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Modification of the HIV-specific CD8$^+$ T-cell response in an HIV Elite Controller after Chikungunya virus infection

Short title: HIV and CHIKV in an Elite Controller

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Abstract

Objective: To evaluate the impact of Chikungunya virus (CHIKV) infection on the quality of the HIV-specific $CD8^+$T-cell (CTL) response in an HIV Elite Controller (EC).

Design: Three blood samples were obtained from an EC at 27 days (EC-CHIKV,Sample 1, S1); 41 days (S2) and one year (S3) after CHIKV infection. Additionally, samples from other nine ECs and nine viremic Chronics were obtained.

Methods: $CD4^+$ T-cell counts, viral load (VL) and immune activation were recorded. NK cells and HIV-specific CTL quality were evaluated. Data was analysed using non-parametric statistics.

Results: A male HIV EC was confirmed for CHIKV infection. At S1, he presented 211 $CD4^+$ T-cells/μl, a HIV VL blip (145 copies/ml) and high T-cell activation. NK cell percentage and activation were higher at S2. All parameters were recovered by S3. CTLs at S1 were exclusively monofunctional with a high proportion (>80%) of degranulating CTLs. By S3, CTL

polyfunctionality was more similar to that of typical EC. The distribution of $CD8^+$T-cell memory subsets also displayed altered profiles.

Conclusions: The results showed that the phenotype and function of HIV-specific CTLs were modified in temporal association with an HIV VL blip that followed CHIKV infection. This might have help to control the transient HIV rebound. Additionally, NK cells could have been involved in this control. These results provide useful information to help understand how EC maintain their status, control HIV infection and alert about the negative impact to the immune function of HIV-infected subjects living in CHIKV endemic areas.

Keywords: HIV; Elite Controller; Chikungunya virus; CD8+ T cell response

INTRODUCTION

Chikungunya fever (CF) is caused by Chikungunya virus (CHIKV), a reemerging arthropod-borne Alphavirus. [1-3] [4]. Since the early 2000s, CF has caused immeasurable morbidity in humans. The first autochthon transmission of CHIKV in America was confirmed in 2013 [3, 5].

The acute phase of CF is characterized by high fever, polyarthralgia, myalgia, high viral load, robust innate response and concomitant abnormalities such as pronounced lymphopenia and moderate thrombocytopenia [3, 6]. *In vitro*, human epithelial and endothelial cells, primary fibroblasts, and monocyte-derived macrophages are susceptible to CHIKV infection [7]. In line with this, CHIKV was detected in the biopsies of muscle, joint, and dermis of infected human patients [8, 9]. Despite not being directly affected by the virus, $CD4^+$ T-cells were described to have a pathogenic role in CHIKV-mediated joint pathology [10]. Also, $CD4^+$ T-cells (and not $CD8^+$ T-cells) seem to be particularly affected due to increased expression of CD95 (FAS) [11]. Interestingly, a case of an opportunistic oral candidiasis in this context of CHIKV-mediated severe $CD4^+$ T-lymphocytopaenia was reported [12].

Despite substantial geographical overlap, there are very few reports that describe subjects coinfected with HIV and CHIKV [13, 14] and none in HIV-infected Elite Controllers (EC) [15, 16]. To our knowledge, we report here for the first time the course of CF in an HIV EC. We envisaged that the opportunity to identify this patient and to perform immunological studies was a unique scenario to deepen in aspects of the HIV-specific T-cell response involved in elite HIV control. Thus, the aim of the study was to evaluate the impact of the transient $CD4^+$ T-cell alteration concurrent to CF on the quality of the HIV-specific $CD8^+$T-cell response in this EC.

METHODS

EC-CHIKV is an HIV-infected EC enrolled by the time that CF was diagnosed. Three samples were obtained at 27 days (S1), 41 days (S2) and one year (S3) after probable day of CHIKV infection. Additionally, samples from nine ECs and nine HIV^+ viremic subjects (Chronics) were obtained according to inclusion criteria previously used by our group [17-19]. Plasma and peripheral blood mononuclear cells (PBMCs) were collected. HIV plasma viral load (VL) was determined by the branched-DNA method (Versant HIV-1 RNA 3.0 assay; SiemensHealthcare,UK). $CD4^+$T-cell count was determined by flow cytometry double platform (BD FACSCanto; BD Biosciences, USA). $CD4^+$ and $CD8^+$ T-cell immune activation (CD38 and HLA-DR co-expression) was determined by flow cytometry. The study was reviewed and approved by the *Comité de Ética Humana, Facultad de Medicina, Universidad de Buenos Aires*. All participants provided written informed consents. Specificity, functionality and phenotype of HIV-specific $CD8^+$ T-cells and Gp120-specific IgG concentration and gp120-specific IgA titers were assayed as previously reported [17-19]. NK cell percentage and activation were evaluated by flow cytometry using the following markers: CD69, NKp30, NKp46, CCR7, CD62L, FasL, CD38 and HLA-DR.

