Title: Cytotoxic response against Epstein Barr virus coexists with diffuse large B-cell lymphoma tolerogenic microenvironment: clinical features and survival impact

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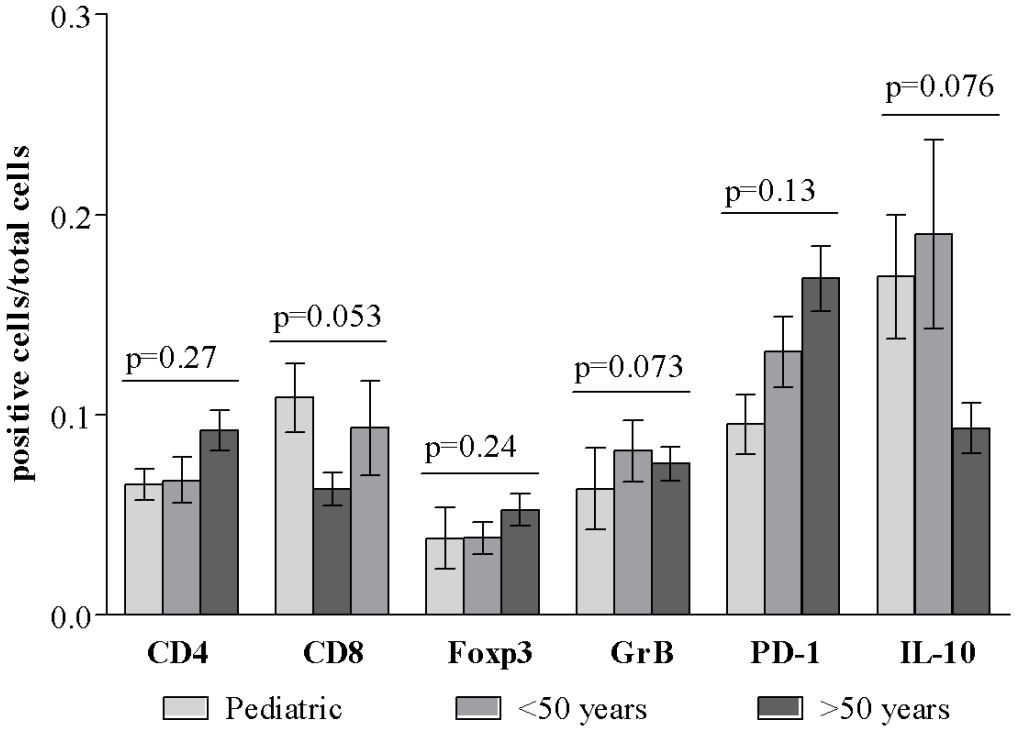
Supplementary Figure 1. Comparative analysis of immunological markers regarding age.



Since immunosenescence has been postulated to be implicated in the pathogenesis, we investigated if there was any difference in tumor microenvironment composition associated with age. All the immunological markers studied were discriminated according to age (pediatric, older and younger than 50 years old patients), and we did not find statistical differences among them (CD4 p=0.27, CD8 p=0.053, Foxp3 p=0.24, GrB p=0.0703, PD1 p=0.13, IL10 p=0.075; Kruskal-Wallis test). Therefore, based on those results, we decided to not discriminate our series into different age groups for cell markers analysis.

Supplementary Table 2. EBERs ISH detection and types of latency pattern expression on EBV+ DLBCL cases

	EBERs[1]	Latency I	Latency II	Latency III	Latency II-III
no. positive cases/total (%)	17/102 (17)	3/17 (18)	7/17 (41)	3/17(18)	4/17 (24)

1EBV status determined by EBERs ISH (≥20% EBERs+ cells as a cutoff value). Latency pattern defined by EBERs ISH, LMP1 and EBNA2 IHC results. Latency II-III was defined when could not be discriminate due to lack of material.

Supplementary Table 3. Types of latency pattern on EBV+ DLBCL cases related to clinical outcome

Patient nº	Age (years)	Immunological status	ISH EBERs[1]	%	Latency expression[2]	Outcome
1	2	IC	+	40	III	Dead
2	9	IS	+	70	III	Dead
3	3	IC	+	30	III	Alive
4	4	IS	+	60	II/III	Dead
5	3	IS	+	80	II/III	Dead
6	14	IS	+	70	II/III	Dead
7	7	IS	+	30	III	Dead
8	8	IC	+	90	I	Alive
9	2	IC	+	50	III	LF
10	18	IC	+	90	III	Dead
11	38	IC	+	90	II	LF
12	35	IC	+	80	II/III	Dead
13	44	IC	+	50	II	LF
14	68	IC	+	80	II	Dead
15	76	IC	+	30	II	Dead
16	76	IC	+	25	II	LF
17	73	IC	+	20	III	LF

Abbreviations: IS, immunosuppressed. IC, immunocompetent. ISH, in situ hybridization. LF, lost at follow-up. 1≥20% EBERs+ tumor cells as a cut-off value. 2 indicates latency pattern defined by IHC.

