

Cross-Reactive Antibodies With the Capacity to Mediate HIV-1 Envelope Glycoprotein–Targeted Antibody-Dependent Cellular Cytotoxicity Identified in HIV-2–Infected Individuals

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Disease progression of human immunodeficiency virus type 1 (HIV-1) is delayed by HIV type 2 (HIV-2) in individuals with dual HIV-1/HIV-2 infection. The protective mechanisms, however, are still to be revealed. In the current study we examined type-specific and cross-reactive antibody-dependent cellular cytotoxicity (ADCC) in HIV-1 and HIV-2 monoinfection or dual infection. Of note, intertype cross-reactive antibodies that mediated HIV-1 envelope glycoprotein (Env)–targeted ADCC were frequently identified in HIV-2–infected individuals. Furthermore, the magnitude of HIV-1 cross-reactive ADCC activity during HIV-2 infections depended on the HIV-1 Env origin and was associated with the duration of infection. These results suggest that preexisting antibodies against HIV-2, which mediate intertype ADCC, might contribute to control of HIV-1 during dual infection.

Keywords. ADCC; HIV-1; HIV-2; HIV-1/HIV-2 dual infection; intertype cross-reactive; anti-HIV antibodies.

Both human immunodeficiency virus type 1 (HIV-1) and HIV type 2 (HIV-2) may cause AIDS development, if not treated. However, HIV-2 is less pathogenic than HIV-1, and

HIV-2–infected individuals remain asymptomatic longer than those with HIV-1 infection [1]. HIV-2 is also less transmissible and mostly confined to West Africa [1]. In this region, AIDS development is also observed in populations dually infected with HIV-1 and HIV-2 [2]. However, our longitudinal studies have shown that HIV-2 can delay subsequent HIV-1 disease progression in individuals with dually HIV-1/HIV-2 infection [3]. The mechanisms behind this natural inhibition of HIV-1 disease by HIV-2 have yet to be revealed and could have implications for future vaccines and therapeutics.

The importance of antibody-dependent cellular cytotoxicity (ADCC) for protective immunity has been implicated in HIV-1 vaccine studies [4]. Moreover, ADCC has been associated with the control of HIV-1 and simian immunodeficiency virus (SIV) infections, and Fc-receptor engagement has been suggested to augment neutralizing antibody effector functions [5]. Similarly to HIV-2 neutralizing antibodies [6], intratype ADCC activity in serum samples from HIV-2–infected individuals has also been shown to be frequent and broad [7, 8].

In the current study, we characterized intratype and intertype ADCC activity in plasma samples from HIV-1, HIV-2 and dually HIV-1/HIV-2–infected individuals. Robust intratype ADCC was observed, independent of type of HIV infection. Interestingly, we also identified cross-reactive ADCC targeting HIV-1 envelope glycoprotein (Env) in plasma samples from HIV-2–infected individuals.

MATERIAL AND METHODS

Study Participants

The study participants were part of a police officer cohort in Guinea-Bissau [9]. Blood samples were obtained from HIV-1–infected (n = 23), HIV-2–infected (n = 30), or dually HIV-1/HIV-2–infected (n = 9) individuals who were either treatment naive or not successfully treated (plasma viral load [VL] >1000 copies/mL). The HIV-2–infected individuals included 21 considered aviremic and 9 considered viremic, based on the plasma VL quantification limit (75 RNA copies/mL). Samples from HIV-2–infected individuals receiving successful antiretroviral therapy (ART) (n = 6) were also analyzed. Details on sex, age, CD4⁺ T-cell levels, and VL can be found in [Supplementary Table 1](#). Informed consent was obtained from the participants and ethical committees of the Ministry of Health in Guinea-Bissau and Lund University, Sweden, approved the study.

