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RESEARCH PAPER

Host genetics contributes to the effectiveness of dendritic cell-based HIV immunotherapy

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ABSTRACT

Systems biological analysis has recently revealed how innate immune variants as well as gut microbiota impact the individual response to immunization. HIV-infected (HIV+) patients have a worse response rate after standard vaccinations, possibly due to the immune exhaustion, increased gut permeability and microbial translocation. In the last decade, dendritic cells (DC)-based immunotherapy has been proposed as an alternative approach to control HIV plasma viral load, however clinical trials showed a heterogeneity of immunization response.

Hypothesizing that host genetics may importantly affects the outcome of immunotherapy in HIV+ patients, genetic polymorphisms' distribution and gene expression modulation were analyzed in a phase I/II clinical trial of DC-based immunotherapy according to immunization response, and quality of vaccine product (DC).

Polymorphisms in genes previously associated with progression of HIV infection to AIDS (i.e.: *PARD3B, CCL5)* contribute to a better response to immunotherapy in HIV+ individuals, possibly through a systemic effect on host immune system, but also directly on vaccine product.

Genes expression profile after immunization correlates with different degrees of immune chronic activation/exhaustion of HIV+ patients (i.e. *PD1*, *IL7RA, EOMES*), but also with anti-viral response and DC quality (i.e.: *APOBEC3G*, *IL8, PPIA*), suggested that an immunocompetent individual would have a better vaccine response.

These findings showed once more that host genetics can affect the response to DC-based immunotherapy in HIV+ individuals, contributing to the heterogeneity of response observed in concluded trials; and it can be used as predictor of immunization success.

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HIV; immunotherapy; dendritic cell; genetics; *PARD3B*

Introduction

HIV-1 infection constitutes a major challenge to global public health. Although antiretroviral therapy (ART) improves survival and quality of life of patients, news strategies are required for viral eradication and functional cure. In the last decade, dendritic cells (DC)-based immunotherapy has been proposed as an alternative approach to control HIV plasma viral load (PVL). This treatment resulted safe and able to increase HIV-1 specific cellular immune response, leading to the destruction of HIV-infected T CD4+ cells and resulting in decrease PVL and increase T CD4+ lymphocytes' blood level.[1] However, since the first published results,[1-3] it appeared that not all the immunized patients uniformly respond to the treatment.

Response to immunotherapy can be considered a multifactorial trait, and host genetics has been hypothesized to contribute for a successful immunization response.[4,5]

Systems biological analysis has recently revealed how innate immune variants as well as gut microbiota impact the individual response to immunization.[6,7]

HIV-infected (HIV+) patients have a worse response rate after standard vaccinations (i.e.: Hepatitis B vaccine[8]), possibly due to systemic immune activation, which is related to persistent infection, increased gut permeability and microbial translocation, but also to the host genetic background. Nevertheless, the molecular mechanism of vaccine effectiveness in HIV+ individuals remains incompletely understood, or even poorly investigated, as in the case of DC-based immunotherapies.

Recently we used both candidate-gene and whole-exome approaches to identify genetic variants associated to DC-immunotherapy response in HIV+ patients of the French-Brazilian clinical trial (phase-I).[2] Single nucleotide polymorphisms (SNPs) in innate immune genes *MBL2* and *NOS1*[9], as well in the HIV-1 restriction factor *PARD3B*, and in mRNA deadenylases complex

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component *CNOT1*[11] resulted associated with a weak or transient response to immunization in these patients. A limit of these studies was the unavailability of biologic samples, other than patients' DNA, to further evaluated how genetic variants could affect vaccine preparation and/or treatment outcome.

In this study, we first investigated the impact of host genetic variants on response to DC-immunotherapy. In particular, how genetics affects PVL and T cells levels after the treatment, but also the quality of DC used for immunization. The second objective consisted in examining the modulation of genes important in vaccine response and/or in HIV infection progression in the peripheral blood of immunized subjects. It could be of practical importance to evaluate the applicability of genetic variants and/or gene expression as biomarkers for successful immunotherapy.

Results and discussion

Host variants in genes previously associated to HIV-1 restriction and/or AIDS progression (*APOBEC3G, CCL4, CCL5, CCR5, CUL5, CXCR6, HLA-C, IFNG, PARD3B, PROX1, SDF-1, TRIM5, ZNRD1*),[12] or to HIV immunotherapy response (*MBL2, NOS2*)[10] were analyzed in 19 HIV+ patients submitted to a DC-based HIV immunotherapy between May 2011 and February 2014 at the Laboratory of Medical Investigation (LIM)-56, Faculty of Medicine, University of Sao Paulo (USP; SP, Brazil) (phase I/II clinical trial; yet unpublished results).