Data analysis was performed using GraphPad Prism 5 (GraphPad Software Inc, USA) and SPICE V5.3 software (https://exon.niaid.nih.gov/spice/). All data were analyzed using nonparametric statistics.

RESULTS

EC-CHIKV was a 46-year-old male at the time of CF. HIV infection had been diagnosed 10 years before CF. He had no history of opportunistic infection and he did not present coinfection with HCV nor HBV (vaccinated). His HLA haplotype was A11/A29, B44/B51 and DR07/DR16. His $CD4^+$T-cell count was always greater than 500 cell/µl (fluctuating around 715 cell/µl). On July 2014, EC-CHIKV travelled to Dominican Republic. He was febrile since July 12th and returned to Argentina on the following day. Three days later, he consulted to our hospital for the persistence of high fever (>39ºC), arthalgias, myalgias and maculopapular rash (face, trunk and upper limbs). Blood samples were taken for molecular assays for CHIKV and dengue virus, and for microscopic diagnosis of Malaria and all were negative. New blood sample for serological studies was then taken. The IgM was then positive for CHIKV infection. S1, S2 and S3 were subsequently taken to initiate the present study. EC-CHIKV showed an HIV VL blip by S2 (145 copies/ml). By S3, VL decreased albeit not to undetectable levels. At S1, $CD4^+$ T-cell count dropped to 211 cells/µl (14%), the lowest values ever recorded for EC-CHIKV. It recovered already by S2 and remained above 500 cells/µl by S3 (Figure 1A). At S1, EC-CHIKV presented considerable $CD4^+$ and $CD8^+$ T-cell activation but it dropped by S3 (Figure 1B). NK percentage and expression of activation and homing markers peaked at S2. Only CCR7 showed a descending pathway from S1 to S3 (Figure 1C).

The magnitude and specificity of the HIV-specific response was analyzed by ELISPOT (Figure 2A, left panel). The magnitude of the ELISPOT response increased three to four times between S1 and S2. Then, it decreased by S3, returning to S1 levels. These changes were even for the three HIV antigens tested, so no changes in immunodominance hierarchy (and presumably not in breadth) were observed. Percentages of HIV-specific $CD8^+$ T-cells observed by ICS paralleled those results obtained by ELISPOT (Figure 2A, right panel).

Then, HIV-specific $CD8^+$ T-cell polyfunctionality was evaluated (Figure 2B). As expected, typical ECs had higher percentages of polyfunctional (i.e. trifunctional and bifunctional) cells than

Chronics. Interestingly, HIV-specific $CD8^+$ T-cells at S1 and S2 were exclusively monofunctional (Figure 2B, upper panel). Of these, >80% were degranulating cells (evidenced by CD107A/B $^+$ movilization). Bifunctional cells were only detected at S3. Finally, the HIV-specific $CD8^+$ T-cell memory differentiation profile was studied. No significant differences were observed at the total $CD8^+$ T-cell subset across S1 to S3. Contrary, the HIV-compartment was severely skewed. (Figure 2C). S1, S2 and S3 showed considerably lower percentages of naïve-like T-cells compared to the median of ECs. This reduction was accompanied by higher percentages of effector memory (T_{EM}) or terminally differentiated (T_{TE}) cells as time progressed. Moreover, $T_{EM}/(T_{EM}+T_{TE})$ ratio at the HIV-specific compartment [17, 20, 21] showed a clear descending pathway from S1 to S3 (Figure 2D), depicting an alteration comparable to Chronics at the time closer to CF and returning to values below the median of ECs one year later.

Regarding gp120-specific plasma antibodies, all three samples from EC-CHIKV had elevated gp120-specific IgG concentration and IgA titers,but no difference was observed among samples (not shown). This suggested that the changes observed in the HIV-specific response after CF were specific of the cellular compartment.