HIV Status, VL, and CD4⁺ T-Cell Determinations

Blood sampling and determination of HIV status, absolute CD4⁺ T-cell count, percentage of CD4⁺ T cells, frequency of CD38⁺HLA-DR⁺ CD4⁺ T cells and plasma VL, was performed

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as described elsewhere [10]. The plasma VL detection limit was 75 RNA copies/mL for HIV-1- or HIV-2-monoinfected and 135 RNA copies/mL for dually HIV-1/HIV-2-infected individuals. Infection status of individuals with HIV-2 monoinfection was also supported by lack of HIV-1 DNA detection, using quantitative polymerase chain reaction primers and probe, as described elsewhere [10].

ADCC Assay

The ADCC-GranToxiLux assay (OncoImmunit) was performed as described elsewhere, with minor modifications [11, 12]. In brief, CEM.NKR_{CCR5} cells were labeled with TFL4 and NFL1 and coated with gp120 Env (Immune Technology) of HIV-1 subtype B, LAI; subtype C, ZA1197MB; subtype CRF02_AG, 01CM_0002BBY (BBY), 1475MV or 98US; and HIV-2 subtype B UC1 or SIVmac239 (all pretested for the ability to bind the target cells). The Env concentration (0.01 mg/mL for ZA1197MB and BBY; 0.02 mg/mL for LAI; 0.08 mg/mL for 1475MV, 98US, UC1 and SIVmac239) was based on competition with anti-CD4 (BD Bioscience) as described elsewhere [11]. The source of effector cells was peripheral blood mononuclear cells, and the effector-target cell ratio was 30:1.

Plasma samples from the study subjects (heat inactivated for 1 hour), the HIV-1 control immunoglobulin (National Institutes of Health AIDS Research and Reagent Program), the HIV-2 control SSI 2335 (in-house) and negative control (in-house pool of HIV-negative serum samples) were tested in 5-fold dilutions starting at 1:300. The thresholds for positive granzyme B responses were established using HIV-seronegative plasma samples ($n = 28$), including samples from seronegative individuals ($n = 10$) within the study cohort, and were defined as the mean plus 3 standard deviations. Thresholds for LAI, ZA1197MB, BBY, 1475MV, 98US, UC1, and SIVmac239 were 5.8, 9.4, 8.5, 6.7, 8.0, 8.0, and 5.7%, respectively. The ADCC activity was assessed as the peak percentage of granzyme B-positive (%GzB⁺) targets, as well as the area under the curve (AUC), and normalized to respective positive control to overcome day-to-day variations.

Statistical Analysis

Nonparametric statistical analysis for comparison between groups (2 or more) and correlations were performed using GraphPad Prism software (version 7.0; GraphPad) and included Mann-Whitney U and Kruskal-Wallis tests and Spearman rank correlation.

RESULTS

Robust Intratype ADCC Activity Frequently Found in Both HIV-1 and HIV-2 Infections

To investigate HIV-1 and HIV-2 intratype and intertype ADCC, plasma samples from HIV-1 ($n = 23$), HIV-2 ($n = 30$) or dually HIV-1/HIV-2 ($n = 9$) infected individuals, either treatment

naive or not successfully treated, were tested in the ADCC-GranToxiLux assay [11]. CEM.NKR_{CCR5} target cells were coated with gp120 originating from HIV-1 subtype CRF02, BBY, or HIV-2, UC1. Intratype ADCC, assessed as peak %GzB⁺ targets, was demonstrated in plasma samples from all HIV-1 and HIV-2-infected individuals. The median percentage was 24.7 (interquartile range [IQR], 18.4–33.3) and 28.8 (21.4–33.7) respectively (Figure 1A and 1B). Plasma samples of dually infected individuals displayed ADCC reactivity against both HIV-1 and HIV-2 Env-coated targets.

