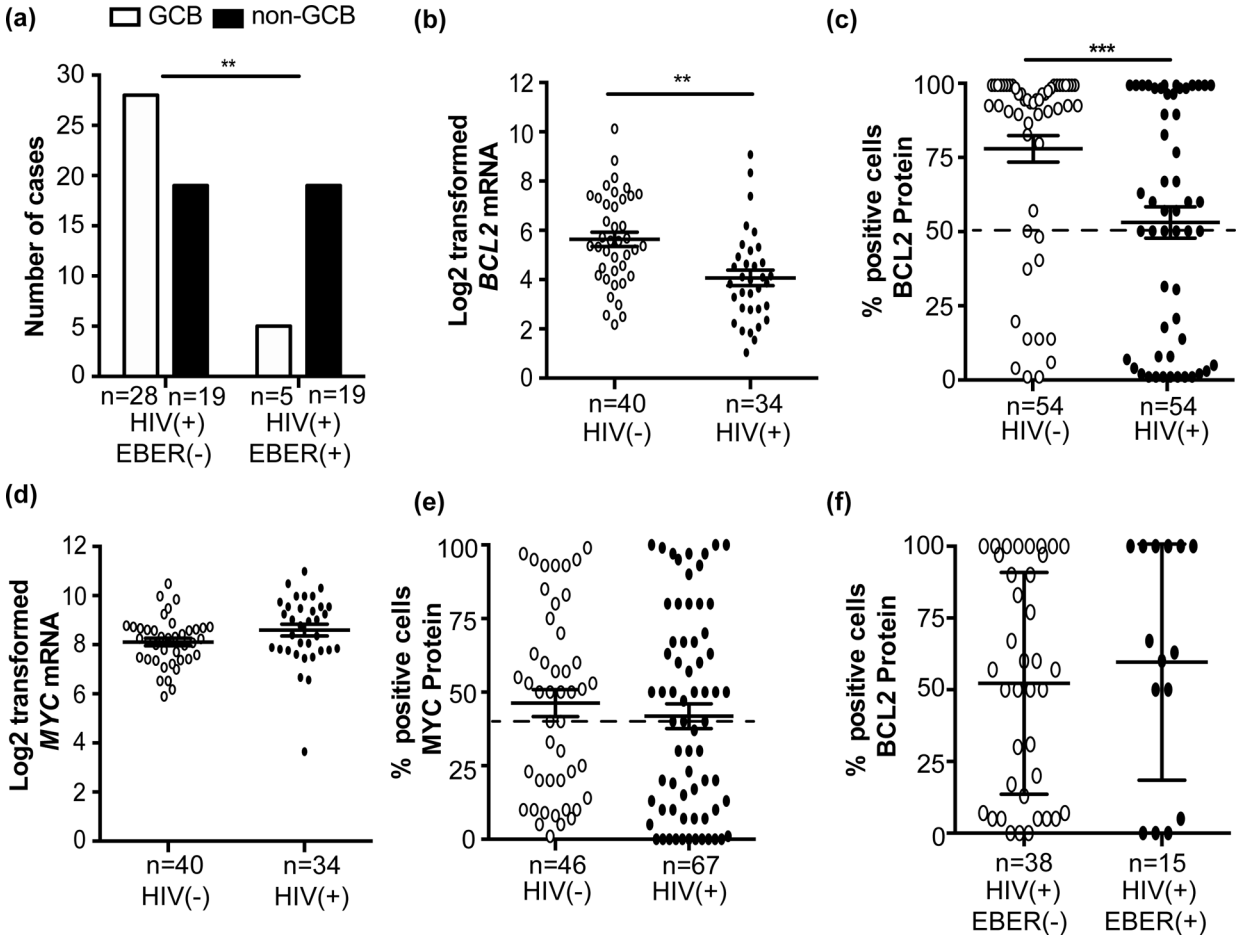

Fig. 1.

