

Distinct antibody profiles in *HLA-B*57⁺*, *HLA-B*57⁻* HIV controllers and chronic progressors

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Objective: Spontaneous control of HIV replication without treatment in HIV-1 controllers (HICs) was associated with the development of an efficient T-cell response. In addition, increasing data suggest that the humoral response participates in viral clearance.

Design: In-depth characterization of Ab response in HICs may help to define new parameters associated with this control.

Methods: We assessed the levels of total and HIV-specific IgA and IgG subtypes induction and their functional potencies – that is, neutralization, phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), according to the individual's major histocompatibility complex class I (HLA)-B*57 status, and compared it with nontreated chronic progressors.

Results: We found that despite an undetectable viral load, HICs displayed HIV-specific IgG levels similar to those of chronic progressors. Interestingly, our compelling multifunctional analysis demonstrates that the functional Ab profile, by itself, allowed to discriminate *HLA-B*57⁺* HICs from *HLA-B*57⁻* HICs and chronic progressors.

Conclusion: These results show that HICs display a particular HIV-specific antibody (Ab) profile that may participate in HIV control and emphasize the relevance of multifunctional Ab response analysis in future Ab-driven vaccine studies.

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Introduction

A rare fraction (<1%) of HIV-infected individuals is able to spontaneously control the viral replication without

combined antiretroviral therapy [1–3]. These individuals, defined as HIV controllers (HICs), provide a unique opportunity for identifying the parameters associated with this control.

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Different cellular or humoral responses may participate in the control of the infection observed in these individuals. Indeed, most HICs display potent cytotoxic CD8 T-cell responses against HIV-infected cells [4,5]. These strong cytotoxic responses are often associated with the expression of the major histocompatibility complex class I allele *HLA-B*57* [1,4,6]. Another study suggested that helper T-cell responses may also contribute to the control of HIV, as HICs' HIV-specific CD4⁺ T-cells express high avidity T-cell receptors [7]. This efficient T-cell response may promote an adequate B-cell response. Indeed, HIV-specific B-cell responses were found to be preserved in HICs [8,9], with a particular B-cell response in *HLA-B*57*⁺ HICs [9].

The triggering of B-cell responses induces an HIV-specific Ab response. This anti-HIV Ab response is mainly of the IgG1 subtype, with variable proportions of other isotypes depending on individuals, HLA status, and clinical parameters [10–13]. In HICs' sera, IgG1s and IgG3s are enriched [13,14]. Others associated anti-HIV IgG2s with long-term nonprogression [15,16] and particularly in the *HLA-B*57*[−] HIC subgroup [12]. These studies highlight the potential roles of Ig isotypes that may depend on the *HLA-B*57* status of HICs.

Concerning Ab function, controversial results on neutralizing activities were published, some showing low cross-neutralizing activities, whereas others reporting higher neutralizing activities in HICs [17–23]. In fact, HICs may represent a heterogeneous group with some individuals controlling their viral load by an efficient Ab response [17–20,24]. The participation of nonneutralizing Ab responses in HIV control was also reported. ADCC levels were shown to be higher in sera from HICs [19] and more particularly in the *HLA-B*57*[−] HIC subgroup [25]. Others found that HICs display multiple effector functions associating antibody-dependent cell-mediated phagocytosis (ADCP), Fc binding, and complement cascade activation, in addition to ADCC activities [13].

In this study, we analyzed the complete Ab profile, that is, the levels of total and HIV-specific IgAs and IgG subtypes in parallel to functional activities: neutralization, phagocytosis, and ADCC. The results were analyzed according to HICs' *HLA-B*57* status and compared with a cohort of nontreated CPs. We found that Ab functions (neutralization versus ADCC) allow to discriminate *HLA-B*57*⁺ HICs, *HLA-B*57*[−] HICs and chronic progressors. The peculiar Ab response induced in HICs may have relevance for future Ab-driven vaccine design.

Materials and methods

Cohorts and samples

The clinical and epidemiological characteristics of the HICs and chronic progressors cohorts are shown in the

Supplemental Table 1, <http://links.lww.com/QAD/C317>.

HICs ($n=36$) were recruited from the CO21 CODEX cohort implemented by the ANRS (Agence nationale de recherches sur le SIDA et les hépatites virales). HICs were defined as HIV-infected individuals maintaining viral loads under 400 copies of HIV RNA/ml without combined antiretroviral therapy for more than 5 years. HICs were divided into two groups: *HLA-B*57*⁺ (*B*57*⁺ HICs, $n=17$) or *HLA-B*57*[−] (*B*57*[−] HICs, $n=19$).

Nontreated, HIV-infected chronic progressors ($n=16$) were recruited from the EP14 cohort, a historic cohort established in 2000, when starting combined antiretroviral therapy immediately after HIV detection was not yet widely practiced. These individuals infected with a median of 8 years had CD4⁺ T-cells levels (median of 696 μl^{-1} at the time of blood sampling) statistically similar to that of HICs (862 and 723 μl^{-1} , for *B*57*⁺ and *B*57*[−] HICs with $P=0.1$ and 0.5 compared with chronic progressors, respectively). However, these patients had significantly higher viral load compared with HICs ($P=0.0003$), failed to control their viremia, and went on therapy shortly after sample collection because of disease progression.

For total IgG and IgA quantification, sera from uninfected individuals (preimmune bleeds of volunteers further enrolled in vaccine trial, $n=30$) were used as HIV-uninfected control.

Total and HIV-specific Ig detection by ELISA

ELISA for total and specific Abs were performed as previously described [9]. For the detection of Env-specific Igs, gp140Yu2b [26] or gp41S30 [27] was used at 5 $\mu\text{g}/\text{ml}$. The concentrations of total Igs were calculated using reference calibrators present as supplier reagent in the IgG and IgA detection kits NK004.S and NK010.S respectively (The Binding Site, Birmingham, UK) according to the international serum protein reference material CRM470. The ratios of HIV-specific Igs to total Igs were calculated using this formula:

$$OD \text{ ratio} = \frac{OD \text{ of } anti - gp41 \text{ or } anti - gp140 \text{ Igs} * \text{serum dilution}}{OD \text{ of } total \text{ Igs} * \text{serum dilution}}$$

Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood received from healthy volunteers at the Blood Transfusion Center (EFS) in Strasbourg using Ficoll-Hypaque sedimentation. Monocytes were isolated from the PBMCs with anti-CD14 beads (Miltenyi Biotec, Paris, France) and differentiated to monocyte-derived macrophages (M_{DM}) by culturing for 5 days with GM-CSF (100 U/ml) (R&D Systems,

Minneapolis, Minnesota, USA). CEM.NKR.CCR5 cell line was kindly provided by G. Ferrari (Duke University, Durham, North Carolina, USA).