To investigate a possible effect of host genetic background on vaccine response, polymorphisms' distribution was first evaluated according to the post-immunization variation (Δ) of plasma viral load and T lymphocytes counts. Multivariate analysis and genotype modelling were performed for the 24 polymorphisms. Sex, age and time of disease were included as confounder variables (adjusted p-value/p_{adj}). Considering the number of studied variants, we applied the Bonferroni multiple-comparison correction, and fixed a more stringent significant threshold ($p \leq 0.002$) than the usual ($p \leq 0.05$). However, taking in account, the limited size of studied cohort, and a possibly relevant biologic effect of selected polymorphisms, all results with a $p_{adj} \leq 0.05$ are reported in Table 1, at least for a descriptive report and discussion (complete results in Supplementary File 1),

Among the 24 polymorphisms analyzed, only *PARD3B* rs11884476 resulted significantly associated to immunization response ($p_{adj} = 5.1 \times 10^{-5}$), whereas polymorphisms in *CCL5, CUL5, TRIM5* and *ZNRD1* resulted differently distributed according to immunotherapy response ($p_{adj} \leq 0.05$), but without reaching significant threshold after Bonferroni correction (Table 1).

Of note, the minor allele of the SNP rs11884476 in *PARD3B* gene resulted more frequent in HIV+ patients with a better response to immunization in term of PVL control (higher reduction of PVL after DC treatment) according to our previously reported study in the phase-I trial.[10]

When considering difference in T CD4+ or CD8+ cells counts after the immunization, none of the studied variants resulted significantly associated to the immunization response after Bonferroni correction ($p_{adj}>0.002$), however it is interesting to observe that *PARD3B* rs11884476 appeared to be more frequent in HIV+ individuals with an augmented T CD4+ cells count (C/G: 207.0 ± 60.0 versus C/C: −4.50 ± 25.82; $p_{adj} = 0.032$), corroborating the above-mentioned association with ΔPVL, and the previously reported association with the "good" response in the phase-I trial.[10] Taking in account the limited size of studied cohorts, these data represent an important replication study for rs11884476 variant in the context of HIV+ patients' response to DC-based immunotherapy.

CCL5 rs2280789 and rs2107538, and *CUL5* rs7103534 and rs7117111, resulted in linkage disequilibrium (D'/LOD > 95) in the cohort (Supplementary File 2), however the derived haplotypes did not result in any association with the components of the immune response that we evaluated ($p_{adj} > 0.05$; data not showed).

Then we performed multivariate analysis according to vaccine product quality (DC viability, DC phenotypic profile, DC ability to *in vitro* activate autologous T lymphocytes). Main results ($p_{adj} \leq 0.05$) are summarized in Table 1. Only p-values adjusted by sex, age and time of disease are reported. We excluded data with only 1 individual for group, independently from the p-value.

HIV+ patients carrying the *CCL5* rs2107538 variant in heterozygosis originate DC with a reduced CD83 expression (2819 ± 373.1 MFI, versus 6614 ± 1103.8 MFI; $p_{adj} = 0.002$). This SNP was previously associated to increased rate of HIV disease progression,[13] suggesting that it could affect DC activation and consequently host immune response.

Of note, *PARD3B* rs11884476 was more frequent in HIV+ patients with a better T lymphocytes activation in co-culture assays. rs11884476_C/G DC induced a higher production of cytokines from T CD4+ cells than DC from C/C or G/G individuals, especially for TNF (0.12 ± 0.04 MFI, versus 0.01 ± 0.01 MFI; $p_{adj} = 0.002$). Moreover, rs11884476_C/G DC induced a significant higher production of IL-2 also from T CD8+ cells compared to homozygotes (0.69 ± 0.26 MFI, versus 0.017 ± 0.011 MFI; $p_{adj} = 5 \times 10^{-5}$). *In vitro* assays suggested that the observed genetic effect of *PARD3B* rs11884476 on immunization response is possible correlated with a better ability of vaccination product (patients' DC) to induce lymphocytes activation.

