A child with perinatal HIV infection and long-term sustained virological control following antiretroviral treatment cessation

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Understanding HIV remission in rare individuals who initiated antiretroviral therapy (ART) soon after infection and then discontinued, may inform HIV cure interventions. Here we describe features of virus and host of a perinatally HIV-1 infected child with long-term sustained virological control. The child received early limited ART in the Children with HIV Early antiRetroviral therapy (CHER) trial. At age 9.5 years, diagnostic tests for HIV are negative and the child has characteristics similar to uninfected children that include a high CD4:CD8 ratio, low T cell activation and low CCR5 expression. Virus persistence (HIV-1 DNA and plasma RNA) is confirmed with sensitive methods, but replication-competent virus is not detected. The child has weak HIV-specific antibody and T cell responses. Furthermore, we determine his HLA and KIR genotypes. This case aids in understanding post-treatment control and may help design of future intervention strategies.
A rapid formation of persistent viral reservoirs follows acute HIV-1 infection. This early establishment of latently HIV-1-infected CD4+ T cells harbouring replication-competent virus remains the major obstacle to HIV cure or remission. As antiretroviral therapy (ART), even when given within days of infection, usually fails to clear these reservoirs, it is unlikely that ART alone can lead to HIV remission. It is, however, hypothesized that ART given very soon after infection may enable a more effective immune response and, together with other strategies, lead to sustained control of viral replication.

Current approaches to HIV cure or remission have focused on either reversing latency (e.g. “shock and kill”), enhancing immune responses or preventing immune activation (e.g. vaccines and other immunotherapies). Central to the question of HIV remission is the interaction between viral reservoir, immune activation, host genetics and immune response.

Several adult cases of post-treatment control have been described. These individuals are unlike elite controllers (<1%) who control HIV-1 to undetectable levels in the absence of ART, probably through distinct immunological mechanisms.

In children, data are extremely limited. In 2013, the report of the “Mississippi baby” suggested that very early ART, here within 30 h of birth, could lead to prolonged (27 months) virological control off-treatment, raising hope for a feasible HIV-1 remission strategy. Unfortunately, this girl “relapsed” after almost 2 years without ART due to return of high levels of viral replication, and required ART. Subsequently, a French girl was reported who started ART at 3 months of age, stopped treatment between 5 and 7 years of age and controlled virus to undetectable levels for over 12 years.

Reports of post-treatment controllers who initiated ART and then discontinued by design or unintentionally may help our understanding of key host determinants of HIV replication control, and inform interventions for HIV remission and cure.

Here we report a detailed virological and immunological analysis of a child at 9.5 years of age, originally enroled in the Children with HIV Early AntiRetroviral therapy (CHER) trial who was randomized to the immediate, time-limited 40 weeks of ART study arm. The CHER trial was initiated at a time when the best understanding of key host determinants of HIV replication control, and inform interventions for HIV remission and cure.

We present a detailed virological and immunological analysis of a child at 9.5 years of age, originally enrolleed in the Children with HIV Early AntiRetroviral therapy (CHER) trial who was randomized to the immediate, time-limited 40 weeks of ART study arm. The CHER trial was initiated at a time when the best understanding of key host determinants of HIV replication control, and inform interventions for HIV remission and cure.

Virus persistence. At 9.5 years, the Roche VL result was reported as target not detected (TND; Fig. 2a). Virus pelleted from 10 mL of plasma, yielded 66 copies and a VL of 6.6 RNA copies per mL (Fig. 2a). Using a highly sensitive RNA nested qPCR on 3 mL of plasma, 2 of 22 replicates were positive (Fig. 2a). Using a semi-nested real-time PCR (sn-qPCR) assay and an input of 1 µg of genomic DNA (gDNA) per well, total cell-associated HIV-1 DNA was estimated at 5 copies per 106 peripheral blood mononuclear cells (PBMCs; six of nine amplifications positive) (Fig. 2b). In a stored sample from ART interruption at 50 weeks of age, this was almost identical (5 copies per 106 PBMCs: 1 of 3 amplifications positive). DNA sequencing of gag from the 9.5 year sample confirmed infection with subtype C virus (Fig. 2c).

Using two virus outgrowth assays (primary CD8-depleted PBMCs and MOLT4/CCR5 cells), no replication-competent virus was detected at 9.5 years (Fig. 2d). However, a weak HIV RNA signal (1 of 24 replicates was positive) was detected in the day 28 supernatant of cultured primary CD4+ cells from the 50-week sample using qualitative real-time PCR nested assay (n-qPCR). The child’s CD4+ T cells could be infected in vitro with the HIV-1 BaL strain (Fig. 2d).