TZM-bl neutralization assays

The conventional TZM-bl neutralization assays were used as previously described [28]. Serial dilution of sera (beginning at 1 : 20 dilution) were tested on two tier-1, 15 tier -2 and one tier-3 (Supplemental Table 2, <http://links.lww.com/QAD/C317>), and inhibitory reciprocal dilution 50% (IRD50) was calculated as described [28]. The capacity of individuals' sera to neutralize murine leukemia virus (MuLV) was assessed as control. The inhibitory reciprocal dilution 50% (IRD50) were defined as the reciprocal sample dilution leading to a 50% reduction in relative luminescence units (RLU). IRD50 more than 20 is recorded as positive neutralization as compared to serum from HIV-negative individuals.

Neutralization and phagocytosis assay

This assay analyzes a combination of neutralizing activity and phagocytosis [29]. Serial dilution of sera (beginning at 1 : 4) or purified IgG (beginning at 200 µg/ml) were tested for their ability to inhibit HIV-1 (neutralization and/or phagocytosis) using MdMs as target cells as previously described [29]. MdMs were infected with SF162.LS. After 36 h, the MdMs were stained for intracellular p24 (KC57-RD1-FITC; Beckman Coulter, Sinsheim, Germany).

The IRD80 were defined as the reciprocal sample dilution leading to an 80% reduction in the percentage of infected cells. IRD80 more than 20 was considered as inhibitory activity.

Luciferase antibody-dependent cellular cytotoxicity assay

The ADCC assay was performed using purified PBMCs as effector cells and the CEM.NKR.CCR5 cell line was infected with 6 HIV-1 expressing the viral envelope and the Renilla luciferase reporter gene (Supplemental Table 2, <http://links.lww.com/QAD/C317>) as previously described [30].

Briefly, after 4 days of infection, 12 500 HIV-1 infected CEM.NKR.CCR5 cells were plated in 12.5 µl of RPMI-1640, 10% FCS, and 50 IU interleukin-2 (R&D Systems) in a U-bottom 96-well plate and mixed with 375 500 PBMCs in 12.5 µl of the same medium (30/1 effector/target ratio). Twenty-five microliter of serial fold diluted samples (beginning at 1/50 for sera or and at 200 µg/ml for purified IgGs) were added. Four hours later, 25 µl of 60 µmol/l Viviren substrate (Promega) was added to determine the luminescence intensity generated by target cells not lysed by ADCC-mediating Abs present in the patient's samples. The percentage of target cell lysis

was normalized to control wells without Abs using the following formula:

$$\% \text{ of lysis} = \left(\frac{\text{RLU of infected target cells with effectors} - \text{RLU of infected target cells with effectors and samples}}{\text{RLU of infected target cells with effectors}} \right) * 100$$

The AUC was calculated as the integrated background-subtracted net activity over a range of dilutions using the trapezoidal method and was truncated above zero.

Radar charts

The radar chart shows a descriptive representation of the Ab profiles from HICs and chronic progressors. For each group, the median statistic was used as summary value. The radar chart was drawn using the *fmsd* R library (<https://cran.r-project.org/web/packages/fmsb>).

Partial least-squares discriminant analysis

In order to identify the Ab features that could potentially explain the differences between the different patients' groups, we performed a discriminant analysis using PLS-DA [31] (partial least-squares discriminant analysis), a multivariate dimensionality-reduction tool.

The graphical representation of the results obtained with PLS-DA are shown with the profiles that could explain the separation, ordered from the most important one at the bottom to the least important one at the top. The contribution of each feature is represented as a bar plot in which each bar length corresponds to the loading weight (importance) of the feature. The loading weight can be positive or negative. These features that are supposed to explain the separation were selected using sPLS-DA (sparsified partial least-squares discriminant analysis) [32]. For accurate estimation, we used a five-fold cross-validation scheme repeated 50 times leading to a global classification error rate of 15%.

Statistical analysis

The statistical significance (*P*-values) was determined using a two-tailed Mann-Whitney test (**P* < 0.05, ***P* < 0.01, ****P* < 0.001) using Prism software (GraphPad).

The associations between continuous variables were evaluated using a Spearman rank order correlation test using 'Visualization of a Correlation Matrix' R package version 0.77 (<https://CRAN.R-project.org/package=corrplot>).

Ethics approval and consent to participate

All individuals provided their written informed consent to give their blood sample for research purposes. The CO21 CODEX cohort and this sub-study were funded

and sponsored by ANRS and approved by the Ile de France VII Ethics Committee under number 11-33. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Results

Similar levels of anti-HIV Igs are induced in HIV-1 controllers and chronic progressors

Immunoglobulin (Ig) class switching is essential for an efficient functional Ab response. We therefore measured total (Supplemental Fig 1, brown dots, <http://links.lww.com/QAD/C317>) and HIV-specific (Fig. 1, brown dots) anti-gp41 and anti-gp140 IgAs, IgGs, IgG2s, and IgG3s in sera of HICs. Historical sera from a cohort of chronic progressors [33] ($n=16$) who were not treated were analyzed in parallel. This chronic progressors cohort, despite a high CD4⁺ level (median 696 cells/ μ l, $P=0.2$ compared with HIC), displayed a significantly higher viral load ($P=0.0003$) (Supplemental Table 1, <http://links.lww.com/QAD/C317>), failed to control their viremia, and went on therapy shortly after sample collection because of disease progression. This chronic progressors cohort is therefore a very interesting group of patients for comparison with the controller status, as their specific Ab response will be analyzed before the profound immunosuppression linked with CD4⁺ cell count decrease.