Even whether our stringent statistical analysis have excluded several other variants with an associated $p_{adj} \geq 0.002$, it interesting to notice that the same SNPs *CUL5* rs7117111 and *TRIM5* rs3740996, that resulted differently distributed according to post-immunization variation in PVL (ΔPVL) and lymphocytes count (ΔT CD4+, ΔT CD8+), seem to affect DC quality (Table 1). *CUL5* rs7117111 resulted more frequent in HIV+ patients with high *in vitro* DC yield ($p_{adj} = 0.012$) and ability to induce TNF production by CD8+ T cells ($p_{adj} = 0.008$), suggesting it could contribute to a better quality of DC. This finding partially explains the previously reported association of this variant with a better response to immunotherapy. On the other hand, *TRIM5* rs3740996, which associated with a worse response to immunization,[14] resulted more frequent in patients with a higher IL-10 production by injected DC ($p_{adj} = 0.030$). Moreover, *MBL2* rs10824, significantly associated to a weak/transient response in the phase-I trial of immunotherapy,[9] appeared here to be related with a poor *in vitro* DC yield ($p_{adj} = 0.012$) as well as DC ability to stimulate IFN-γ production by T CD8+ cells ($p_{adj} = 0.011$).

Table 1. Host genetic variants' distribution according to immunization response and vaccine product quality. 24 polymorphisms in 16 genes related with HIV-1 restriction and/or DC-based HIV immunotherapy response were genotyped in 19 HIV+ patients submitted to a phase I/II clinical trial of DC-based HIV immunotherapy. Multivariate analysis of polymorphisms' distribution was performed according to "immunization response" (ΔPVL, ΔT CD4+, ΔT CD8+), "dendritic cells quality" (DC yield, CD83+ cells, IL-10 production), "ability of DC in inducing a T CD4+ or CD8+ cell response" (IL-2+, IFN-γ+, TNF+ and MIP-1ß+ cells, and cell proliferation after 24 or 96 hours of DC/lymphocytes co-culture). Sex, age and time from diagnosis were included in the analysis as confounder variables. Herein only main results are presented ($p_{adj} \leq 0.05$). Statistical significant p-values, according to Bonferroni correction ($p_{adj} \leq 0.002$), are indicated in bold characters Gene name, polymorphism identification number (ID), minor allele, genotypes and their distribution (n), variables' mean value and standard error (mean ± SE), as well as p-values adjusted for sex, age and time from diagnosis (p_{adj}). ΔPVL: variation in plasma viral load after the immunization; ΔT CD4+: variation in peripheral blood T CD4+ cells count after the immunization; ΔT CD8+: variation in peripheral blood T CD8+ cells count after the immunization. PVL is expressed in log (RNA copies/μL). T cells count is expressed as number of cells/μL. Flow cytometry data are expressed as percentage of positive cells (%) or median fluorescence intensity (MFI) for the used markers. Cytokines production by DC is expressed as concentration in culture supernatant (pg/mL). T lymphocytes proliferation is expressed as percentage (%) of CSFE cell staining dye.