HIV-specific antibodies. At 9.5 years of age, HIV-specific antibodies were undetectable by enzyme-linked immunosorbent assay (ELISA) (optical density (OD) 0.056; cut-off 0.263), and the western blot was indeterminate with weak reactivity to Gag proteins (p24++, p40++ and p55/51+) (Fig. 3a). By multiplex bead arrays the child showed a substantial IgA2 response to gp41, and weak but detectable responses to gp120 and Vpu (IgG1), Gag (IgG2), Tat (IgG2, IgG3, IgA1) and Vpu9 peptide (IgM) (Fig. 3b).

Cellular immune responses. The child possessed strong T cell responses to staphylococcal enterotoxin B (SEB; 9.03% CD4, 6.35% CD8) and a very strong anti-CD16-induced natural killer (NK) cell response (27.79% interleukin-2/interferon-γ (IL-2/IFN-γ)+CD3−CD56+ cells) (Fig. 3c). A weak (0.116%) CD4+ T cell response to Gag was detected, without detectable CD8+ T cell responses to any peptide pool (Fig. 3d).

Host genotyping. All HLA loci were heterozygous: A*30:02/01: 66:01; B*08:01/01:44:03:01; C*04:01/01:07:01:01; DPB1*01:01/18:01; DQB1*05:01/01:06:09:01; DRB1*12:01/01:13:02:01 (Fig. 4a). The child’s KIRAA1 genotype, the most common genoype globally, included both full-length (f) and truncated (v) KIR2DS4 alleles (Fig. 4f). These are among features that we have associated with greater risk of transmission/acquisition of HIV-1 infection in mother-to-child transmission studies. HLA B*44:03:01 has a threonine (T) at position 80 as part of the Bw4
Immunophenotyping. The CD4:CD8 T cell ratio was 1.9, higher than all uninfected control children (Fig. 5a). Measurement of T cell subsets representing various stages of differentiation highlighted that 9.5–10-year-old HIV-uninfected children do not display adult-like proportions of the different subsets, particularly for naive, central memory and effector memory CD4+ T cell subsets and central memory CD8+ T cells (Fig. 5b). The child had high proportions of naive, central memory and effector memory CD8+ T cells compared to uninfected children. CCR5 density on CD4+ and CD8+ T cells was similar for children and adults, with the child having lower CCR5 expression than 14 of the 15 uninfected children/adults (Fig. 5c). Proportions of the child’s CCR5-expressing CD4+ and CD8+ T cells were comparable to those of uninfected children. Of note, CCR5-expressing CD8+ T cells were significantly higher in adults than epitope (NLRTALR) of the α1 helix 2 of the molecule—and therefore will bind to KIR3DL1 on NK cells. HLA- Cw*07:01:01 (allotype C1) and Cw*04:01:01 (C2) have a lysine (K) and asparagine (N) at position 80, respectively—allowing for interaction with both KIR2DL3 (C1) and 2DL1 (C2) on NK cells. Collectively, these genotyping data suggest the potential for diverse interactions that may include engagement of CD4 and CD8 T cells and NK cells in antiviral responses.
children \((p = 0.0013; \text{Mann–Whitney } U \text{ test})\). Levels of immune activation, measured by HLA-DR, were similar to those of uninfected children and adults. Expression of TIGIT in the child’s CD4+ and CD8+ T cells was similar to uninfected children, and significantly higher in adults \((p = 0.027 \text{ and } p = 0.0027, \text{respectively; Mann–Whitney } U \text{ tests})\). PD-1 expression on the child’s CD4+ T cells was substantially above the median values for children or adults. However, two adult outliers had similarly high levels. Similar to TIGIT, PD-1 expression on CD8+ T cells was significantly higher in adults than in children \((p = 0.0007; \text{Mann–Whitney } U \text{ test})\), with the child having high levels similar to the adult median. Flow cytometry data are provided in Supplementary Table 3.

**Discussion**

We report virological and immunological characteristics in a South African child of 9.5 years of age in long-term HIV...
remission. He initiated ART at 8.7 weeks of age and discontinued ART after 40 weeks in accordance to his randomization in the CHER trial. This is the only child to achieve this outcome among 227 who stopped ART (at 40 or 96 weeks) in the trial (0.4%). The French case of long-term remission was one of 15 children in the French paediatric cohort who stopped ART (6.7%)\(^2\).