We found that total IgG concentrations were significantly increased in chronic progressors (mean 39 mg/ml) compared with HICs (mean 30 mg/ml, $P=0.02$) (Supplemental Fig 1, <http://links.lww.com/QAD/C317>). This hypergammaglobulinemia is likely driven by IgG1 isotype as total IgG2 and IgG3 isotypes did not differ between the different groups (Supplemental Fig 1, <http://links.lww.com/QAD/C317>). We further subdivided the HICs group according to their *HLA-B*57* allele: either positive (*HLA-B*57*⁺: *B*57*⁺ HICs, purple dots) or negative (*HLA-B*57*⁻: *B*57*⁻ HICs, orange dots). The increased IgG concentration was mainly observed in *B*57*⁺ HICs (mean 34 mg/ml). By contrast, total IgA concentrations were similar between HICs and CPs ($P=0.1$), or between *B*57*⁺ and *B*57*⁻ HICs ($P=0.6$) (Supplemental Fig 1, <http://links.lww.com/QAD/C317>).

Despite HICs' undetectable viral loads, we were unable to find statistically significant differences in the induction of HIV-specific IgAs, IgGs, IgG2s, or IgG3s between HICs and chronic progressors (Fig. 1). When analyzing the *B*57* subgroups, only *B*57*⁻ HICs had a significantly lower ratio of anti-gp140 IgAs to total IgA than chronic progressors ($P=0.03$) (Fig. 1a).

Although we found lower HIV-specific IgAs in *B*57*⁻ HICs and lower levels of total IgGs in HICs, the overall

HIV-specific Ab response detected in HICs was very similar to that in chronic progressors.

HIV-1 controllers induce low neutralizing activities against HIV strains

Next, the ability of the sera from HICs and chronic progressors to neutralize various HIV-1 strains was determined. In this experiment, we used two reference (tier-1) strains, 15 difficult-to-neutralize tier-2 strains (including six T/F strains) and one highly difficult-to-neutralize tier-3 T/F strain (Fig. 2).

As expected, the overall median neutralizing activity was significantly lower in HIC patients (median IRD50 = 1) than in chronic progressors (median IRD50 = 28) ($P < 0.0001$, not shown). However, for seven out of 18 strains (REJO, CH058, CH077, YU2, QH0, X1632, and CH119) (Fig. 2a), the neutralizing activity was comparable indicating that functional Ab neutralizing response was somehow maintained in HICs for these particular strains. These neutralizing activities were not specifically assigned to a subgroup of *B*57*⁺ or *B*57*⁻ HICs (Fig. 2b).

Moreover, chronic progressors demonstrated broader neutralizing activities compared with HICs with 81% of chronic progressors compared with about 50% of HICs displaying neutralizing activities against 40% of the total strains or tier-2 strains analyzed (Fig. 2).

These results show that, although the overall neutralizing response is lower than in chronic progressors, HICs have developed and maintain a neutralizing response against some HIV-1 tier-2 strains.

High antibody-dependent cellular cytotoxicity activities are detected in HIV-1 controllers

As the Fc-mediated inhibitory activities may participate in the overall control of HIV replication, we analyzed the ADCC and phagocytosis activities with assays using viruses and infected cells. The ADCC assay was performed on six different HIV-1 tier-2 strains and the phagocytosis activity was determined concomitantly to neutralization by analyzing the inhibition of the HIV-1 SF162 strain replication on primary monocyte-derived macrophages (M ϕ M) [29] (Fig. 3).

ADCC activities were detected in all HICs. The median ADCC activities in HICs was significantly higher for two out of six viruses (X1632: $P=0.02$) and CE1176: $P=0.004$), compared with chronic progressors (Fig. 3a). When HICs were separated according to their *HLA-B*57* status, the median ADCC activity was significantly higher for three viruses in *B*57*⁺ HICs, than in chronic progressors (Fig. 3b). *B*57*⁺ HICs also demonstrated increased ADCC activity compared with *B*57*⁻ HICs on the six tested strains with a significant increase for the virus TRO11 and CH119 (Fig. 3b). These results indicate