Immunization response				
Characteristic	Gene/Polymorphism ID (minor allele)	Genotypes (n)	Mean ± SE	p_{adj}
ΔPVL (log RNA copies/μL)	*CCL5* rs2280789 (G)	A/A (13)	−0.31 ± 0.30	0.004
		A/G (4)	0.18 ± 0.15	
		G/G (2)	−3.83 ± 0.11	
	CCL5 rs2107538 (T)	C/C (7)	0.11 ± 0.13	0.031
		C/T (10)	−0.43 ± 0.42	
		T/T (2)	−2.04 ± 1.80	
	CUL5 rs7117111 (A)	G/G (7)	−1.19 ± 0.68	0.006
		G/A (6)	0.07 ± 0.11	
		A/A (6)	0.14 ± 0.14	
	PARD3B rs11884476 (G)	C/C (16)	−0.03 ± 0.17	5.1×10^{-5}
		C/G (3)	−3.40 ± 0.44	
ΔT CD4+ (cells/μL)	*PARD3B* rs11884476 (G)	C/C (16)	−4.50 ± 25.82	0.032
		C/G (3)	207.0 ± 60.0	
	TRIM5 rs3740996 (A)	G/G (16)	40.40 ± 27.85	0.046
		A/G (3)	−88.0 ± 84.30	
	ZNRD1 rs3869068 (T)	C/C (14)	47.0 ± 31.17	0.009
		C/T (3)	17.33 ± 39.63	
		T/T (2)	−160.50 ± 74.50	
ΔT CD8+(cells/μL)	*ZNRD1* rs3869068 (T)	C/C (14)	−92.92 ± 62.97	0.017
		C/T (3)	133.67 ± 147.16	
		T/T (2)	−637.50 ± 231.50	
DC quality				
DC yield (%)	*CUL5* rs7117111 (A)	G/G (7)	1.07 ± 0.43	0.012
		G/A (6)	4.41 ±1.32	
		A/A (6)	1.43 ± 0.71	
	MBL2 rs10824 (C)	T/T (4)	4.15 ± 2.07	0.007
		C/T (13)	1.69 ± 0.46	
		C/C (2)	0.75 ± 0.15	
CD83 (MFI)	*CCL5* rs2107538 (T)	C/C (7)	5901.0 ± 1032.0	0.002
		C/T (10)	2819.0 ± 373.1	
		T/T (2)	10179.0 ± 100.1	
IL-10 (pg/mL)	*CXCR6* rs2234358 (T)	T/T (5)	45.68 ± 42.33	0.030
		G/T (11)	26.73 ± 9.79	
		G/G (3)	141.59 ± 56.77	
	TRIM5 rs3740996 (A)	G/G (16)	33.04 ± 14.52	0.030
		A/G (3)	141.59 ± 56.77	
T CD4+ cell activation				
IFN-γ 24h (MFI)	*PARD3B* rs11884476 (G)	C/C (16)	0.31 ± 0.30	0.047
		C/G (3)	3.12 ± 3.04	
IFN-γ 96h (MFI)	*PARD3B* rs11884476 (G)	C/C (16)	0.12 ± 0.10	0.030
		C/G (3)	1.21 ± 1.15	
TNF 24h (MFI)	*PARD3B* rs11884476 (G)	C/C (16)	0.01 ± 0.01	0.002
		C/G (3)	0.12 ± 0.04	
MIP-1ß 24h (MFI)	*APOBEC3G* rs3736685 (C)	T/T (16)	0.01 ± 0.01	0.029
		T/C (3)	0.45 ± 0.45	
IL-2 96h (MFI)	*PARD3B* rs11884476 (G)	C/C (16)	0.002 ± 0.001	0.022
		C/G (3)	0.44 ± 0.43	
T CD8+ cell activation				
IL-2 24h (MFI)	*PARD3B* rs11884476 (G)	C/C (16)	0.02 ± 0.01	5×10^{-5}
		C/G (3)	0.69 ± 0.26	
IFN-γ 24h (MFI)	*MBL2* rs10824 (C)	T/T (4)	0.047 ± 0.032	0.011
		C/T (13)	0.002 ± 0.002	
		C/C (2)	0.005 ± 0.005	
TNF 24h (MFI)	*CUL5* rs7117111 (A)	G/G (9)	0.002 ± 0.002	0.011
		G/A (5)	0.030 ± 0.014	
		A/A (5)	0.043 ± 0.006	
MIP-1ß 24h (MFI)	*PARD3B* rs11884476 (G)	C/C (16)	0.08 ± 0.03	0.027
		C/G (3)	0.52 ± 0.42	
T CD8+ proliferation (CSFE, %)	*APOBEC3G* rs3736685 (C)	T/T (16)	1.19 ± 0.34	0.031
		T/C (3)	7.99 ± 6.71	

Altogether these findings confirmed the hypothesis that host genetic background can contribute to a better response to immunotherapy in HIV+ individuals, possibly through a systemic effect on host immune system, but also directly on vaccine product.

In a second moment, we questioned if exists an expression signature in peripheral blood mononuclear cells (PBMC) of immunized HIV+ patients, as reported for other vaccines,[4] and whether this signature could be indicative of immunization success. Our objective was to identify some gene whose expression could be significantly correlated to HIV immunotherapy response or to vaccine preparation. For this purpose 86 genes were selected based on previous studies about blood cells profile after immunization[4,5,15,16] or HIV disease progression,[10,11,17] and analyzed in PBMC isolated from of HIV+ patients before (t0) and after (t1) the DC-based immunization. We have defined as differently modulated those genes with a fold change (FC) expression in t1 higher than 1.2 (or, in case of down-regulation, fold regulation/FR <-1.2) compared to t0.

A correlation analysis was performed between genes' modulation (FC or FR values of different modulated genes, DEGs) and post-immunization variation in PVL (ΔPVL) and T lymphocytes (ΔT CD4+ or ΔT CD8+). Moreover, the correlation was performed between genes' modulation and vaccine product quality. The vaccine quality was defined by the means of patients' DC cell viability and activation state, measured by surface level of HLA-DR and co-stimulatory molecules (CD80, CD86, CD83, CD40), migration capacity (surface level of chemokines receptors) and ability to *in vitro* activate autologous T lymphocytes (T cells proliferation and production of IFN-γ). Statistical significant results required Spearman correlation parameters $r^2>0.95$ and $p<0.05$. Significant results are reported in Table 2, and briefly described here below.