EBV co-infection in HIV(+) DLBCL and comparison of BCL2 and MYC expression in HIV(−) and HIV(+) DLBCL. (a) Number of GCB and non-GCB DLBCL cases according to EBV co-infection in HIV(+) DLBCL samples. EBV status was determined by EBER. Differences between the proportions were determined by Fisher's exact test with $*P < 0.05$. Percent tumor cells stained positive for BCL2 (b) and MYC (c) protein in HIV(−) and HIV(+) DLBCL. Log2 transformed *BCL2* (e) and *MYC* (f) mRNA levels in HIV(−) and HIV(+) DLBCL. Each dot represents an individual tumor with mean ± SEM. Differences were determined by the Student's t-test and *P*-values were adjusted using Sidak's multiple test correction with $** P < 0.01$ and $*** P < 0.001$.

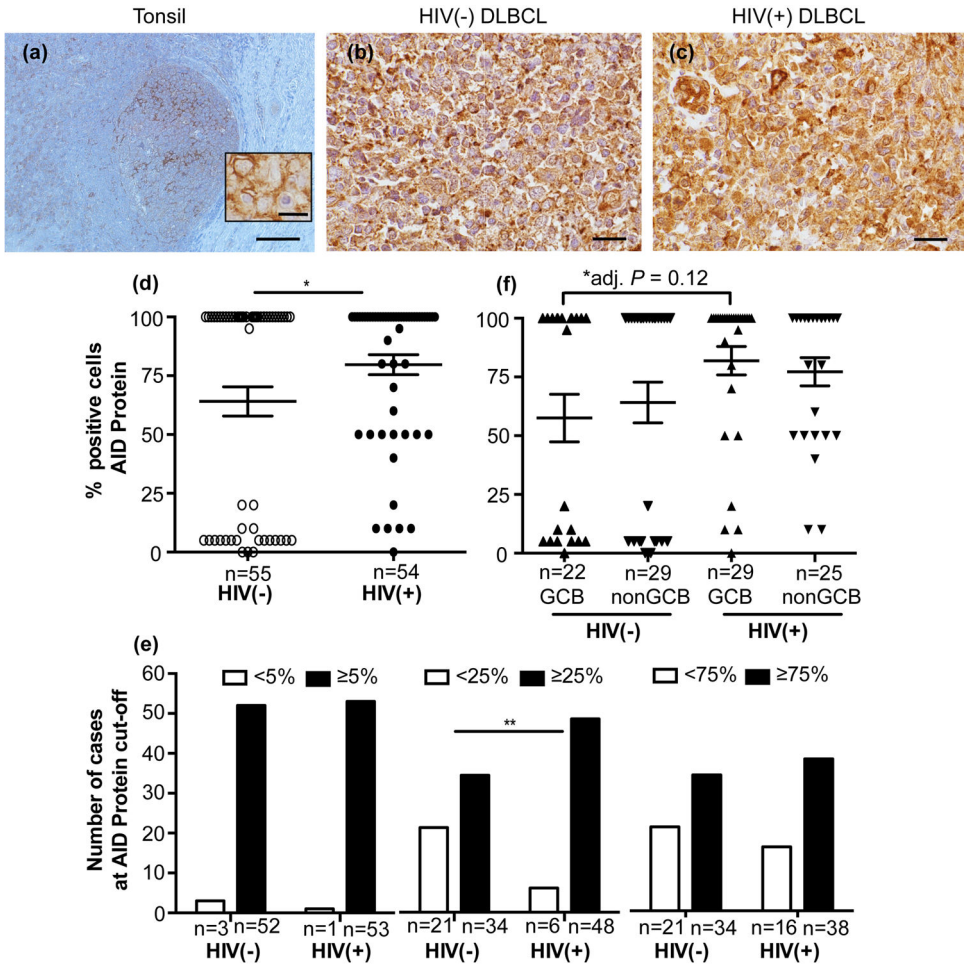

Fig. 2.