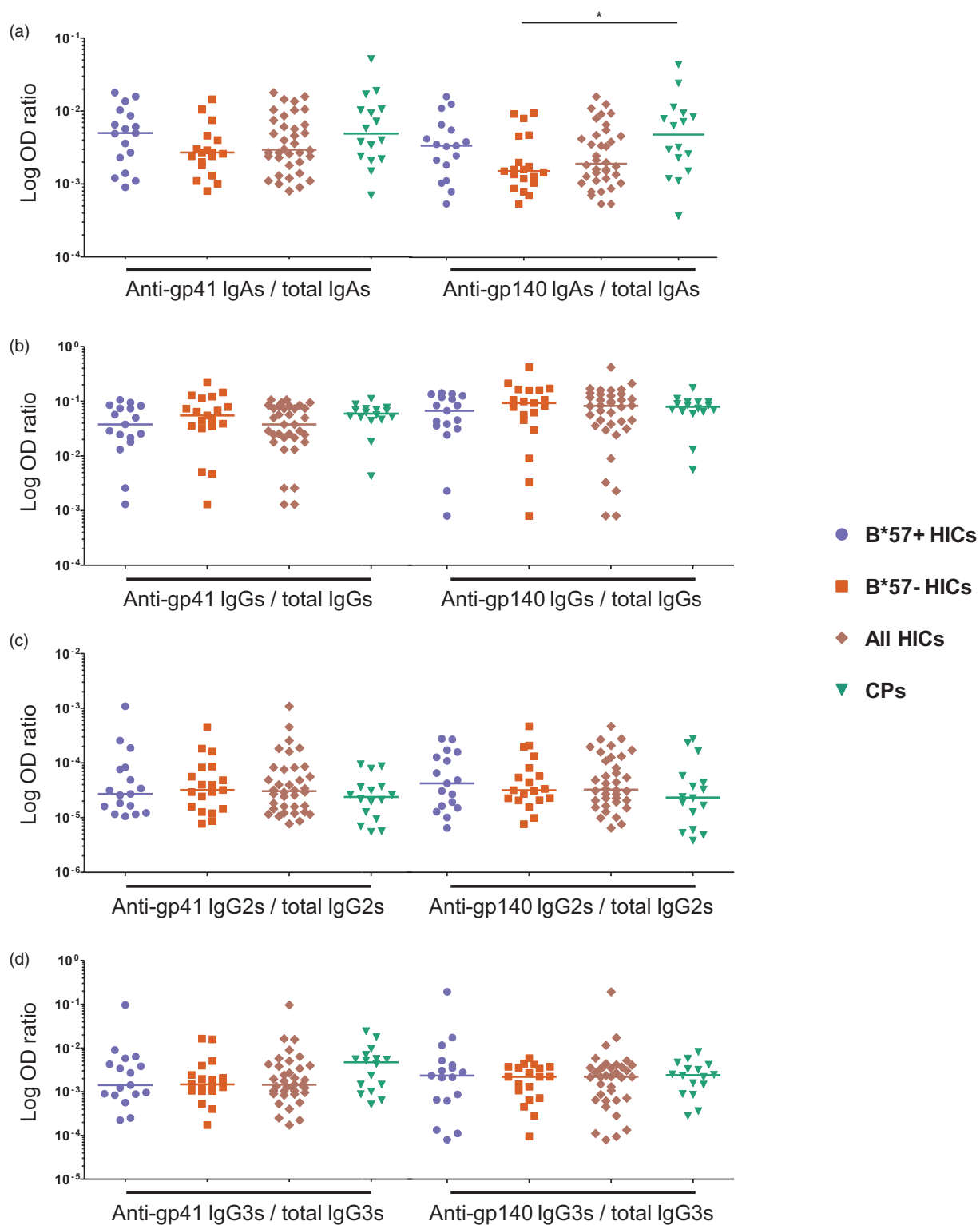


Fig. 1. Similar levels of anti-HIV Igs are induced in HICs and CPs. (a) Optical density (OD) ratio (Log₁₀) of anti-gp41 IgAs (left panel) or anti-gp140 IgAs (right panel) to total IgAs for B*57+ HICs ($n = 17$, purple circles), B*57- HICs ($n = 19$, orange squares), all HICs ($n = 36$, brown diamond-shaped) and CPs ($n = 16$, green triangles). (b) Same for IgGs, (c) IgG2s and (d) IgG3s. The statistical significance is indicated (*: $P < 0.05$). The horizontal bars represent the median of OD ratios for each patient group.