The increased expression of *APOBEC3G* (FC = 1.37 ± 1.42) positively correlated with ΔPVL ($r^2 = 1$; $p = 0.017$), according to the well-known function of this protein in contrasting HIV-1 infection.[18]

An increased expression of exhaustion marker *PD1* (FC = 1.55 ± 1.44), as well as a diminished expression of *EOMES* (FR = −1.31 ± 0.47), positively correlated with an increased level of T CD4+ lymphocytes after immunization. Although the role of EOMES in T $CD4^+$ cells is not well characterized, it has been implicated in driving the production of IFN-γ in Th1 cells.[19] Together these data evidenced whether PBMC were adequately activated by immunization.

None of the analyzed genes resulted significantly correlated to T CD8+ cells variation.

When correlation analysis was performed taking in account the quality of vaccination product (DC) (Table 2), we observed that the diminished expression of *CNOT1* (FR = −2.26 ± 0.32) and *IL7RA* (FR = −2.13 ± 0.28) are inversely correlated with DC activation markers CD83 ($r^2 = -1.0$; $p = 0.017$) and CD40, respectively. These results indicated that a better DC quality, in terms of maturation and lymph node migration ability, is related with a diminished expression of this factor, corroborating our previously published data about the association of *CNOT1* with a poor response to DC-based immunotherapy in HIV+ individuals.[11] On the other hand, the decreased expression of activation marker *IL7RA/CD127* reinforced the idea that chronically activated and exhausted PBMC could not generate effective vaccine.

The decreased expression of *EOMES* (FR = −1.31 ± 0.47) positively correlated with the CD86 level ($r^2 = 0.97$; $p = 0.033$). It is interesting to note that, as above mentioned, a diminished expression of *EOMES* positively affects also CD4+ T cells count after immunization, emphasizing that a reduction of *EOMES* could be beneficial to the vaccine response.

Table 2. PBMC gene expression profile according to immunization response and vaccine product quality. The expression of 86 genes related with HIV progression/AIDS or immunization response was evaluated in peripheral blood mononuclear cells (PBMC) isolated from 19 HIV+ patients before (t0) and after (t1) immunization with autologous HIV-stimulated DC (phase I/II trial of DC-based immunotherapy). The modulation of gene expression was calculated as Fold Change/FC ($2^{-\Delta\Delta Ct} > 1$) or Fold Regulation/FR ($2^{-\Delta\Delta Ct} < 1$) according to Schmittgen and Livak,[25] and genes with a FC > 1.2 or FR < −1.2 were considered differentially expressed after the treatment (differential expressed genes, DEGs). Non-parametric Spearman test of correlation was performed between DEGs and "immunization response" (ΔPVL, ΔT CD4+, ΔT CD8+), "dendritic cells quality" (DC yield, CD83+ cells, IL-10 production), or "ability of DC in inducing a T CD4+ or CD8+ cell response" (IL-2+, IFN-γ+, TNF+ and MIP-1ß+ cells, and cell proliferation after 24 or 96 hours of DC/lymphocytes co-culture). Herein only main results are presented ($r^2 \geq 0.95$ and $p \leq 0.05$). Variable name, gene name, FC or FR mean value and standard error (mean ± SE), as well as Spearman correlation parameters (r^2 and p-value) were reported. ΔPVL: variation in plasma viral load after the immunization; ΔT CD4+: variation in peripheral blood T CD4+ cells count after the immunization; ΔT CD8+: variation in peripheral blood T CD8+ cells count after the immunization. PVL is expressed in log (RNA copies/μL). T cells count is expressed as number of cells/μL. Flow cytometry data are expressed as percentage of positive cells (%) or median fluorescence intensity (MFI) for the used markers. Cytokines production by DC is expressed as concentration in culture supernatant (pg/mL). T lymphocytes proliferation is expressed as percentage (%) of CSFE cell staining dye.

Immunization response				
Characteristic	Gene	Gene expression modulation (FC or FR)	r^2	p
ΔPVL (log RNA copies/μL)	*APOBEC3G*	1.37 ± 1.42	1.0	0.017
	SLFN11	−1.20 ± 0.47	0.97	0.033
ΔT CD4+ (cells/μL)	*EOMES*	−1.31 ± 0.47	1.0	0.003
	PD1	1.55 ± 1.44	1.0	0.017
DC quality				
CD83 (MFI)	*CNOT1*	−2.26 ± 0.32	−1.0	0.017
CD86 (%)	*EOMES*	−1.31 ± 0.47	0.97	0.033
CD40 (%)	*IL7RA/CD127*	−2.13 ± 0.28	−1.0	0.017
CCR6 (%)	*IL8*	1.33 ± 0.92	1.0	0.017
T CD4+ cell activation				
IFN-γ 24h (MFI)	*PPIA*	−1.28 ± 0.53	0.97	0.033
	ISG15	1.25 ± 1.10	−0.97	0.033
T CD8+ cell activation				
T CD8+ proliferation (CSFE, %)	*IL8*	1.33 ± 0.92	1.0	0.017

Table 3. Main characteristics of immunized HIV+ individuals.