DISCUSSION

CF is associated with a marked lymphopenia. Some evidence indicates that CD95-mediated apoptosis of $CD4^+$T-cells could account for at least part of this phenomenon [11, 22].The abrupt drop in $CD4^+$T-cell count may pave the way to opportunistic infections as already reported [12].This scenario could be even worst in subjects infected with HIV, who already experience HIV-mediated depletion and dysfunction of $CD4^+$ T-cells. In this line, a recent report suggested that, in coinfected subjects, there would be no worsening in either condition [14]. Contrary, another group presented evidence of a more pronounced immunosupression in coinfected subjects, compared to CHIKV-infected HIV-negative subjects [13].

Data accumulated over the past years have established that specificity, functionality and phenotype of the CD8 T-cell response plays a critical role on HIV control [23]. However, controversies still

exist regarding the causalities, i.e. if the quality of the $CD8^+$ T-cell response is the cause of virus control or if, instead, the immune exhaustion observed in viremic subjects is the result of constant antigen stimulation. The infection of an HIV EC with CHIKV prompted an unique scenario to investigate how HIV-specific $CD8^+$ T-cell response adjust in the so-far inexperienced and transient $CD4^+$T-cell depletion and how it associates with HIV control.

It has been widely documented that immunodominance of Gag-specific $CD8^+$T-cells is associated with virus control [19, 24-32]. In line with this, Gag-specific T-cells dominated the response in all three samples from EC-CHIKV, showing the pattern of a typical EC and indicating that CHIKV infection did not change specificity hierarchy. Rather, the observed picture was reflecting the expansion of specific memory cells after the re-emergence of the antigen. On the other hand, some reports suggested that $CD8^+$ T-cell polyfunctionality would be a functional correlate of virus control [33, 34]. However, later studies claimed that lack of T-cell polyfunctionality would be the consequence of constant antigen stimulation, cell exhaustion and functional impairment [19, 35, 36]. Moreover, not all the functions exerted by a polyfunctional cell would be equally relevant in mediating antiviral control. In this sense, we have previously demonstrated that the simultaneous capacity to degranulate and to secrete IFN-□was the function better related to higher $CD8^+$ T-cell antiviral activity, instead of polyfunctionality as a whole [19].The study of polyfunctionality in EC-CHIKV provided further support to this notion. In S1 and S2, the samples concurrent with the HIV VL blip, there is a dominance of monofunctional degranulating cells. This pattern is not characteristic of an EC. Instead, it could be reflecting the preferential proliferation of specific cells able to rapidly eliminate HIV-producing cells. This was followed by a partial re-gain in HIV control by S3, which, in turn, showed a $CD8^+$ T-cell polyfunctional pattern more similar to ECs. Finally, it is also known that the differentiation of total and HIV-specific $CD8^+$ T-cells into the different memory subsets is severely altered in HIV-infection but not in ECs [17, 20, 37-39]. HIV-specific $CD8^+$ T-cell maturation was severely skewed in EC-CHIKV S1 but showed an ongoing recovery towards S3. The major alteration (at S1) was concurrent with the point of lower $CD4^+$ T-cell count

and presumably sub-optimal $CD4^+$ T-cell help, which is consistent with previous publications in HIV infection [31]. However, it did not prevent the development of an effective response. Alternatively, this alteration could reflect differential half life of HIV-specific CD8+ T_{TE} cells between ECs and Chronics and across EC-CHIKV S1 to S3 [40].

On the other hand, NK cells sense CHIKV and play a key role in viral clearance [41]. In EC-CHIKV, activation of NK cells could have had a role in controlling both CHIKV and the HIV rebound contributing to its control.

One limitation of this study is the lack of pre-CF sample. This precludes drawing definite conclusion about the evolution of immune responses before, during and after the co-infection. However, EC-CHIKV restores characteristic of a typical EC by S3 which allows to postulate that the alterations observed at S1 and S2 are the consequence of the immunological modifications introduced by CHIKV infection.

In sum, here we report about the dynamics in HIV-specific $CD8^+$ T-cell responses after a transitory alteration of the $CD4^+$ T-cell subset in an HIV EC. Results indicate that, in this subject, the $CD8^+$ T-cell response dynamically reshaped in order to control HIV replication after CHIKV infection. Also, our results alert on the detrimental immune consequences that CHIKV could cause in HIV-subjects with already low $CD4^+$ T-cell counts. Thus, close monitoring of HIV^+ subjects during CF for the possible development of opportunistic infection should be considered. Also, HIV-positive travelers to CHIKV endemic areas should be alerted of the risk of a possible worsening of their immunosupression.

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YG, GT and NL conceived the study. NL, YM, PP and OS enrolled participants. YG, MJR, JS and CT performed experiments. YG, MJR, GT and NL analyzed the data. YG, GT and NL drafted the report. All authors provided input into the report and approved the final version.