Cross-Reactive HIV-1 Env-Targeted ADCC Activity Identified in HIV-2-Infected Individuals

Further analyses revealed that most of the HIV-2-infected individuals (27 of 30) had plasma with cross-reactive ADCC against HIV-1 BBY Env-coated targets, median 20.4 (IQR, 10.7–26.0) %GzB⁺ cells (Figure 1A). On the contrary, intertype ADCC against the HIV-2 UC1 Env-coated targets in plasma from HIV-1-infected individuals was infrequent (7 of 23) and with limited magnitude, median 6.1 (IQR, 4.6–10.2) %GzB⁺ (Figure 1B). Intertype ADCC assessed according to AUC corroborated findings noted for peak %GzB⁺ targets (Supplementary Figure 1A and 1B).

Magnitude of HIV-1 Cross-Reactive ADCC is Elevated in Long-Term HIV-2 Infections

To investigate modulators of intratype and intertype ADCC during HIV-2 infection, we divided the group into viremic ($n = 9$) and aviremic ($n = 21$) individuals. HIV-2-infected individuals that had successful ART were also included ($n = 6$). The results showed that the strength of intratype ADCC against HIV-2 Env did not differ according to level of viremia, or ART (Figure 1C). Instead, magnitude of intertype ADCC targeting HIV-1 BBY Env tended to be reduced in HIV-2-infected subjects on ART ($P = .10$; Figure 1D). Assessing ADCC according to AUC further supported the finding that viral control during ART tends to reduce intertype ADCC, while the intratype activity is not affected (Supplementary Figure 1C and 1D). Elevation of immune activation, detected as frequency of CD4⁺ T cells expressing CD38⁺HLA-DR⁺, was also associated with stronger intertype ADCC ($P = .04$; $r = 0.385$; Figure 2A). No such correlation was seen for the intratype ADCC (Supplementary Figure 2A).

Because information on duration of infection was available for 11 of the HIV-2-infected participants, we compared ADCC between those with longer or shorter infection duration (mean, 13.5 years). Individuals with longer HIV-2 infection had intertype ADCC with higher magnitude than those with shorter infection (median 24.2 vs 9.8 %GzB⁺ targets respectively; $P = .03$; Figure 2B). The magnitude of intratype ADCC did not differ in relation to infection duration (Supplementary Figure 2B).