Sex	Age	Time from diagnosis	PVL_{t0}	T CD4+$_{to}$	T CD8+$_{to}$	PVL_{t1}	T CD4+$_{t1}$	T CD8+$_{t1}$	ΔPVL	ΔT CD4+	ΔT CD8+
M	40.0	4.0	4.09	566.00	776.00	4.55	633.00	885.00	0.46	67.00	109.00
M	32.0	11.0	4.36	320.00	525.00	4.11	377.00	713.00	−0.25	57.00	188.00
M	24.0	1.0	4.39	569.00	868.00	4.26	444.00	878.00	−0.13	−125.00	10.00
M	55.0	5.0	4.46	473.00	1058.00	5.09	519.00	1458.00	0.62	46.00	400.00
F	39.0	6.0	3.54	543.00	1007.00	3.49	456.00	844.00	−0.05	−87.00	−163.00
M	42.0	6.0	5.02	351.00	1569.00	2.07	498.00	1039.00	−2.96	147.00	−530.00
M	40.0	2.0	4.66	400.00	1580.00	4.99	314.00	1174.00	0.33	−86.00	−406.00
M	27.0	14.0	3.45	502.00	1139.00	3.47	559.00	1050.00	0.02	57.00	−89.00
M	41.0	1.0	4.30	329.00	1165.00	1.99	276.00	754.00	−2.31	−53.00	−411.00
M	32.0	6.0	4.53	306.00	482.00	4.72	421.00	551.00	0.19	115.00	69.00
F	28.0	3.0	3.71	376.00	490.00	3.47	388.00	595.00	−0.24	12.00	105.00
M	35.0	9.0	4.77	471.00	1212.00	4.69	236.00	343.00	−0.08	−235.00	−869.00
F	32.0	1.5	4.21	580.00	1536.00	4.6	629.00	1614.00	0.39	49.00	78.00
M	22.0	6.0	3.20	544.00	822.00	3.00	613.00	783.00	−0.2	69.00	−39.00
M	28.0	1.0	4.20	423.00	785.00	4.64	456.00	848.00	0.44	33.00	63.00
M	39.0	4.5	3.57	608.00	781.00	4.05	780.00	848.00	0.49	172.00	67.00
F	39.0	15.0	3.54	480.00	1371.00	3.71	411.00	949.00	0.18	−69.00	−422.00
M	26.0	1.0	5.60	291.00	877.00	1.77	558.00	806.00	−3.83	267.00	−71.00
F	32.0	12.0	3.43	456.00	763.00	3.6	395.00	655.00	0.17	−61.00	−108.00
M/F (n)	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD
14/5	34.37±7.97	8.37±12.16	4.16±0.62	452.0±102.1	989.8±349.4	3.80±1.01	471.7±135.8	883.5±298.9	−0.36±1.25	19.74±117.1	−106.3±300.3

ID = identification number; M = male. F = female; CD4+ = CD4+ T lymphocytes.t0 = before of the treatment t1 = after the start of the treatment.

As expected, higher *IL8* expression positively correlated with vaccine quality, in term of T CD8+ cells proliferation ($r^2 = 1.0$; $p = 0.017$) as well as with CCR6 expression on DC ($r^2 = 1.0$; $p = 0.017$), corroborating the role of the chemokine in both DC activation,[20,21] as well as in decreasing HIV-1 replication.[22] A diminished PBMC expression of the peptidyl-prolyl isomerase cyclophilin A/*PPIA* (FR = −1.28 ± 0.53) resulted positively correlated with T CD4+ cells activation (T CD4+ IFN-γ+ cells) ($r^2 = 0.97$; $p = 0.033$), according to its previously reported role, not only as a HIV-1 restriction factor, but also as a TCR signaling regulator.[23]

Altogether, these findings corroborated previously published data about a PBMC expression profile consistent with a chronic activation and exhaustion of HIV+ patients (i.e. *PD1*, *IL7RA*, *EOMES*),[15] but also suggested that the DC-based immunotherapy was able to promote a protective immune response (i.e.: *APOBEC3G*, *IL8, PPIA*), reflecting at least in part the expected induction of an anti-HIV immune response. Moreover, our findings emphasize that among immunized HIV+ patients, the ones with a more exhausted PBMC expression profile originated dendritic cells with a poor ability to activate lymphocytes, and vice-versa, suggesting that a pre-immunization *in vitro* assay of patient' DC could be useful for the improvement of an individualized immunotherapy for HIV-1.