AID expression in HIV(+) DLBCL tissues compared to HIV(−) DLBCL. (a-c) Representative images of positive AID immunohistochemical staining in a tonsil control tissue (a), and in HIV(−) and HIV(+) DLBCL tissues (b and c, respectively). Original magnification, 200x (a, inset at 600x) and 600x (b,c) with scale bars, 200 and 20 μm. (d) Comparison of AID expression in HIV(−) and HIV(+) DLBCL as percent tumors cells staining positive. The Student's t-test was used to determine a significant difference with $*P < 0.05$. Each dot represents an individual tumor with mean ± SEM. (e) Number of HIV(−) and HIV(+) DLBCL tissues with positive AID staining at 5%, 25%, and 75% cutoffs. Differences between the proportions were determined by Fisher's exact test with $**P < 0.01$. (f) Percent tumor cells positive for AID in GCB and non-GCB DLBCL tissues according to HIV sero-status. Each dot represents an individual tumor with mean ± SEM.

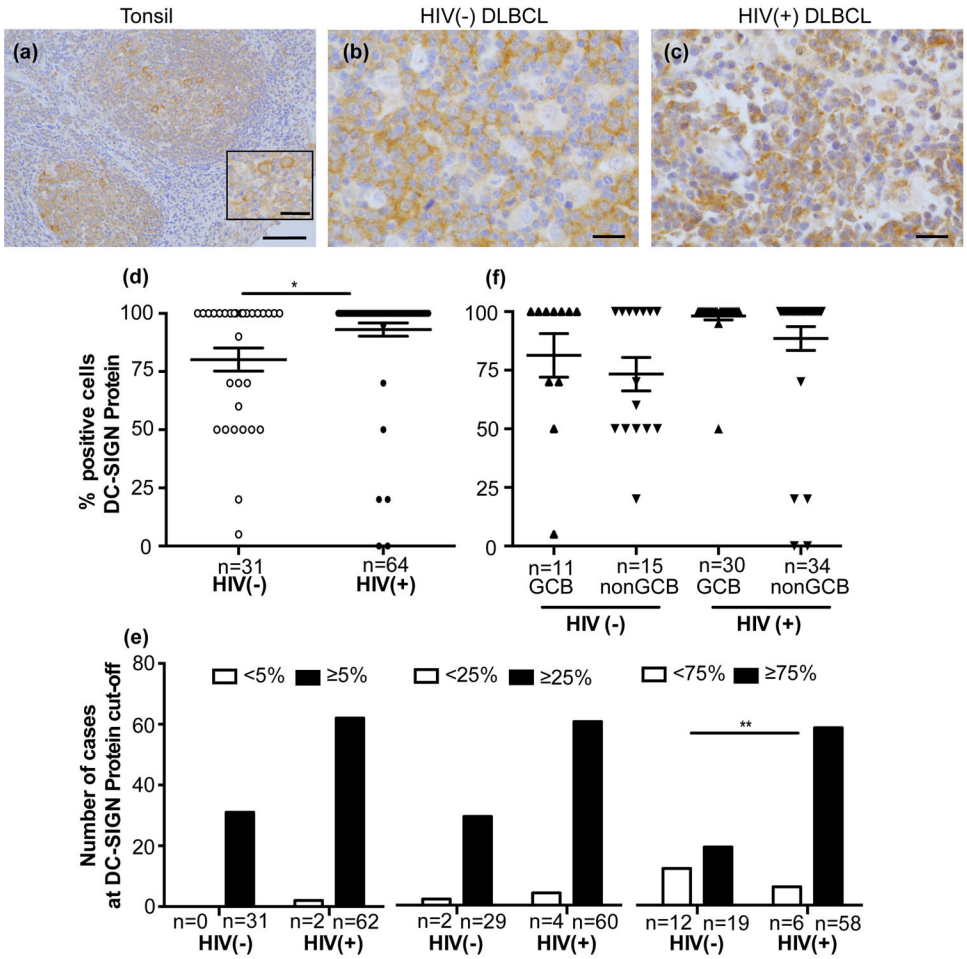

Fig. 3.