Studies have shown that earlier ART initiation results in a smaller HIV reservoir size\(^26-28\). Treatment was very early (within 30 h of birth) for the Mississippi baby who achieved 27 months of virological control off-treatment before experiencing virological rebound\(^19,20\). This delay in rebound may have been attributed to a small size of latent replication-competent reservoir. Both the French case with >12 years of remission\(^21\) and this South African case started ART later, at 3 and 2 months, respectively. HIV transmission was likely intrapartum in the French child and in utero in the Mississippi baby. The timing is unknown for the South African child. Timing of transmission may have been a key factor affecting the different outcomes of remission. Of note, subtype of virus (B, H and C, respectively), treatment duration (18 months, 6 years, 10 months, respectively) and ethnicity were different in these children. Both earlier cases were girls, while this is the first report of a boy with HIV remission. Unlike the South African child, using similar methods and number of CD4+-T cells, replication-competent virus was readily found in the French case\(^21\). In contrast, no replication-competent virus was found in the Mississippi baby when testing 22 million CD4+-T cells\(^19\), highlighting that this measure poorly predicts long-term likelihood of remission. Furthermore, recent reports of very early treatment in adult cases (within 2 weeks of infection) described multiple sensitive tests for HIV-1 persistence, which supported the absence of detectable virus during ART—these cases all rebounded shortly after ART was stopped (n = 8 patients\(^8\), median 26 days, range 13-48 days; n = 1 patient\(^8\), 225 days). Collectively, these findings suggest some limited viral replication may in fact be required for durable long-term remission.

Early in infection the child’s VLs were high, indicative of a highly replicating virus. The VL declined from >750,000 to 150,000 RNA copies per mL prior to beginning treatment, suggesting an immune response attempting to control virus replication. After ART initiation, viral decline was biphasic, with the expected initial sharp decline followed by a more gradual decline. After ART cessation, VLs remained below the detection threshold for 8.75 years.

More sensitive methods for VL measurement confirmed the presence of low amounts of virus produced in vivo (6.6 RNA copies per mL). Inability to detect replication-competent virus in vitro may be because of assay sensitivity (2 million CD4+ cells tested), or defective virus that cannot accumulate to detectable levels.

Early initiation of ART is associated with non-reactive HIV antibody results in many HIV-1-infected children and adults\(^29-31\). On standard assays the child is seronegative; however, results from bead arrays revealed footprints of historical adaptive responses that have either waned or are being maintained through ongoing antigenic priming. The substantial IgA2 (mucosal) response to gp41 could be primed by microbial antigens sharing homology with gp41\(^32-34\). However, a recent study reported stronger gp41-specific IgA responses in elite controllers, which could not be well explained by responses to microbial antigens\(^35\). HIV-1 Env- and Vpu-specific NK cell antibody-dependent cellular cytotoxicity (ADCC) responses, including against C-terminal peptide Vpu19 (our Vpu9 peptide), are associated with elite HIV-1 control\(^36\). Using a whole blood assay, we have shown similar responses associated with reduced maternal–infant HIV-1 transmission and lower VLs in HIV-1-infected mothers\(^37,38\). The child lacked a detectable NK cell response to any HIV-1 peptide pool, likely due to very low levels of antibodies that target Env and Vpu. The Vpu9-IgM antibody response suggests a potential for interaction with complement. Anti-Tat IgG responses have also been associated with natural HIV-1 control and improved immune function in ART-treated patients receiving Tat vaccine\(^39,42\). Interestingly, an African study highlighted that persistent anti-Tat IgM in addition to IgG might be protective against disease progression\(^41\).

An IgG2 antibody response to Gag has been associated with CD4+-T cell response and long-term nonprogression\(^43\). The child had both responses. The weak Gag-specific CD4+-T cell response without a detectable CD8+ T cell response is intriguing. Such responses are reported in HIV-exposed uninfected individuals and some HIV-uninfected individuals, only when using sensitive cultured ELISPOT assays\(^44\). However, the opposite exists in untreated HIV-1-infected infants where we readily demonstrated Gag-specific CD8+, but not CD4+-HIV-specific T cell responses in the first few months of life—using the same whole blood intracellular cytokine assay as for the present study\(^45\). Furthermore, there is an absence of sustained HIV-specific T cell responses in early ART-treated HIV-1-infected children\(^46\). The CD4+ Gag response demonstrated in the child seems remarkable given early treatment and long duration of viral suppression. The CD4+ T cell response may be maintained by small amounts of virus (replication-competent or replication-defective) or Gag protein produced in vivo. Lack of CD8+ T cell response suggests that CD8+ T cells might not be currently involved in controlling levels of viraemia, supporting the possibility that HIV may be
defective for further infection of permissive cells. In keeping with this, we hypothesize the HIV-1 reservoir in the child may be maintained through homeostatic, antigen-stimulated or integrase site-dependent proliferation of CD4+ T cells harbouring transcription/translation-competent but not necessarily replication-competent HIV-1. These cellular proliferative mechanisms present a challenge for HIV-1 eradication strategies. In contrast, the French case showed weak but broader mechanisms present a challenge for HIV-1 eradication strategies. In contrast, the French case showed weak but broader mechanisms present a challenge for HIV-1 eradication strategies. In contrast, the French case showed weak but broader mechanisms present a challenge for HIV-1 eradication strategies. In contrast, the French case showed weak but broader mechanisms present a challenge for HIV-1 eradication strategies.