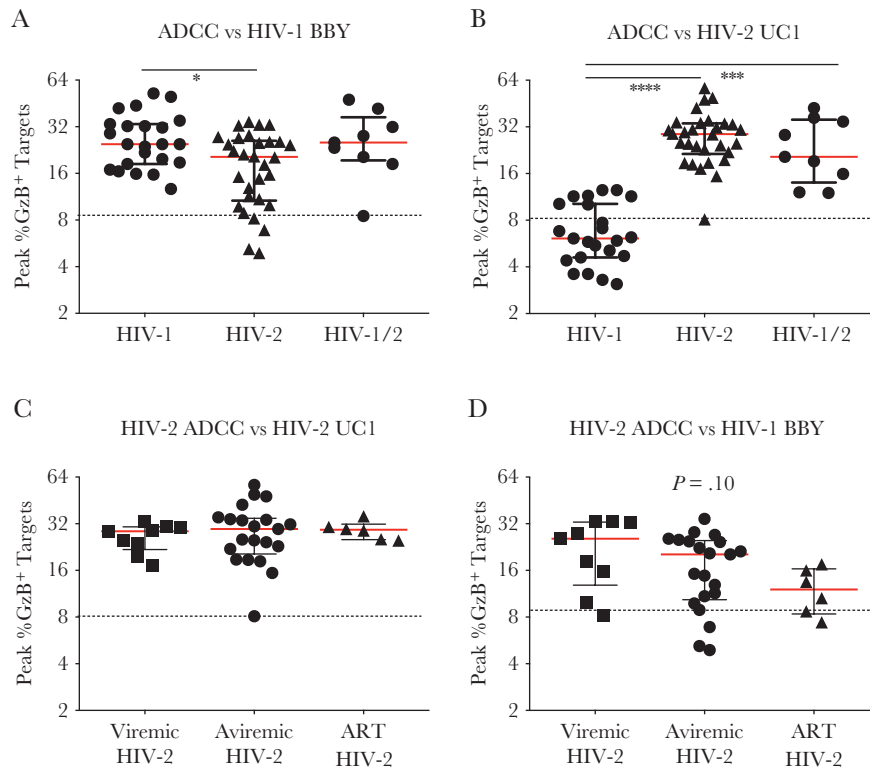


Figure 1. Intratype and intertype antibody-dependent cellular cytotoxicity (ADCC) in human immunodeficiency virus type 1 (HIV-1), HIV type 2 (HIV-2), and dual HIV-1/HIV-2 infections. ADCC against HIV-1 gp120 01CM_0002BBY (BBY) envelope glycoprotein (Env) (A, D) and HIV-2 UC1 Env-coated CEM.NKR_{CCR5} target cells (B, C), mediated by plasma samples from treatment-naïve or unsuccessfully treated HIV-1 (n = 23), HIV-2 (n = 30), and dually HIV-1/HIV-2 (n = 9) infected individuals (A, B) or samples from HIV-2-infected individuals, subdivided according to plasma viral load, above (viremic; n = 9) or below (aviremic; n = 21) 75 RNA copies/mL, and HIV-2-infected subjects receiving virus-controlling antiretroviral therapy (ART; n = 6) (C, D). Dotted lines indicate the threshold for positive peak percentage of granzyme B–positive (%GzB⁺). Statistical differences were calculated according to nonparametric Kruskal-Wallis analysis with Dunn posttest. **P* < .05; ****P* < .001; *****P* < .0001.

Strength of Intertype ADCC Activity in HIV-2 Plasma Depends on the Origin of Targeted HIV-1 Env

We next analyzed the breadth of intertype ADCC activity in plasma samples from HIV-2-infected individuals. Eleven such samples with potent ADCC activity against HIV-1 BBY (>20% GzB⁺ targets) were tested against a panel of gp120-coated targets, representing HIV-1 subtype B (LAI), subtype C (ZA1197MB), subtype CRF02_AG (98US_MSC5007 and 01CM_1475MV) and SIVmac239. Eight of the 11 plasma samples from HIV-2-infected individuals displayed intertype ADCC against additional (≥1) HIV-1 Env. Three of the samples mediated intertype ADCC against all 5 HIV-1 Envs. We also noted that magnitude of intertype ADCC varied depending on Env origin, with BBY Env-coated targets being the most sensitive and those coated with LAI Env the most resistant (median, 25.6 [IQR, 21.2–33.0] vs 5.3 [4.4–7.8] %GzB⁺, respectively; *P* < .001; Figure 2C). ADCC targeting SIVmac239 was strong in all 11 plasma samples tested from individuals with HIV-2 infection (median, 21.5 [IQR, 15.4–27.3] %GzB⁺ targets; Figure 2C).

Plasma samples from HIV-1-infected individuals were also tested against the panel of Env-coated targets. Again, the cells coated with the BBY Env were found to be the most sensitive

(Supplementary Figure 2C). As for the breadth of HIV-2 intertype ADCC, it both correlated with lower CD4⁺ T-cell level (*P* = .004, *r* = –0.8064; Figure 2D) and higher VL (*P* = .03; *r* = 0.6518; Supplementary Figure 2D), suggesting that prolonged HIV-2 infection may trigger broader ability to mediate cross-reactive intertype ADCC.

DISCUSSION

We show that robust intratype ADCC can be detected in plasma samples from both HIV-1-infected and HIV-2-infected individuals. ADCC targeted against Env of both HIV-1 and HIV-2 origin was also detected in individuals with dual HIV-1/HIV-2 infection. Moreover, the current study reveals that a majority of HIV-2-infected individuals harbor antibodies that can mediate intertype cross-reactive ADCC against targets coated with a specific HIV-1 Env protein. The magnitude of this HIV-1 cross-reactive ADCC activity, identified in plasma samples from HIV-2-infected individuals, was in the range of that previously correlated with protective immunity [4]. In addition, some HIV-2-infected individuals had ADCC-mediating antibodies broadly cross-reactive against HIV-1 Envs representing different subtypes.