DC-SIGN expression in HIV(+) DLBCL tissues compared to HIV(−) DLBCL. (a-c) Representative images of positive DC-SIGN immunohistochemical staining in a tonsil control tissue (a), and in HIV(−) and HIV(+) DLBCL tissues (b and c, respectively). Original magnification, 200x (a, inset 600x) and 600x (b,c) with scale bars, 200 and 20 μm. (d) Comparison of DC-SIGN expression in HIV(−) and HIV(+) DLBCL as percent tumors cells staining positive. The Student's t-test was used to determine a significant difference with $*P < 0.05$. Each dot represents an individual tumor with mean ± SEM. (e) Number of HIV(−) and HIV(+) DLBCL tissues with positive DC-SIGN staining at 5%, 25%, and 75% cutoffs. Differences between the proportions were determined by Fisher's exact test with $** P < 0.01$. (f) Percent tumor cells positive for DC-SIGN in GCB and non-GCB DLBCL tissues according to HIV sero-status. Each dot represents an individual tumor with mean ± SEM.

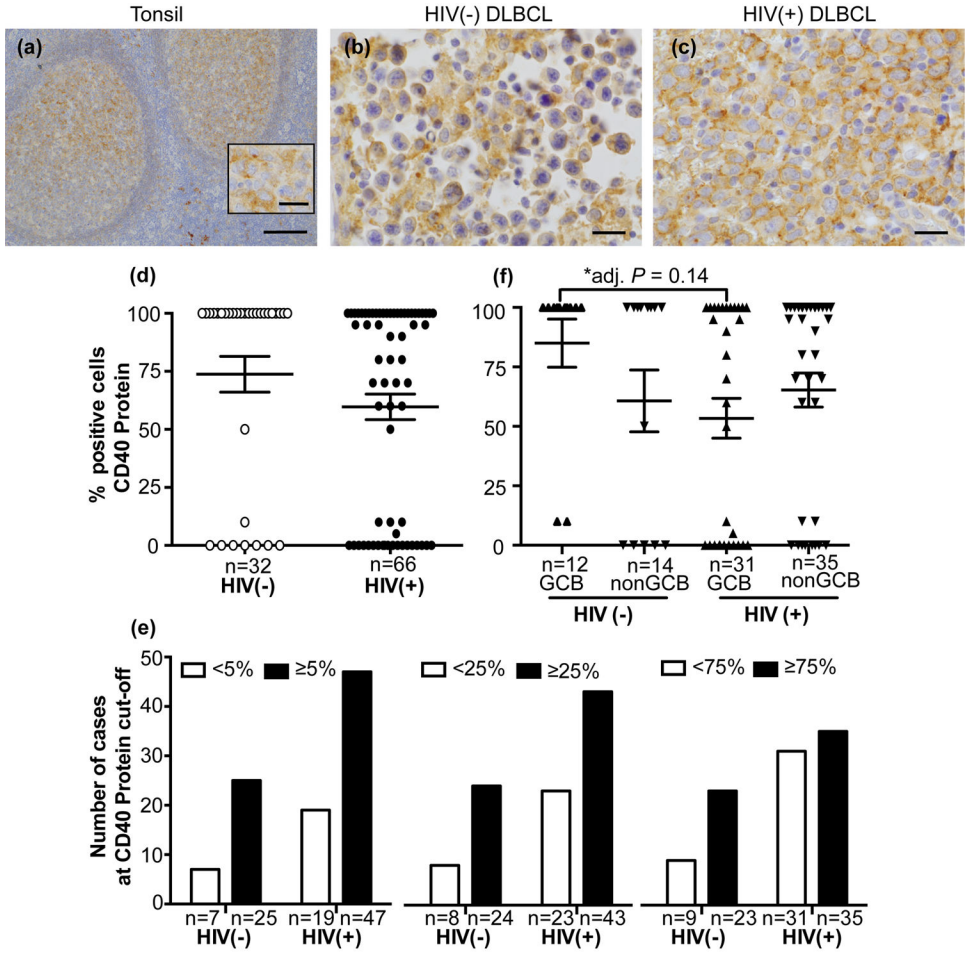

Fig. 4.