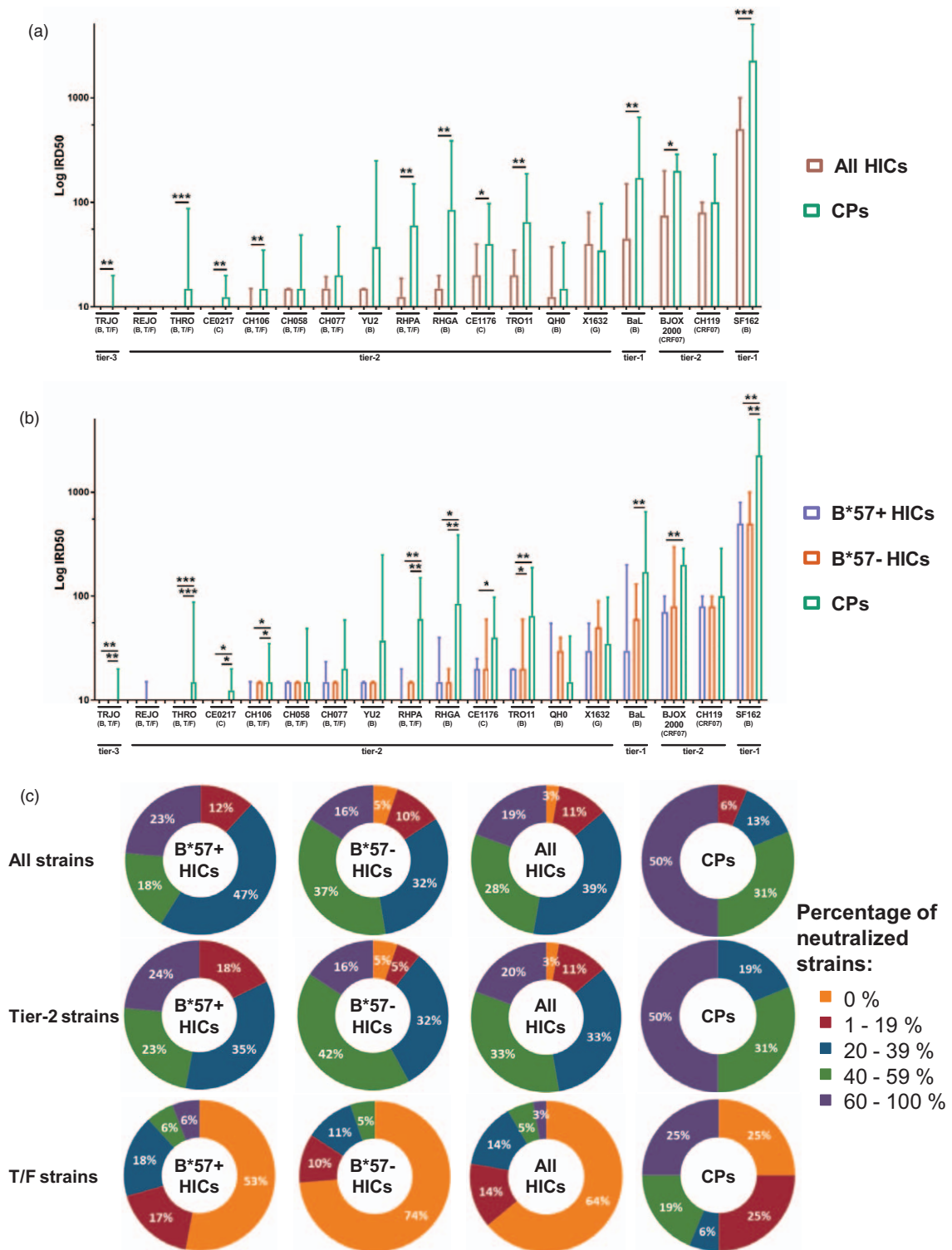


Fig. 2. Neutralizing activities against tier-2 strains are detected in HICs. Sera from B*57+ HICs ($n = 17$), B*57- HICs ($n = 19$) and CPs ($n = 16$) were tested against 18 HIV-1 strains. CH058, CH077, CH106, RHPA, THRO4156.18, REJO4541.67 and TRJO4551.58 are T/F viruses. (a) The median neutralization IRD50s with interquartile ranges are represented for all HICs (brown) and CPs (green). (b) Same for B*57+ HICs (purple), B*57- HICs (orange) and CPs (green). The statistical significances are indicated ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). (c) The neutralization breadth is expressed as the percentage of patients indicated in white in each colored section able to neutralize 0%, 1 to 19%, 20 to 39%, 40 to 59% and 60 to 100% of all strains (upper panel), tier-2 strains (middle panel) and T/F strains (lower panel) for B*57+ HICs, B*57- HICs, all HICs and CPs.

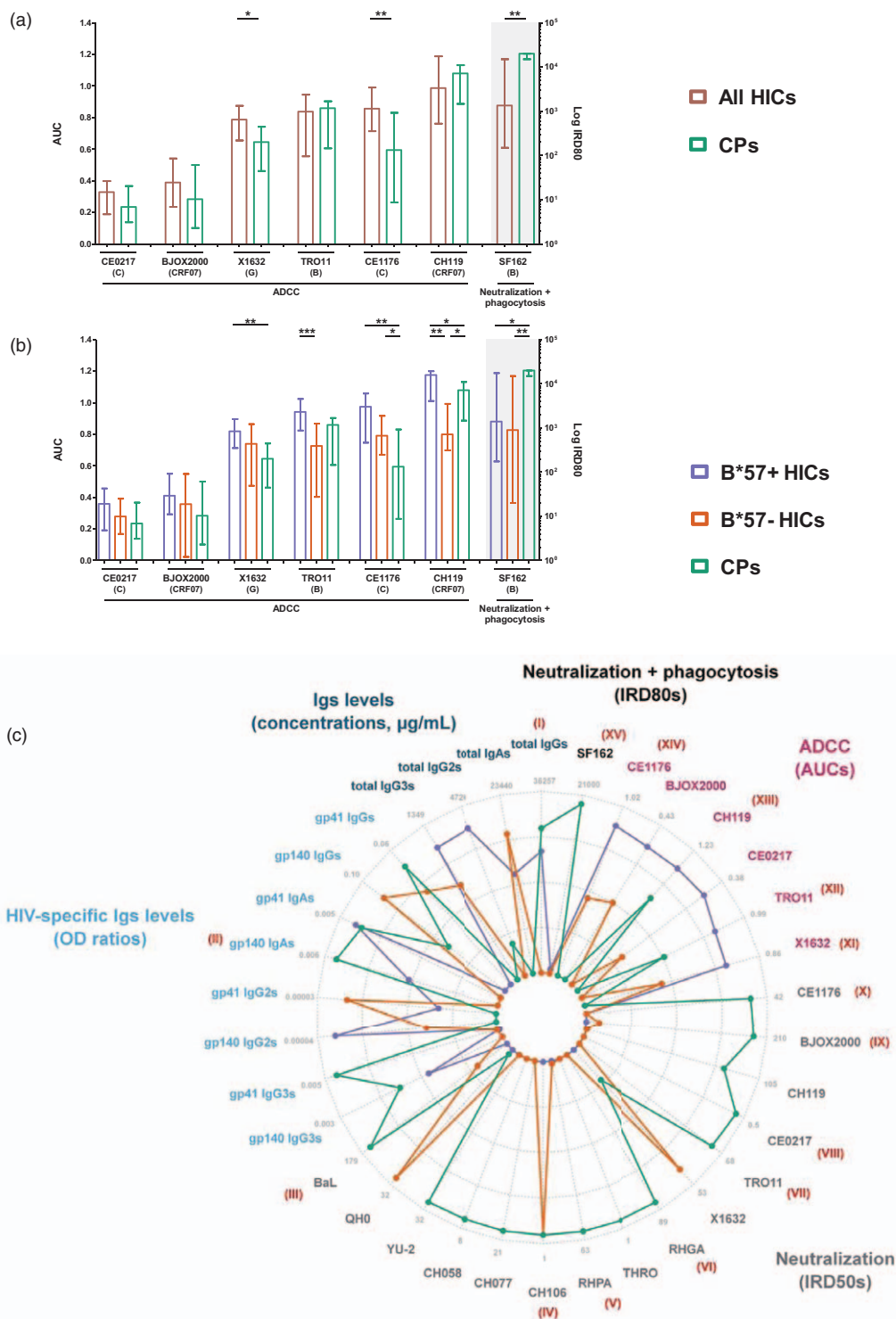


Fig. 3. HICs have stronger ADCC activities than CPs. Sera from B*57+ HICs ($n = 17$), B*57- HICs ($n = 19$) and CPs ($n = 16$) were tested against 6 HIV-1 LucR strains for ADCC and 1 HIV-1 strain for neutralization + phagocytosis activities. (a) The median ADCC AUC with interquartile ranges are represented on the left axis and the median neutralization + phagocytosis IRD80 with interquartile ranges are represented on the right axis (Log₁₀) for all HICs (brown) and CPs (green). (b) Same for B*57+ HICs (purple), B*57- HICs (orange) and CPs (green). (c) Radar plot illustrates the median Ig isotype levels (concentrations, dark blue), the median HIV-specific Ig isotype levels (OD ratios, light blue), the median neutralization IRD50 against 18 HIV-1 strains (grey), the median ADCC AUC against 6 HIV-1 strains (pink) and the median neutralization + phagocytosis IRD80s against 1 HIV-1 strain (black). The red Roman numerals (I to XV) indicate the statistically different median values.

that the increased ADCC activity in HICs is virus-dependent and mainly driven by B^*57^+ HICs.