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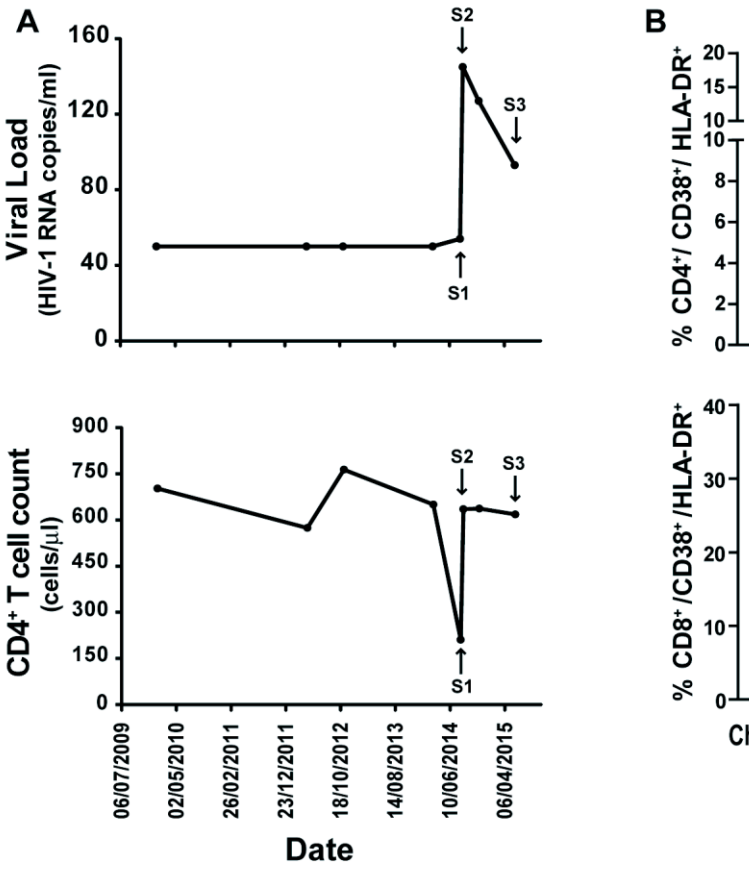
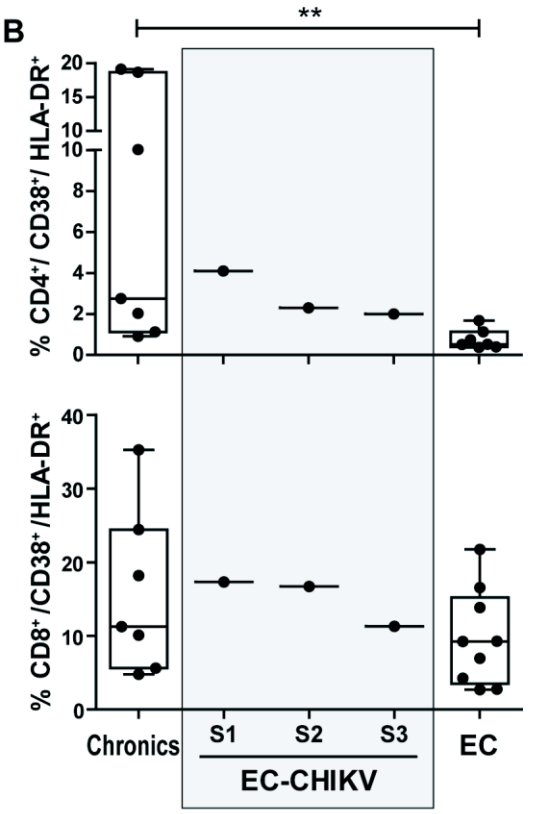
FIGURE LEGEND

Figure 1: HIV Viral load (VL), $CD4^+$T-cell count and immune activation corresponding to EC-CHIKV. A. Longitudinal progression of VL (upper panel) and $CD4^+$T-cell count (lower panel) in EC-CHIKV. Data depicts the period between September 2009 and July 2015. Time-points when S1, S2 and S3 were obtained are highlighted. **B.** Immune activation in Chronics, ECs and the three longitudinal samples from EC-CHIKV, evaluated as the percentage of $CD38^+HLA\text{-}DR^+$ $CD4^+$ (upper panel) and $CD8^+$ (lower panel) T-cells. For Chronics and ECs, data is presented as overlaid box-and-whisker and dot plots. Horizontal lines stand for median values. For samples corresponding to EC-CHIKV (S1, S2 and S3) data is presented as one dot representing the media of triplicates. P value was calculated using Mann-Whitney test. Asterisks denote P value <0.01. **C**. NK cell ($CD3^-CD56^+$ cells) expression of activation and homing markers CD69, NKp30, NKp46, CCR7, CD62L, FasL, CD38 and HLA-DR. Each line represent the expression of one marker across S1 to S3.