CD40 expression in HIV(+) DLBCL tissues compared to HIV(−) DLBCL. (a-c) Representative images of positive CD40 immunohistochemical staining in a tonsil control tissue (a), and in HIV(−) and HIV(+) DLBCL tissues (b and c, respectively). Original magnification, 200x (a, inset at 600x) and 600x (b,c) with scale bars, 200 and 20 μm. (d) Comparison of CD40 expression in HIV(−) and HIV(+) DLBCL as percent tumors cells staining positive. The Student's t-test was used to determine there was no significant difference. Each dot represents an individual tumor with mean ± SEM. (e) Number of HIV(−) and HIV(+) DLBCL tissues with positive CD40 staining at 5%, 25%, and 75% cutoffs. Differences between the proportions were determined by Fisher's exact test. (f) Percent tumor cells positive for CD40 in GCB and non-GCB DLBCL tissues according to HIV sero-status. Each dot represents an individual tumor with mean ± SEM.

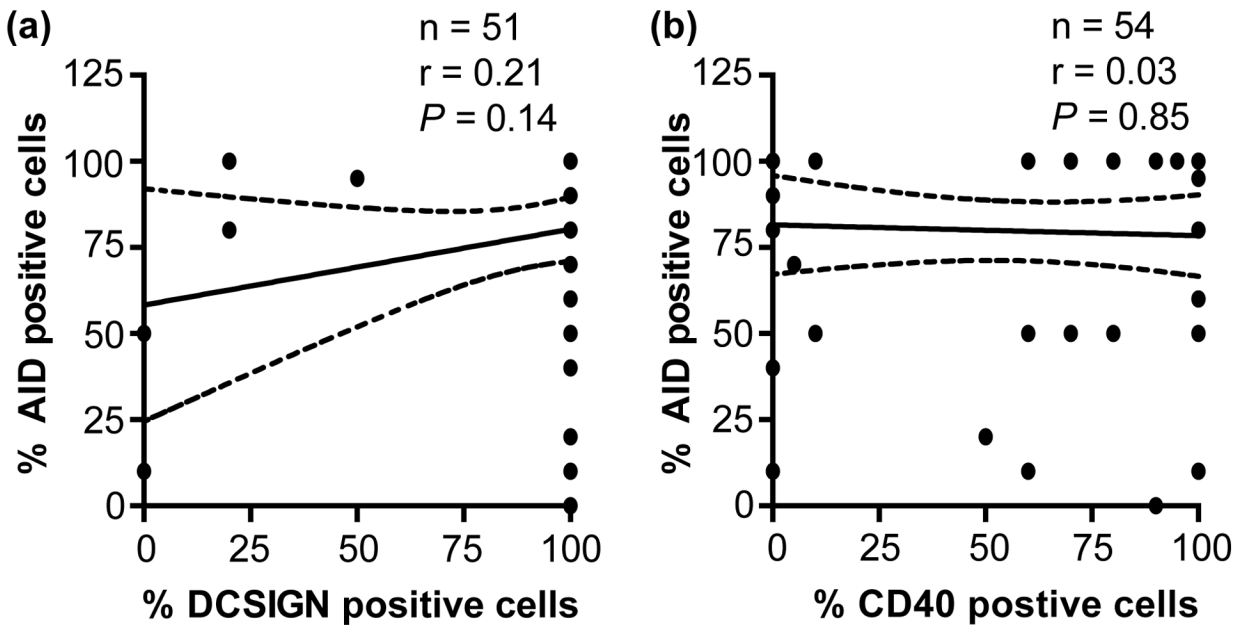

Figure 5.

AID expression in HIV(+) DLBCL according to DC-SIGN and CD40. (a,b) Spearman correlations between percent AID positive tumor cells in HIV(+) DLBCL and either percent DC-SIGN (a) or percent CD40 (b) positive tumor cells within the same DLBCL tissue. Each dot represents an individual tumor and the dashed lines show the 95% confidence interval.