Conclusion

Host genetics affects the response to DC-based immunotherapy in HIV+ patients, and can, at least partially, explicate the heterogeneity of response observed in concluded trials.

In this study *PARD3B* rs11884476 not only associated with ΔPVL, as previously reported by our group,[10] but also with DC activation state, corroborating the key role of this gene in HIV-1 infection progression to AIDS,[24] as well as genetic predictor of immunization success.

PBMC expression profile after immunization correlates with different degrees of immune chronic activation/exhaustion of HIV+ patients but also with the vaccine (DC) quality, clearly showed that a better vaccine is originated from a less immunocompromised individual.

Material and methods

Clinical study

This study is comprised of 19 HIV+ patients vaccinated with DC-based immunotherapy (phase I/II trial) between May 2011 and February 2014 at the Laboratory of Medical Investigation (LIM)-56, Faculty of Medicine, University of Sao Paulo (USP; SP, Brazil).

All patients were adult (22 to 55 years old), seropositive for at least 5 years, naïve for antiretroviral therapy for at least 1 year, and without clinical AIDS or other chronic diseases, with blood $CD4^+$ cells count > 350 cells/μl, and PVL > 3 log (1000 RNA copies/mL) before the start of the immunization.

HIV+ individuals were vaccinated with three doses of 3–30 × 10^6 autologous HIV-pulsed DC in 2-week intervals (according to phase-I trial),[2] and followed-up for 1 year.

Written informed consent was obtained from each subject according to the protocol of the USP Ethical Committee (reference number: 21170713.0.0000.5467).

Complete data of the trial are not yet published, however the immunization product resulted safe, with no adverse events, but with a modest effect on PVL and T cell count (yet unpublished data). Main characteristics of the participants (sex, age, time from diagnosis), PVL and T lymphocytes counts before (t0) and 1 year after the immunization (t1), as well as PVL and T lymphocytes variations after immunization (Δ) are summarized in Table 3.

Biologic samples

Peripheral blood mononuclear cells (PBMC) were isolated from fresh blood at t0 and two weeks after the third vaccine dose by

Ficoll-Paque gradient (*GE Healthcare*), following the manufacturer's protocol. PBMC were used for DNA and RNA isolation as well as for monocytes and lymphocytes obtainment for the *in vitro* assays.

Monocytes were cultured at 1.5–2 × 10^6 cells/ml in AIM-V medium (*Gibco, Thermo Fisher Scientific*) containing 50 ng/ml GM-CSF (*Cell-Genix*) and 50 ng/ml IL-4 (*Cell-Genix*) for 5 days to differentiate in DC. Lymphocytes were frozen and stored to be used for autologous DC-lymphocytes co-culture assays.

Genomic DNA and total RNA from 1 × 10^6 PBMC were isolated using Trizol® (*Invitrogen, ThermoFisher Scientific*) according to the manufacturer's instructions. Nucleic acids concentrations and purity were measured with the NanoDrop® ND-1000 spectrophotometer (*Nanodrop Technologies*). 50–100 ng of genomic DNA were used for genotyping assays. 250 ng of total RNA was used for RT-PCR.

Candidate-gene genotyping

Twenty-two polymorphisms in 13 genes involved in HIV-1 host restriction genes (*APOBEC3G, CCL4, CCL5, CCR5, CUL5, CXCR6, HLA-C, IFNG, PARD3B, PROX1, SDF-1, TRIM5, ZNRD1*) and 2 in *NOS1* and *MBL2* genes, were selected based on previously studies realized by our group in the context of phase-I DC-based HIV immunotherapy trial.[9,10] Genotyping was performed by commercially available allele-specific TaqMan assays (*Applied Biosystems, Thermo Fisher Scientific*) and qPCR on a QuantStudio 3.0 real-time PCR instrument (*Applied Biosystems)* Allelic discrimination was performed using the QuantStudio 3.0 software (*Applied Biosystems*).

RT-PCR experiments

250 ng of total RNA was reverse-transcribed using *RNAeasy ® mini kit* (*Quiagen*) and used for both customized 86-genes PCR array (*Quiagen*) and gene-specific Taqman assays (*Applied Biosystems*) in qPCR experiments.