These collective findings raise questions of whether cross-reacting antigens might, in addition to small amounts of virus production in vivo, contribute to maintaining some of these memory responses—and, if such responses contributed to remission in this child. Importantly, lack of detection does not preclude having CD8+ T cell responses, or other protective responses such as ADCC/phagocytosis in the presence of maternal/child antibody, that may have been active early in life and possibly essential to the outcome of HIV-1 remission. The rapid decline in VL over a month prior to ART initiation may be of considerable importance in understanding the reasons behind virological control in the child. ART may have protected an early pre-ART antiviral response that may otherwise have been compromised by continued viral replication.

The child had no HLA class I alleles shown in adults to associate with HIV-1 control, except for heterozygosity at all HLA loci that is considered advantageous. In contrast, the French case was homozygous at three loci, considered disadvantageous. The child possesses HLA class II alleles already associated with the robust mucosal CD4+ T cell responses in elite
controllers (DQB1*06 and DRB1*13:02:01)\(^{31}\). DQB1*06 has also been associated with IgA responses to V8V2 and increased risk of acquisition in the RV144 HIV vaccine trial\(^{32}\). Possession of these class II alleles may explain why the CD4+ Gag response remains detectable at 9.5 years, despite the low-level antigenic exposure. KIR genotyping revealed some features already associated with greater risk of vertical transmission in mother-to-child HIV infection\(^{33,34}\). In this regard TIGIT, another exhaustion given no evidence of high immune activation in the children. High PD-1 expression is unlikely from immune CD8+ T cells were comparable to adults but much higher than in uninfected children. Overall, these features show an immune system that closely resembles that of an uninfected child of similar age, making this child an ideal example of post-treatment HIV-1 control.

The ability of this child to both control virus to levels below detection on standard assays and to control immune activation presents the best of both worlds; these features have been described separately for elite controllers\(^{18,55}\), non-pathogenic nonhuman primate Simian immunodeficiency virus infection and long-term non-progressing children\(^{56,57}\). Events that may have led to sufficient silencing of virus replication will be explored by whole-genome sequencing of virus/provirus. Further investigation will expand our understanding of how the immune system controls HIV-1 replication with accompanying low immune activation, and inform future strategies for ART interruption and other interventions for HIV remission.

**Methods**

**The case and study participants.** In the CHER trial (ClinicalTrials.gov Identifier: NCT00102960; 2005–2011), HIV-1–infected treatment-naive, asymptomatic infants aged ≤12 weeks with a CD4% ≥25% were randomized to early limited ART for 40 (ART–40W; \( n = 143 \)) or 96 (ART–96W; \( n = 143 \)) weeks or to deferred ART (\( n = 125 \))\(^{32}\). In the ART–40W group, 122 met criteria to stop ART, together with 105 in the ART-96W group. Re-initiation of ART was based on a CD4% decrease to <20% and on clinical criteria\(^{23}\). By the trial end, median follow-up was 4.9 years (IQR: 3.7–5.3). The child in this report was randomized to the ART-40W arm. Follow-up after the trial continued as part of PEPFAR-supported routine health services and later in an observational study\(^{36,39}\). CD4+ T cell counts were determined using the FACSCount System (BD Biosciences, San Jose, CA, USA).

Plasma HIV-1 RNA levels were quantitated retrospectively on stored samples by Roche Amplicore/COBAS\(^{®}\) Taqman HIV-1 Test, v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a lower detection limit of 20 RNA copies per mL (except for three time points where detection limits of assays used were 50 or 400 RNA copies per mL).

To gain further insight into specific characteristics of this case, detailed virological, immunological and genetic studies were undertaken at 9.5 years of age. Some viral studies were conducted on stored samples from 50 weeks of age of ART initiation. The mother is deceased and no stored maternal samples are available for study.

For comparisons of immune cell phenotypes, we included samples from five healthy age-matched HIV-infected children (3 females, 2 males; median 9 years, range 9.3–10 years) and 10 HIV-uninfected adults (5 females, 5 males; median age 44 years, range 35–55 years) from the same population.

**Ethics statement.** The CHER trial was approved by the Ethics Committees of the University of the Witwatersrand and Stellenbosch University. Thereafter, the Human Research Ethics Committee of the University of the Witwatersrand provided approval for all subsequent observational studies and investigations of the Case. All participants provided written informed consent. For all minors, a parent or guardian gave written informed consent and the child gave written assent. We have complied with all ethical regulations.