In contrast to what was observed with ADCC, HICs presented significantly lower IRD80s on infected macrophages compared with chronic progressors ($P=0.002$) (Fig. 3a). This decrease was also observed when HICs were separated in B^*57^+ and B^*57^- HICs ($P=0.01$ and $P=0.003$, respectively) (Fig. 3b). Considering that this assay records a combination of both neutralization and phagocytosis activities [29] and that the median neutralization of the SF162 strain was decreased in HICs, the decreased phagocytosis activity observed in HICs may potentially be attributed to a decreased neutralizing activity.

Distinct antibody profiles in HIV-1 controllers and chronic progressors

The study of the different Ab features gave an extensive profile for B^*57^+ HICs, B^*57^- HICs, and chronic progressors cohorts. The radar plot in Fig. 3c, showing the median values of each Ab feature, highlights the difference between the different patient groups. For this representation, we excluded the high median neutralization values of SF162 to avoid constriction of all other values. Significant median differences between groups (annotated by red Roman numbers I–XV) were recorded mainly for neutralization of tier-2 strains, phagocytosis, and ADCC, highlighting the essential role of Ab functions for the discrimination of these groups.

Induction of HIV-specific antibody isotypes correlates with functional activities in B^*57^+ HIV-1 controllers

To further characterize the different Ab profiles in the three cohorts of patients, we searched for potential correlations between the different analyzed parameters (Fig. 4). Correlations (blue) or inverse correlation (red) were represented by color-coded intensities.

The general correlation pattern only differed slightly between HICs and chronic progressors (Fig. 4 a,b). There was no correlation between HIV-specific Ab detection and functional activities (neutralization or ADCC). However, the levels of total IgGs positively correlated with neutralization in the cohort of chronic progressors (Fig. 4b, blue rectangle) and this correlation was not found in HICs. Moreover, all the neutralizing activities highly correlated with each other in chronic progressors (Fig. 4b, blue triangle), suggesting that the total IgG response induced by the high viral loads typically detected in these patients is overall beneficial for the development of an efficient neutralizing response. Interestingly, ADCC and phagocytosis activities did not correlate with neutralization in HICs (Fig. 4a) and even correlated negatively in chronic progressors (Fig. 4b, red rectangle). This highlights the distinct skills for Fc-mediated Ab functions as compared with classical Ab neutralization.

More surprisingly, we found very distinct Ab correlations when comparing B^*57^+ and B^*57^- HICs (Fig. 4c, blue triangle and rectangle; Fig. 4d, light colored dots in green triangles and rectangles). The correlation between HIV-specific Ab isotypes and Ab function, exclusively observed in B^*57^+ HICs, suggests that preservation of the HIV-specific B cell response observed in B^*57^+ HICs may lead to particular HIV-specific isotype commutation associated with efficient Ab function [9]. In B^*57^- HICs, the nonstatistical decrease of total IgG2s detected (Supplemental Fig 1, <http://links.lww.com/QAD/C317>) conveyed a strong negative correlation (dark red dots) between this subtype and the other HIV-specific isotypes or the neutralizing activities (Fig. 4d, red rectangle). This inverse correlation raises the question of the role of IgG2s in this group of patients. These results highlight the different Ab profiles of HICs according to *HLA-B*57* allotype.

The antibody profile discriminates HIV-1 controllers from chronic progressors and B^*57^+ HIV-1 controllers from B^*57^- HIV-1 controllers

To further discriminate Ab profile between these different groups, we generated a bi-dimensional representation of multicomponent characteristics of their Abs using the partial least-squares discriminant analysis (PLS-DA) model. We found that HICs clustered in a different area than CPs (Fig. 5a) with the strongest correlate of distinction for neutralizing activities ($P=0.007$ for virus RHGA) (Fig. 5a). Noteworthy, samples from patients CP4 and CP8 clustered with HICs. These chronic progressors differ from other chronic progressors by their early time of sample collection after infection. However, whether the time of infection plays a role in this clustering will need additional investigations. In addition, B^*57^+ HICs clustered in a different area than B^*57^- HICs (Fig. 5b), with the strongest correlates of distinction for ADCC activities (Fig. 5b). These three cohorts, that is, B^*57^+ HICs, B^*57^- HICs, and chronic progressors, clustered separately, based only on their Ab profile (Supplemental Fig 2, <http://links.lww.com/QAD/C317>). These data clearly demonstrate that in each group, chronic progressors versus HICs and B^*57^+ HICs versus B^*57^- HICs have developed a specific HIV-specific Ab profile that allows to discriminate them based solely on the Abs they have induced.

Discussion

In this study, we performed an analysis of the Ab profile, assessing Ab types and isotypes, neutralization and Fc-mediated functions found in a large cohort of HICs and compared these characteristics to those found in patients who do progress to disease. By correlating these different parameters, we demonstrate that HICs can be differentiated from chronic progressors by their functional Ab