Customized RT2 Profiler PCR array

86 genes were selected according to their reported role in protection against HIV infection and/or in immunotherapy response for the customization of a specific array (RT2 Profiler PCR Array: CAPH112563; *Qiagen*). Complete list of genes and relative references are reported in Supplementary File 3.

Gene-specific amplification was performed with the customized array and qPCR using the ABI 7300 Real-Time PCR System (*Applied Biosystems*). The SDS 2.3 software was used to obtain cycle threshold (Ct) values for relative gene expression analysis (t1 versus t0) according to Fold Change (FC) method . When FC<1, expression data are presented as Fold Regulation (FR = −1/FC) as suggested by Schmittgen and Livak.[25] We arbitrary assumed that differentially expressed genes (DEGs) have a FC>1.2 or FR <−1.2. Quality controls confirmed the lack of DNA contamination and were successfully tested for RNA quality and PCR performance. Average Ct of 4 housekeeping genes (*ACTB*, *B2M*, *GAPDH*, *HPRT1*) for each sample was used for ΔCt calculation. Standardization was applied by subtracting the mean of normalized data for a gene from each ΔCt and dividing by the respective standard deviation. All statistical analysis was performed after this step.

Gene-specific Taqman assays

Selected genes, namely *NOS1* and *MBL2* were amplified with TaqMan® gene-specific assays (*Applied Biosystems*) and qPCR using the QuantStudio 3.0 Real Time PCR equipment. The QuantStudio 3.0 software was used to obtain cycle threshold (Ct) values for relative gene expression analysis according to Fold Change (FC) method.

DC phenotypic and functional assays

DC were pulsed with autologous aldrithiol-inactivated HIV-1[26] (10^6 viral particles/30 × 10^3 DC) for 4 hours, and then treated with 50 ng/ml IL-4, 50 ng/ml GM-CSF, 50 ng/ml TNF, 10 ng/ml IL-1ß, 100 ng/ml IL-6 for 48 hours.[2]

DC maturation and activation were analyzed by the meaning of phenotypic profile (CD14, CD11c, HLA-DR, CD80, CD86, CD83, CD40, DCSIGN, CCR6) and flow cytometry (FACScalibur) *(BD Biosciences*).

Lymphocytes were cultured (2 × 10^5 cells/well) with autologous DC (lymphocytes/DC ratio: 5/1) in duplicates for 24 and 96 hours. 20μg/ml of brefeldin A (BFA; *Sigma-Aldrich*) was added 6 hours before the end of co-culture assay. Lymphocytes proliferation was measured by the mean of CSFE dilution (*Thermo Fisher Scientific*). Staphylococcal enterotoxin B (SEB; *Sigma-Aldrich*) and phytohemagglutinin (PHA; *Sigma-Aldrich*) were used as positive control of lymphocytes activation and proliferation, respectively. IFN-γ, MIP-1β, IL-2 and TNF production, and CD107a expression were measured by flow cytometry (FACScalibur) *(BD Biosciences*).

Data analysis

R-project software (www.r-project.org) was used to calculate allelic, genotypic and haplotypic frequencies, to performed multivariate analysis (general linear model/GLM) as well as for genotypes modelling ("SNPassoc" package, version 1.9-2). Multivariate analysis was used to analyze polymorphisms' association with clinical or laboratorial variables, and including sex, age and time of disease as confounder variables (adjusted p-value/p_{adj}). Formal multiple-comparison Bonferroni correction for the number of polymorphisms would require a significance threshold of p = 0.002 (p_0/n, p_0 = 0.05, n = 24 polymorphisms). Genotype modelling was performed according to three inheritance models (dominant, recessive, overdominant) for the polymorphisms' minor allele. The best inherited model was then chosen (and presented) by selecting the model with the minor value of Akaike information criterion (AIC).

Haploview software[27] was used to investigate the linkage disequilibrium (LD) pattern and for deriving the haplotypes.

Correlation between gene expression modulation (FC or FR) and clinical or laboratory variables was performed using non-parametric Spearman coefficient (r) using GraphPad Prism

software (version 7.0). Statistical significant results required Spearman correlation parameters $r^2 > 0.95$ and $p < 0.05$.

Abbreviations

DC	dendritic cells
DEGs	differential expressed genes
HIV+ individuals	HIV-1 chronically infected individuals
PBMC	peripheral blood mononuclear cells

Disclosure of potential conflicts of interest

The authors declare that they have no competing interests.

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Ethical statement

Written informed consent was obtained according to the protocol of Ethical Committee of the University of Sao Paulo (reference number 21170713.0.0000.5467).

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