**Assays to detect HIV-1 DNA and RNA.** An sn-qPCR assay targeting reverse transcriptase (RT) was developed for subtype C proviral DNA quantitation based on methods of Pasternak et al.\(^{60}\) and Kiselinova et al.\(^{61}\), and described in Kuhn et al\(^{28}\). The sn-RT-qPCR is a two-step PCR in which the first round of amplification was carried out using conventional PCR and allowed to proceed for 15 cycles only and the total product of the first-round PCR was subsequently used in the second round of PCR, a real-time hydrolysis probe-based PCR using a fluorescently labelled TaqMan probe, one primer identical to the forward primer used in the first-round PCR and a second reverse primer designed to bind "deeper" within the amplicon. The second-round PCR was carried out for the standard cycle numbers used in real-time PCR (40 cycles). The numbers of HIV-1 proviral DNA copies were determined by using the standard curve method. To construct the standard curve, known copies of linearized HIV-1 pNL4-3 plasmid DNA were serially diluted and amplified in a background of HIV-1-negative human gDNA (the same amount used in the experimental wells) and subjected to the same cycling conditions. Sequences of primers and probe are as follows: first-round PCR, sn-RT-forward primer 1-5'-CAT GTT GGA TGG GGT GAT A-3' and sn-RT-reverse primer 1-5'-CTT GTC TTC GCA TTA TTC TCT TCC-3'; second-round PCR, forward primer identical sn-RT-forward primer 1-5' and sn-RT-reverse primer 2-5'-TGG GCC AGT ATT TTA TTC CCA TTA-3'; sn-RT-probe: 5'-6 FAM-AGC TGG AGC AGT GCA AAC GAC-3' and gDNA was extracted using the QIAamp DNA Blood kit (Qiagen, Hilden, Germany). Conventional PCR was performed using the Roche Expand High-Fidelity (HiFi) PCR System (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) and the qPCR was carried out using LightCycler 480 Probes Master Mix (Roche Diagnostics). Cycling conditions were standard for both Expand HiFi and TaqMan hybridisation probe qPCR. At 9.5 years of age, a total of 9 µg of gDNA extracted from isolated PBMCs (cell equivalent is 1.36 × 10⁶ cells) was tested at an input of 1 µg per well. The standard curves were run in triplicate at each plasmid dilution, except for the 1 copy standard where five replicates were done. At 50 weeks of age, 3 µg of PBMC DNA (cell equivalent is 4.54 × 10⁶ cells) was tested.
An in-house ultrasensitive n-qPCR, targeting the highly conserved integrase (IN) gene designed using all subtype C sequences available on the Los Alamos HIV Database (https://www.hiv.lanl.gov/), was used to detect subtype C HIV-1 DNA and RNA. Briefly, the n-qPCR assay is a two-step PCR with the first PCR being a standard PCR allowed to proceed to end-point (30 cycles) using the Roche Expand HiFi PCR system and the second PCR a hydrolysis probe real-time PCR using Roche LightCycler 480 Probes Master Mix. The total product generated in the first-round PCR is used in the second round. Negative controls include both water and gDNA or RNA extracted from healthy HIV-1-uninfected donors. Positive controls include gDNA/RNA extracted from known HIV-1-infected individuals. Sequences of primers and probe are as follows,

**first-round PCR:**
- n-IN-forward primer-1 5′-TGG CAG TRT TCA TTC ACA ATT TTA-3′
- n-IN-reverse primer-1 5′-TCC TGT CYA CYT GCC ACA CAA TCA-3′

**second-round qPCR:**
- n-IN-forward primer-2 5′-CGG GTT TAT TAC AGR GAC AGC AGA G-3′
- n-IN-reverse primer-2 5′-ACT ACT GCY CCT TCA CCT TTC CA-3′
- n-IN-probe 5′−6 FAM-TTG GCT GGT CCT TTC MGB-3′

**gDNA** was extracted using the QIAamp DNA Blood kit (Qiagen). **RNA** was extracted using the QiAamp RNA Blood kit (Qiagen).

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**Fig. 5** Immunophenotyping of CD4+ and CD8+ T cells. 

(a) Cell parameters determined by flow cytometry for the child at 9.5 years are compared with those of uninfected age-matched children (n = 5) and uninfected adults (n = 10): **a** CD4:CD8 ratio. **b** Percentages of CD4+ and CD8+ T cell subsets according to stage of differentiation. Naïve (CD45RO−CCR7+CD62L+CD95−); SCM: stem cell memory (CD45RO−CCR7+CD62L+CD95+); CM: central memory (CD45RO+CCR7+CD62L+CD95+); TM: transitional memory (CD45RO+CCR7−CD62L+CD95+); EM: effector memory (CD45RO−CCR7−CD62L−CD95+). **c** CCR5 density and percentages of cells expressing CCR5, HLA-DR (immune activation), TIGIT and PD-1 (immune checkpoint inhibitory molecules) on CD4+ and CD8+ T cells. Uninfected children were compared to adults using two-tailed non-parametric Mann–Whitney U tests; individual data points are shown as coloured circles; medians are shown as solid lines; statistical significance at P < 0.05 is indicated. One adult outlier (extreme) data point was omitted from the CD8+ HLA-DR+ graph; the adult vs. children comparison was not statistically different with or without this data point (P > 0.05).
extracted from plasma or culture supernatant using the QiaAmp Viral RNA kit. Complementary DNA was synthesized using the Invitrogen SuperScript III First-Strand Synthesis System for RT-PCR (Thermo Fisher Scientific, MA, USA) and a combination of a gene-specific primer (reverse primer-1) and random hexamers. DNase treatment of extracted RNA was carried out using the Ambion Turbo DNA-free kit (Thermo Fisher Scientific). Cycling conditions were standard for both Expand HiFi and TaqMan hybridization probe qPCR.