Supplementary Table 4. Demographic and histological characteristics of DLBCL series related to EBV status (positive vs. negative)

Patients' characteristics	EBV+[1] n (%)	EBV- n (%)	p[2]
Age (years)			
pediatric	4 (21)	15 (79)	
< 50	4 (17)	20 (83)	0.26
≥ 50	4(8)	48(92)	
Gender			
male	10 (20)	40 (80)	0,43
female	7 (14)	45 (86)	
Histologicalsubtype			
GC	7 (21)	27(79)	0,075
post-GC	9 (23)	30 (77)	
ND	1	28	
Clinicalstage			
I-II	1 (5)	21 (95)	0,23
III-IV	5 (20)	20 (80)	
ND	11	44	
Primarysite			
nodal	11 (21)	42 (79)	0,30
extranodal	6 (12)	43 (88)	

1EBV status determined by EBERs ISH (≥20% EBERs+ cells as a cutoff value).2p as determined by Fisher's or Chi square exact test. Abbreviations: GC: germinal-centre; ND: not determined (insufficient material).

Supplementary Table 5. Cytokines and chemokines gene expression related to EBV expression status

Gene	EBV status†	Mean‡	SE	Median‡	Range	*p**
IL-10	Negative	5.1	0.35	5.2	(1.0-8.8)	0.042*
	Positive	6.3	0.43	6.2	(3.9-9.2)	
TGFβ	Negative	1.2	0.056	1.1	(1.0-2.9)	0.80
	Positive	1.2	0.076	1.0	(1.0-2.0)	
IFNγ	Negative	1.4	0.066	1.4	(1.0-2.5)	0.18
	Positive	1.6	0.15	1.5	(1.0-3.0)	
CCL20	Negative	1.0	0.0025	1.0	(1.0-1.1)	0.69
	Positive	1.0	0.0084	1.0	(1.0-1.1)	
CCL22	Negative	6.7	0.44	7.4	(1.0-10)	0.11
	Positive	8.2	0.58	8.3	(4.7-11)	

SE: standard error †EBV status determined by EBERs ISH (≥20% EBERs+ cells as a cutoff value). ‡The number represents the mean log2 transformed of the gene expression data. *p as determined by Mann Whitney

Supplementary Table 6. TIL subsets quantification in tumor microenvironment related to EBV status

TIL subsets	EBV status†	Mean‡	SE	Median	Range	*p**
CD4	Negative	0.082	0.007	0.069	(0-0.29)	0.77
	Positive	0.076	0.02	0.062	(0-0.22)	
CD8	Negative	0.074	0.009	0.049	(0-0.51)	0.042*
	Positive	0.11	0.02	0.12	(0-0.24)	
Foxp3	Negative	0.045	0.006	0.024	(0-0.23)	0.61
	Positive	0.050	0.02	0.018	(0-0.29)	
GrB	Negative	0.062	0.006	0.041	(0-0.26)	0.0007*
	Positive	0.15	0.02	0.17	(0.02-0.28)	
PD-1	Negative	0.14	0.01	0.12	(0-0.53)	0.38
	Positive	0.15	0.02	0.13	(0.07-0.29)	
IL-10	Negative	0.128	0.02	0.0984	(0.02-0.39)	0.41
	Positive	0.162	0.03	0.162	(0.05-0.32)	

SE: standard error †EBV status determined by EBERs ISH (≥20% EBERs+ cells as a cutoff value). ‡The number represents the mean of the n°+ cells / nº total cells.*p as determined by Mann Whitney test.

Supplementary information 7. Definitions of common terms in survival analysis

Event: Death, disease occurrence, disease recurrence, recovery, or other experience of interest. In this particular study an Event is defined as non-response, death from any cause, tumor progression or second malignancy.

Time: The time from the beginning of an observation period (such as surgery or beginning treatment) to (i) an event, or (ii) end of the study, or (iii) loss of contact or withdrawal from the study.

Censoring / Censored observation: If a subject does not have an event during the observation time, they are described as censored. The subject is censored in the sense that nothing is observed or known about that subject after the time of censoring. A censored subject may or may not have an event after the end of observation time.

Event-free survival: According to the National Cancer Institute (NCI), after primary treatment for a cancer ends, EFS is the measurement of the length of time that the patient remains free of certain complications or events that the treatment was intended to prevent or delay. These events may include the return of the cancer or the onset of certain symptoms, such as bone pain from cancer that has spread to the bone. In a clinical trial, measuring the event-free survival is one way to see how well a new treatment works.

Follow-up: the date when the patient go to the medical consultation for any cause.

Time to event: the quantity of time (generally in months) from the diagnosis to the observation defined previously as event (non-response, death from any cause, tumor progression or second malignancy).