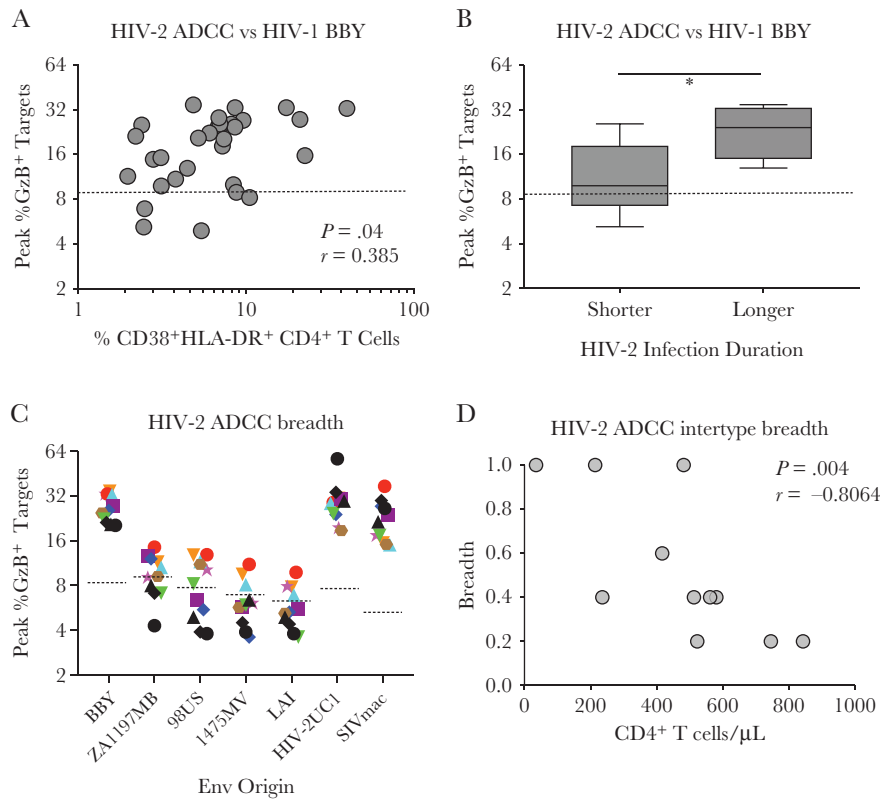


Figure 2. Modulators of intertype antibody-dependent cellular cytotoxicity (ADCC) magnitude and breadth in human immunodeficiency virus type 2 (HIV-2) infection. Cross-reactive ADCC against envelope glycoprotein (Env)-coated CEM.NKR_{CRF5} targets, mediated by plasma samples from HIV-2-infected treatment-naïve or unsuccessfully treated individuals, was analyzed. *A, B*, Intertype HIV type 1 (HIV-1) gp120 01CM_0002BBY (BBY) directed ADCC activity in plasma samples from HIV-2-infected individuals. *A*, Correlation with the percentage of CD38⁺HLA-DR⁺ CD4⁺ T cells. *B*, Comparison between shorter ($n = 5$) and longer ($n = 6$) durations of infection, with the cutoff considered the mean duration (13.5 years), as estimated from the midpoint between the last HIV-2 seronegative and the first seropositive samples. *C, D*, Plasma samples from HIV-2-infected individuals ($n = 11$), selected on the basis of having >20% granzyme B-positive (GzB⁺) HIV-1 BBY Env-coated targets, tested against an extended panel of targets pulsed with Env of HIV-1 BBY, ZA1197MB, 98US, 1475MV, LAI, HIV-2UC1, and SIVmac origin. *C*, ADCC activity depicted with symbols specific to each study participant). *D*, Correlation between breadth of HIV-1 cross-reactive ADCC, as assessed against the 5 HIV-1 Envs (ie, breadth of 1 indicates ADCC against all 5 Env-coated targets), in relation to CD4⁺ T-cell count. Dotted lines represent the threshold for positive peak percentage of GzB⁺ targets (%GzB⁺). Statistical correlations were calculated using nonparametric Spearman rank correlations, and differences between 2 groups using the Mann-Whitney *U* test. * $P < .05$.