HIV-1 proviral gq sequencing. The near full-length HIV-1 gq gene encompassing partial p17 to 5′ coding regions to the Gag stop codon was PCR-amplified from gDNA isolated from CD4+ T cells in an in-house nested PCR assay. A first-round PCR amplification was performed using outer PCR primers gag6F (5′-TGT AAA ACG ACT TAG TAT GGG GAA C-3′) and gag9or (5′-TTA CCT TGA TAA AAC CTT CTC C-3′) using the FastStart HighFi DNA System (Roche Diagnostics). A second-round PCR amplification was performed using a tenth volume of first-round PCR product and second-round PCR primers gagginF (5′-TTG CAC TTA ACC CGC TTT TAG A) and gagrin (5′-ATT ATT TCC TTA AAC TGT ATC TTC G-3′) PCR products were purified using Agencourt Ampure XP magnetic bead separation (Becton-Dickinson, Franklin lakes, NJ, USA), cloned into pCRI-TOPO plasmid vectors (Invitrogen, Carlsbad, CA, USA) and transformed into Escherichia coli bacteria. Plasmid inserts were sequenced using BigDye Terminator v3.1 Cycle Sequencing Master Mix (Life Technologies, Carlsbad, CA, USA) and eight sequence primers. The two inner PCR primers were employed, followed by overlapping internal sequence primers (gaginF: 5′-GCT CTT CAG ACA GGA ACA GA-3′, gaggeqF: 5′-GGG CAT CAA GGA GCC ACG GTC C-3′, gaggeqE: 5′-GAA GAA ATG ATG ACA GCA TG-3′, gaggeq8H: 5′-GCC CTT AGT CTA CCT CC-3′, gaggeq8R: 5′-TTT CCC CTA CTT CAA CAG C-3′, gaggeq8s: 5′-TCC CTA CTT CCA CAG CC-3′) were used to generate gq sequencing fragments. Sequencing reactions were resolved on an ABI PRISM 3730 DNA Analyzer instrument (Life Technologies, Carlsbad, CA, USA). DNA sequences were analysed using Sequencher v4.5 software (Gene Codes Corporation, Ann Arbor, MI, USA). A consensus sequence (Supplementary Fig. 1) was derived from sequencing multiple cloned gq fragments (Genbank accession numbers: MH789553–MH789572), and viral subtype determined by phylogenetic analysis with reference subtypes A–D using MEGA version 4.58.