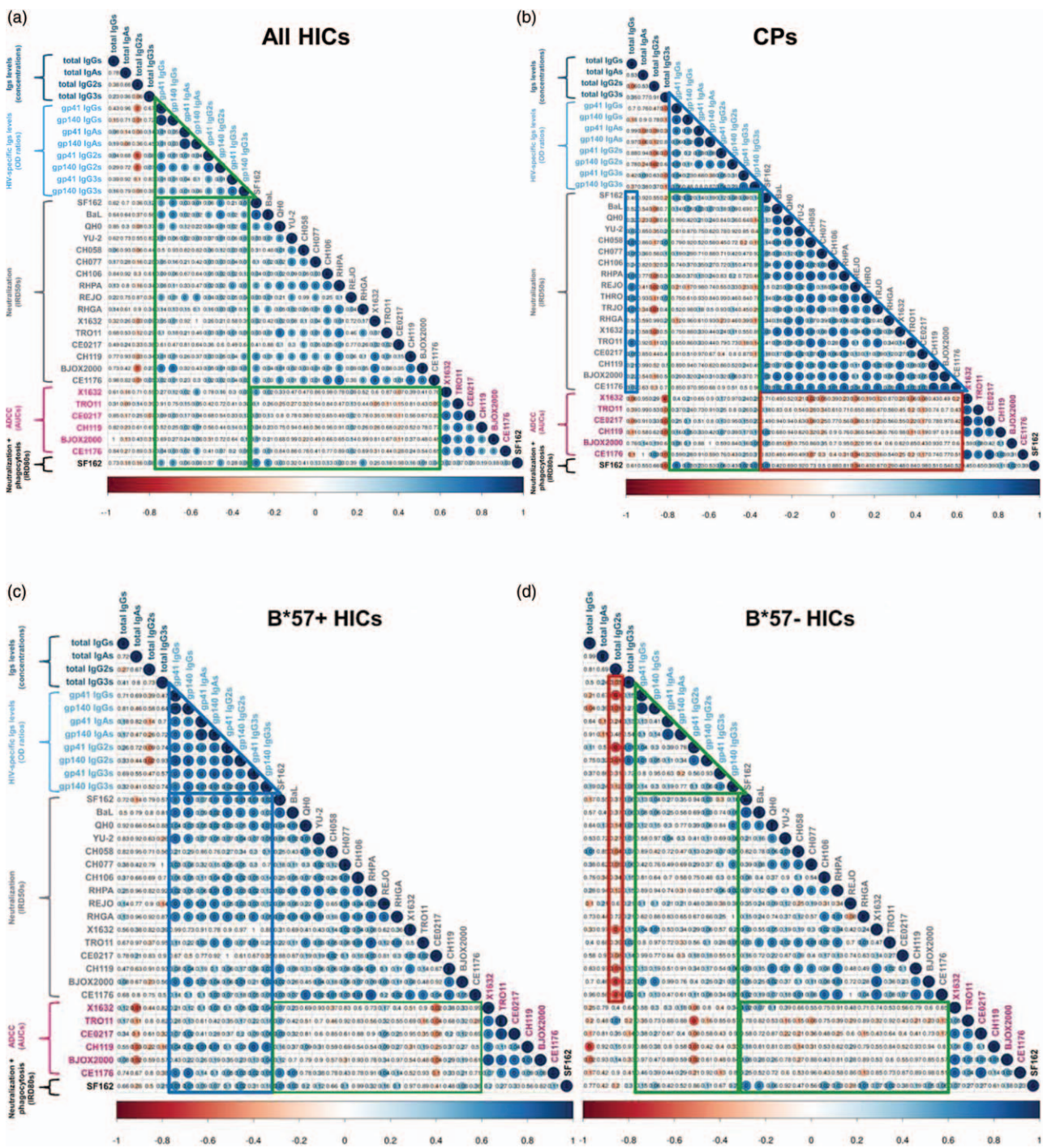


Fig. 4. Correlation between HIV-specific Ab isotypes and functional activities in B*57+ HICs. Spearman correlation matrices between the concentrations of various total Ig isotypes (dark blue), the levels of various HIV-specific Ig isotypes (OD ratios, light blue), the neutralization efficacy (IRD50s, grey) against 18 HIV-1 strains, the ADCC activity (AUCs, pink) against 6 HIV-1 strains and the neutralization plus phagocytosis activity (IRD80s, black) against 1 HIV-1 strain for (a) all HICs, (b) CPs, (c) HLA-B*57+ HICs (B*57+ HICs) and (d) HLA-B*57- HICs (B*57- HICs). Strength and significance are represented as size and color intensity: blue for positive correlation and red for negative correlation. p-values are indicated in the matrices.