In line with the current study, the few previous studies that analyzed ADCC in HIV-2 infections also found frequent HIV-2 intratype ADCC activity [7, 8]. However, unlike in our study, intertype cross-reactive ADCC against HIV-1 was not detected [7, 8, 13]. This difference may be due to differences between assays; previous studies were based on chromium 51 release from chronically infected monocytic cells [7, 8, 13], whereas we assessed %GzB⁺ Env-coated CD4⁺ T cells. Exposure of Env epitopes may also differ; for example, epitopes in the CD4-bound confirmation have been reported to depend on the level of CD4 modulation by HIV-1 accessory proteins [5].

Another difference between the current study and previous ones might be related to Env antigen variation, because earlier studies on HIV-1 ADCC activity within HIV-2 serum samples exclusively used target cells infected with the HIV-1 IIIB strain [7, 8, 13], with the exception of 2 serum samples also tested against the RF strain [13]. Interestingly, we found that cells pulsed with Env originating from the IIIB homolog LAI

were the most resistant against HIV-2 antibody-mediated intertype ADCC. Thus, our results largely reproduce earlier results [7, 8] but also for the first time reveal that HIV-2 infection can trigger antibodies mediating ADCC against cells presenting other HIV-1 Env variants. Interestingly, the strongest HIV-1 cross-reactive ADCC was seen against targets coated with one of the Envs originating from CRF02_AG, an HIV-1 subtype frequently found in the area of the study participants and also suggested to influence the strength of intratype ADCC in HIV-1-infected populations in this region [14].

In HIV-1-infected individuals, initially strain-specific antibodies evolve over time to acquire neutralization activity against heterologous HIV-1 variants [15]. In line with this observation, our study of a subset of plasma samples, uniquely obtained from HIV-2-infected individuals in whom infection duration was known, suggests that HIV-1 cross-reactive ADCC activity increases with longer HIV-2 infection duration. Also, correlations between elevated intertype ADCC and declining

CD4⁺ T-cell counts, as well as elevated frequencies of activated CD4⁺ T cells, imply that prolonged infection duration stimulates development of cross-reactive antibodies.

In addition, because plasma samples from HIV-2–infected individuals receiving ART tended to mediate weaker intertype ADCC, the level of virus replication may influence production of such antibodies. This has also been noted for intratype ADCC in HIV-1–infected individuals during ART [12]. On the other hand, the ability to mediate ADCC also depends on natural killer cell function, which improves during ART [12]. Still, we did not find any statistically significant difference in cross-reactive activity between plasma samples from viremic and aviremic HIV-2–infected subjects. Thus, low-level viremia seems to sustain cross-reactive antibody producing B-cells in HIV-2 infection. Intriguingly, we did not find any correlation between level of HIV-2 intratype ADCC and infection duration, nor virus replication, which is different from findings described elsewhere for HIV-1 intratype ADCC activity [12].

The broad ADCC activity against HIV-1 seen in plasma samples from HIV-1–infected, as well as some HIV-2–infected individuals, is encouraging in the light of vaccine development. Antibody responses cross-reactive between different HIV-1 strains are essential for an effective vaccine, and broad ADCC responses have been associated with virus control [5]. Furthermore, our results suggest that intertype ADCC-mediating antibodies that preexist in HIV-2 infection, at least in part, may contribute to reported delay in HIV-1 disease progression during dual infection [3]. Thus, this observation could have implications for the design of HIV disease–preventing interventions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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