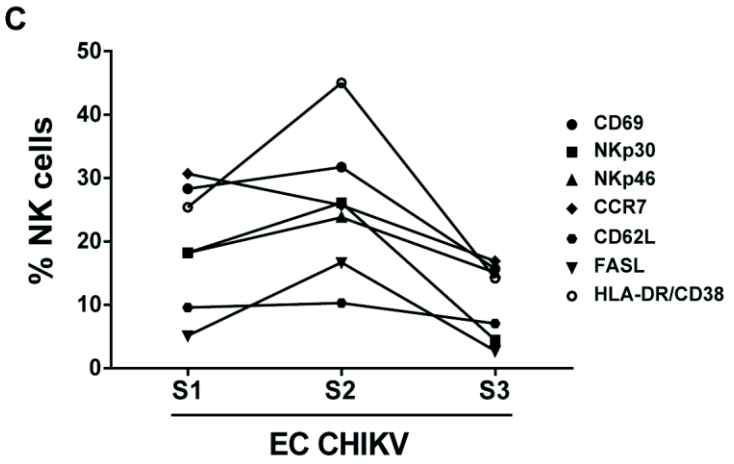


Figure 2: Specificity, functionality and memory phenotype of HIV-specific immune response in EC-CHIKV. A. Magnitude of total anti-Nef, anti-Gag, and anti-Env T-cell responses evaluated by Elispot and expressed as SFU/10^6PBMCs (left panel), or by intracellular staining(ICS) expressed as the proportions out of total $CD8^+$T-cells (right panel). In the latter, specific cells were added-up regardless its specific function. Bars represent the media of triplicates. **B.** Polyfunctionality of the HIV-specific $CD8^+$T-cells in EC-CHIKV, ECs and Chronics (CH). Pies (upper panel) represent the media proportion of cells expressing one (white), two (gray), or three (black) functions, independently of any particular function in ECs, Chronics and the three longitudinal samples from EC-CHIKV. Bar chart (lower panel) depicts the decomposition of the response into the different functional profiles. Black dots represent the percentage out of the total specific $CD8^+$T-cells expressing the particular function or combination of functions indicated on the x-axis, for each subject. Chronics N=7 (17 specific responses corresponding to different stimuli for each subject). ECs N=7 (nine specific responses). In the case of EC-CHIKV S1, S2 and S3, each dot represents different stimuli for the corresponding sample. Boxes represent IQR. Data correspond to background-subtracted results using the CD28/49d control. Values corresponding to bi- and monofunctional cells were corrected by subtracting the values corresponding to triple-positive events and double- and triple-positive events, respectively. In addition to black dots, box-and-whisker plot are shown. All combinations of one (white), two (gray) or three (black) functions are indicated matching the colour code used in pie charts. **C.** Distribution of memory sub-populations within HIV-specific $CD8^+$ T-cells. Proportion of $CD8^+$ T naïve-like cells ($CCR7^+/CD45RO^-$); central memory T-cells ($CCR7^+/CD45RO^+$); effector memory T-cells (EM, $CCR7^-/CD45RO^+$) and terminal effector T-cells (TE, $CCR7^-/CD45RO^-$) corresponding to ECs, Chronics(CH) and the three longitudinal samples from EC-CHIKV, are shown in pie and bar charts (upper and lower panel, respectively). Colour code indicated in the bar chart for each population matches the pie chart. In the bar chart, black dots represent the percentage of a given subset out of the total specific $CD8^+$T-cells. In addition to black dots, boxes depicting IQRs are shown. Chronics N=7 (13 specific

responses corresponding to different stimuli for each subject). ECs N=7 (17 specific responses). In the case of EC-CHIKV S1, S2 and S3, each dot represents different stimuli for the corresponding sample. **D.** HIV-specific $T_{EM}/(T_{EM}+T_{TE})$ ratio in ECs, Chronics and the three longitudinal samples from EC-CHIKV. In the case of EC-CHIKV S1 S2 and S3, individual data points corresponding to different stimuli to identify specific cells were used to construct the plot. Box-and-whisker plots depict the median (horizontal line) and error bars extend from min to max. P value was calculated using Mann-Whitney test. Asterisk denotes P value <0.05.