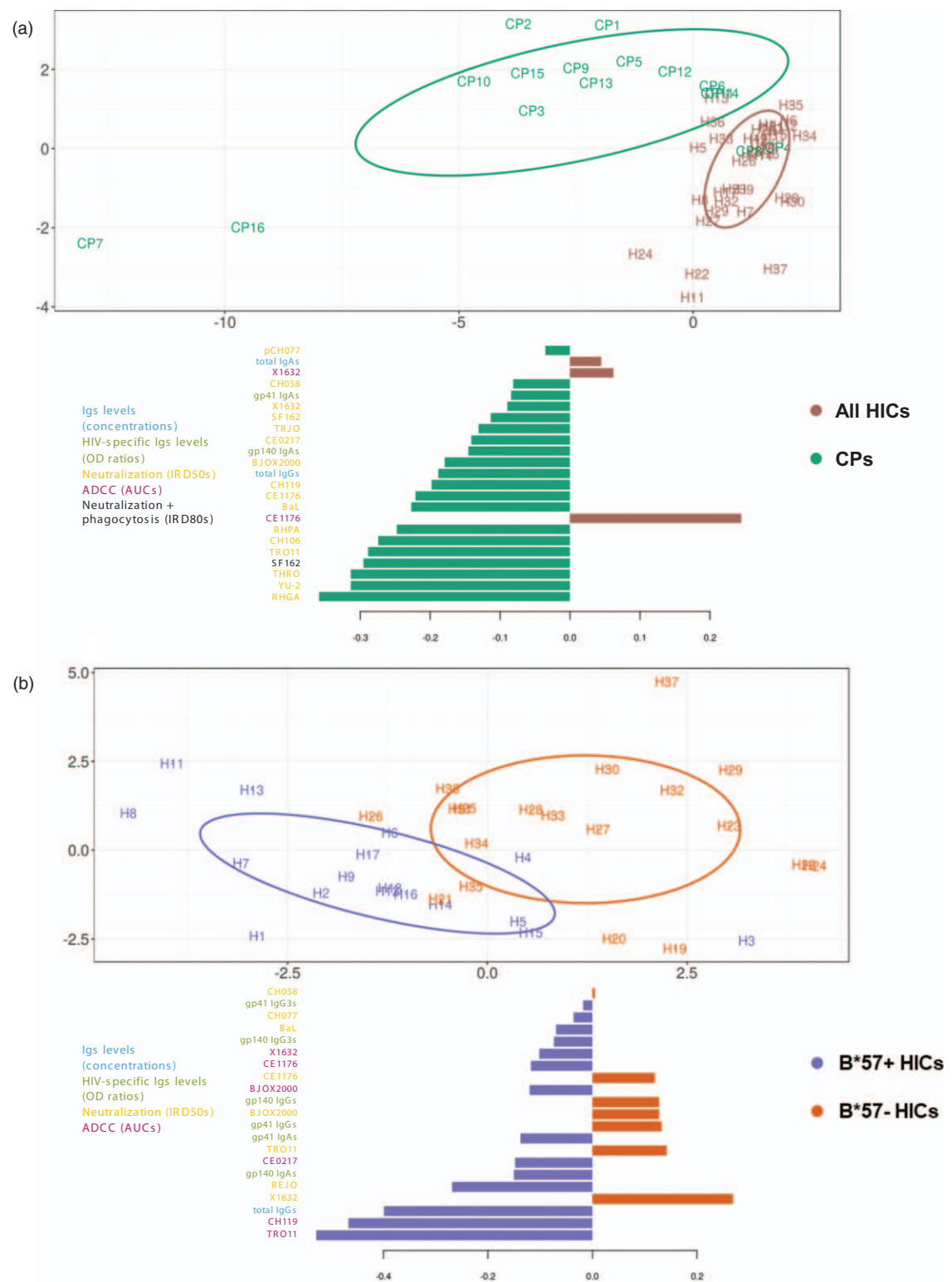


Fig. 5. Partial least-squares discriminant analysis (PLS-DA) between a) HICs and CPs and b) B*57+ and B*57- HICs. Separation between groups based on their Ab characteristics. The ellipses correspond to the estimated 95% confidence intervals. The variable importance in the projection plots corresponding to the relative importance (longer bar: more important) of selected features: levels of Ig isotypes (concentrations, blue), levels of HIV-specific Ig isotypes (OD ratios, green), the neutralization efficacy (IRD50s, yellow), the ADCC activity (AUCs, purple) and the neutralization + phagocytosis against one HIV-1 strain (IRD80s, black). The colors of the bars correspond to the group (all HICs, brown/CPs, green, B*57+ HICs, purple/B*57- HICs, orange).

profile and that B^*57^+ HICs display a different Ab response from B^*57^- HICs. This is the first study connecting all the distinct Ab characteristics to obtain a large comprehensive view of the Ab specificities associated with HIV control.

Concerning total and HIV specific Ab responses, we found that the overall total and HIV-specific isotype levels did not differ drastically between the different patient groups, even though the highest difference was found in the B^*57^- HIC subgroup. Recently, Kant *et al.* [34] also showed that HIV-specific Abs were similar in HICs and nontreated progressors.

Previous studies analyzing HIV-specific isotypes in HICs reported contradictory results, with either higher [12,15,16,35–37] or lower [13,14], levels of HIV-specific Abs compared with viremic controllers and noncontroller patients. In addition to differences in protocol and antigen/epitope used for the detection of HIV-specific Abs, the discrepancy in HIV-specific Ab response may be attributed to the progressor group selected for comparison. Here, we used a historical group of samples collected from patients before the decline of their $CD4^+$ T-cells levels (Supplemental Table 1, <http://links.lww.com/QAD/C317>), implying that they were collected before profound immunosuppression.

The similar concentration of HIV-specific Ab found in the sera from HICs and chronic progressors allows an accurate comparison of the functional inhibition in these polyclonal specimens. As for previous studies, we found that the overall neutralizing profile in HICs was lower than in chronic progressors. This lower neutralization breadth was associated with a decreased viral quasispecies in HICs [38] that drive the humoral response to select B cells directed to conserved epitopes [39–42].

Nonetheless, some HICs were able to mount a neutralizing Ab response against tier-2 and T/F strains. Recently, Martin-Gayo *et al.* [24] identified a subgroup of HIV-1 controllers with distinct transcriptional signatures who mount bnAbs against tier-2 viruses. Whether the efficient T-cell response associated with the *HLA-B*57* allele or whether a unique inflammatory profile [42] lead to a preservation of HIV-specific memory B cells and to functional neutralizing Abs needs to be further investigated. Especially, the epitope recognized on the tier-2 strains by the bnAbs induced in the presence of low viral load in HICs may give new insights on the functional Ab profile associated with protection or control of the infection.

An increased body of evidence demonstrate the participation of nonneutralizing Fc-mediated Ab activities in the control of HIV replication [19,25,35,43–46]. ADCC is a complex phenomenon. Different in-vitro assays have been developed that will influence the results observed [47]. In this study, we infected

CEM.NKR.CCR5 cell line with six distinct primary strains and used Renilla luciferase detection for readout. We found higher median ADCC activity in HICs than in chronic progressors for two virus infections. These results clearly show that the ADCC activity from HICs is virus-dependent. Moreover, we found higher ADCC activity in B^*57^+ compared to B^*57^- HICs that did not correlate with the neutralizing activity. Previously, Ackerman *et al.* [13] did not find enhanced ADCC activities among HICs by using different experimental procedures, that is, CFSE loss from gp120 pulsed target cells or CD107a-induced NK activation. However, they found that HICs possess polyfunctional attributes with multiple functions including ADCP and complement deposition, suggesting a qualitatively superior functional Ab response. Here, we analyzed the phagocytosis of opsonized HIV-1 SF162 virus using an assay detecting FcγRI opsonized virus phagocytosis together with neutralization, as previously described [29]. We did not detect an increased phagocytic activity in HICs under these conditions. Our data point to an increased ADCC activity for 2 of 6 and 3 of 6 HIV strains tested in B^*57^- and B^*57^+ HICs, respectively, further demonstrating that, in addition to the type of ADCC assay, the virus used also strongly influences the experiment outcome.

Overall, this extensive analysis of Ab features in HICs allowed dissection of the interactions and relative contributions of the distinct Ab characteristics. Although differences between HICs and chronic progressors were not conclusive when analyzing each Ab characteristics alone, when combined, functional Ab profiles on their own allowed discriminating HICs from chronic progressors. This group separation indicates that virus control with decreased viral replication enables the induction of a distinct functional Ab response in HICs, prioritizing ADCC to the detriment of neutralizing function. More importantly, the functional Ab profile by itself allows discriminating B^*57^+ from B^*57^- HICs. This shows that the *B*57* allele not only differ in their specific T lymphocytes response [48] but also influences the B cell response [49] and the Ab profile. This virus control and the efficient immune response developed in B^*57^+ HICs may enable the generation of improved Ab response in these patients. The precise humoral immune response associated with the *HLA-B*57* allele gives additional hints on the relevant functional Abs that should be induced in future Ab-driven vaccine design.

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Conflicts of interest

The authors declare no competing interests.

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