Viral outgrowth assays. The presence of replication-competent virus was assessed by flow cytometric analysis of CD4+ T cells isolated from a stored sample of the case at 50 weeks of age (2 × 10⁶ cells) and from a fresh sample at 9.5 years (2 × 10⁶ cells) were activated with phytohaemagglutinin (PHA) (5 µg mL⁻¹) for 2 days in media supplemented with IL-2 (200 U mL⁻¹), Roche Diagnostics). Proliferation was added and 8 µL of PHA-stimulated DB4-depleted PBMCs (5 × 10⁶ cells) from a healthy HIV-1-uninfected donor (with high CCR5 expression as determined by flow cytometry) or 3 × 10⁵ MOLT4/CRC5 cells. Culture supernatants were harvested after HIV-1 p24 by ELISA (5 µL) (10 × 10⁶ cells) from an HIV-1-uninfected donor (high CCR5 expression as determined by flow cytometry) or 3 × 10⁵ MOLT4/CRC5 cells. Culture supernatants were harvested at days 5 and 7, and then inoculated into a suspension of beads (supplementary Table 4). To each well of a 96-well plate (Bio-Rad), 50 µL of working microsphere mixture prepared in assay buffer (PBS, 1% BSA; 2500 beads from each bead region per well), 40 µL of assay buffer and 25 µL of virus (protein A coated with human immunoglobulin G) was added. After 2 h at 37 °C, the plate was washed three times with washing buffer (PBS, 0.1% BSA, 0.05% Tween-20) with a final wash in assay buffer. HIV-specific antibody immobilized to bead, detected by addition of 1 µg of the costimulatory antibody CD28 and 1 µg of the costimulatory antibody CD49d. Incubation buffer (500 µL) 0.05% sodium azide), counted using a haemocytometer and stored in the −20 °C. HIV-specific immunoglobulin isotypes were detected by preparing a microsphere mixture comprising the seven HIV antigen-coupled beads (gp41, GAG, RT, gp120, Nef, Tat, Vpu, peptide Vpu9 and V1V2 scaffold; Supplementary Table 4). To each well of a 96-well flat bottom Greiner plate (Bio-Rad, 50 µL of working microsphere mixture prepared in assay buffer (PBS, 1% BSA; 2500 beads from each bead region per well), 40 µL of assay buffer and 25 µL of virus (protein A coated with human immunoglobulin G) was added. After an incubation period (on a shaker in the dark at room temperature; 2 h), the plate was washed three times with washing buffer (PBS, 0.1% BSA, 0.05% Tween-20) with a final wash in assay buffer. HIV-specific antibody immobilized to bead, detected by addition of 1 µg of the costimulatory antibody CD28 and 1 µg of the costimulatory antibody CD49d (Bio-Rad) by ELISA (Bio-Rad Laboratories) and a beacon ssDNA probe representing Gag, Nef, Pol, Nef, Env, Tat, Rev, Vpu, Vpr and Vps proteins. Briefly, 200 µL of whole blood, collected in sodium heparin, was stimulated with a final concentration of 10 µg mL⁻¹ of peptide (synthesized as 15–18 mers overlapping by 10 amino acids), with the exception of Nef, which overlaps by 11 amino acids, NMI, Germany) together with 1 µg of the costimulatory antibodies CD28 and CD49d (biotin-conjugated mouse anti-human IgG2, to IgG2, (Cat. No. 90150–09, Southern Biotech, USA), mouse anti-human IgM (Cat. No. 9020–09, Southern Biotech, mouse anti-human IgA (Cat. No. 9130–09, Southern Biotech) with shaking (in the dark at room temperature) followed by three washes. Beads were finally resuspended in Bio-Rad sheath fluid and analysed using the Bio-Plex 200 instrument (Bio-Rad) by measuring the fluorescence signal for 50 beads per analyte. Background signal, defined as the mean fluorescence intensity (MFI) for each microsphere when set with PE detection in the absence of patient plasma, is subtracted from the fluorescent intensity of each sample. The cut-off for positivity for each analyte is calculated as the average MFI value from pooled plasma from HIV-1-uninfected patients plus 3 standard deviations. HIV-1 IgG (Cat. No. 3957; NIH AIDS Reagent Program) served as a positive control for each plate and the MFI values tracked with a Levy–Jennings plot.

Intracellular cytokine assays. HIV-1-specific T cell (CD4+, CD8+) and NK cell responses were measured using a whole blood intracellular cytokine assay (IFN-γ and IL-2) performed with overnight stimulation using the 7-HLA-1 plus 3 standard deviations. HIV-1 p24 in pooled and pelleted supernatant samples. MOLT4/CCR5 cells (Cat. No. 340975 and 340976, respectively, BD Biosciences) and the transport inhibitor brefeldin A (10 µg mL⁻¹; Sigma-Aldrich). Positive controls were stimulated with 1 µg/mL SEB (Cat. No. 54881, Sigma-Aldrich) for T cells or with 5 ng mL⁻¹ anti-CD26 antibody (Clone ebco8B16, Cat. No. 16–0168–05, ebioscience, San Diego, CA, USA) for NK cells. The latter measures reverse ADCC, therefore providing a measure of the capacity of NK cells to perform ADCC through engagement with FcyRIIA/FcγRIIa receptors. To monitor spontaneous cytokine release, the equivalent amount of dimethyl sulfoxide, as in the peptide tubes, together with the costimulatory antibodies was prepared. Samples were incubated for 6 h at 37 °C, after which they were maintained at 18°C overnight. Twenty microlitres of ethylene-diaminetetra-acetic acid (EDTA) was added for 15 min, following which red blood cells were lysed for 10 min at room temperature using 2 mL FACs lysing solution (BD Biosciences). FACS permeabilizing solution (500 µL; 5 min; room temperature; BD Biosciences) was added to centrifuged samples which were washed twice before staining with CD3 PerCP (clone SK7, Cat. No. 345766, 5 µL), CD4 FITC (clone SK1, Cat. No. 343713, 5 µL), CD4 BV786 (clone 120, Cat. No. 563914, 0.8 µL), CD56 APC (clone NCAM16.2, Cat. No. 341025, 5 µL of a 1:6 dilution), IL-2 phycoerythrin (PE) (clone 344411, Cat. No. 340450, 10 µL) and IFN-γ PE (clone 237231, Cat. No. 340652, 5 µL) for 60 min at room temperature in the dark. Stained cells were acquired using a 4-laser BD LSRFortessa® X-20 flow cytometer (BD Biosciences) and analysed (gating strategy in Supplementary Fig. 2) using the FlowJo Software (Tree Star Inc, Ashland, OR, USA).

Multiparameter flow cytometry. Whole blood immunophenotyping by multicolour flow cytometry (to determine proportions of naive/memory CD4+ and CD8+ T cell subsets) included measures of CCR5 expression (density and %), immune activation (HLA-DR) and immune exhaustion (TIGIT and PD-1). The following antibodies were used, in three antibody panels: CD19 APC-7H (clone SK7, Cat. No. 560176, 2 µL), CD8 PerCP (clone SK1, Cat. No. 347314, 6.5 µL), CD62L APC (clone NCAM16.2, Cat. No. 341025, 5 µL of a 1:6 dilution), IL-2 phycoerythrin (PE) (clone 344411, Cat. No. 340450, 10 µL) and IFN-γ PE (clone 237231, Cat. No. 340652, 5 µL) for 60 min at room temperature in the dark. Stained cells were acquired using a 4-laser BD LSRFortessa® X-20 flow cytometer (BD Biosciences) and analysed (gating strategy in Supplementary Fig. 2) using the FlowJo Software (Tree Star Inc, Ashland, OR, USA).
gating strategies in Supplementary Fig. 3-5) using the FlowJo Software (Tree Star Inc.).

**Host genotyping.** HLA class I and II and KIR genotyping was conducted on gDNA extracted from blood cells (QiaAmp DNA blood mini kit, Qiagen). The sequence-based typing resolver kits (Conexio Genomics, Fremantle, Australia) were used to generate HLA-A, HLA-B, HLA-C, DRB1, DPB1 and DQB1 amplicons for DNA sequencing as described by the manufacturers. The exons included for DNA sequencing are: exons 1–4 for HLA-A and HLA-B; exons 1–8 for HLA-C; exons 2 and 3 for HLA-DRB1 and HLA-DQB1; exons 1–5 for HLA-DPB1.

The presence or absence of the 16 KIR genes (14 functional and 2 pseudogenes 2DPI and 3DPI) were determined using allele-specific (AS) primers (Supplementary Table 5) in a real-time PCR assay27. Briefly, PCR reactions were performed in a 5 µL volume, containing 2X Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Burlington, ON, Canada), 0.2 µM of KIR-specific primers, 0.2 µM of galactosylceramide-specific primers and 5 ng of DNA. Thermocycling was performed using the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95 °C for 10 min, followed by 30 cycles of 95 °C for 15 s and 60 °C for 1 min, with subsequent melt-curve analysis.

Published primers and probe specific for KIR2DS4 full-length (f: `001`) or truncated (r: `005`), KIR3DL1/S/S/S` were used in combination with the primers and probe specific for the human β-globin (BGB) reference gene28. (Supplementary Table 6) in a probe hydrolysis-based relative quantification real-time allele-specific (AS)-PCR assay to determine gene copy number variation (KIR2DS4 and KIR3DL1/DS1). To facilitate target and reference gene multiplexing, KIR-specific probes were labelled at the 5′ end with the fluorochrome VIC, while BGB probes were labelled with the fluorochrome FAM. Control samples of known gene copy numbers were run concurrently with unknowns and gene copy numbers inferred using a delta Ct method (difference between Ct values obtained for KIR and BGB genes). All samples were run in duplicate. Real-time AS-PCR amplification was performed in 96-microwell PCR plates using an ABI7500 real-time PCR system (Life Technologies, Carlsbad, CA, USA). Reaction volumes were 5 µL containing 5 ng of genomic DNA, 2X LightCycler 480 Probes Master Mix (Roche), 0.5 µM KIR3DL1/S or KIR2DS4/f forward/reverse primers (Inqaba Biotec, Pretoria, RSA), 0.5 µM BGB forward/reverse primers (Inqaba Biotec, Pretoria, RSA), 0.1 µM VIC-labelled KIR3DL1/S or KIR2DS4/f probe and 0.1 µM FAM-labelled BGB probe (Life Technologies, Carlsbad, CA, USA). Cycling conditions were an initial incubation of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 10 s and 60 °C for 30 s.

**Data availability**

The data that support the findings in this study are available from the corresponding authors upon reasonable request. The source data underlying Figs 1a–c and 3a–c are provided in Supplementary Tables 1 and 3, respectively. Genbank accession numbers for cloned gag proviral sequences are MH789953–MH789972.

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Author contributions

A.V. and C.T.T. conceived, designed and obtained ethical clearance for the study. A.V., M.F.C., L.K., A.L., J.M.B. and D.G. contributed the clinical data. K.O. analysed the clinical data. M.P. performed and analysed data from sensitive HIV RNA and DNA assays. D.B.S. performed and analysed data from viral growth assays and from HIV-specific antibody profiling. D.B.S. and S.S. performed and analysed data from intracellular cytokine assays, respectively. S.S. developed the immunophenotyping flow cytometry panels. B.D.C.D. and S.S. conducted immunophenotyping assays and analysed the flow data. S.L. performed host genotyping and virus subtyping. C.T.T. interpreted the collective scientific data and wrote the manuscript, which was reviewed and edited by all authors.

Additional information

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