Nonprogressing HIV-infected children share fundamental immunological features of nonpathogenic SIV infection

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Disease-free infection in HIV-infected adults is associated with human leukocyte antigen–mediated suppression of viremia, where the sooty mangabey and other healthy natural hosts of simian immunodeficiency virus (SIV), viral replication continues unabated. To better understand factors preventing HIV disease, we investigated pediatric infection, where AIDS typically develops more rapidly than in adults. Among 170 nonprogressing antiretroviral therapy–naïve children aged >5 years maintaining normal-for-age CD4 T cell counts, immune activation levels were low despite high viremia (median, 26,000 copies/ml). Potent, broadly neutralizing antibody responses in most of the subjects and strong virus-specific T cell activity were present but did not drive pediatric nonprogression. However, reduced CCR5 expression and low HIV infection in long-lived central memory CD4 T cells were observed in pediatric nonprogressors. These children therefore express two cardinal immunological features of nonpathogenic SIV infection in sooty mangabeys—low immune activation despite high viremia and low CCR5 expression on long-lived central memory CD4 T cells—suggesting closer similarities with nonpathogenetic mechanisms evolved over thousands of years in natural SIV hosts than those operating in HIV-infected adults.

INTRODUCTION

Without antiretroviral therapy (ART), HIV infection in >99% of cases inevitably results in the development of AIDS. However, despite undetectable viral loads in a small subset of ART-naïve adults (elite controllers) or in ART-treated individuals, systemic immune activation levels remain higher than in uninfected individuals (1, 2). This gives rise to an increased risk of non-AIDS mortality and morbidities normally linked with aging, including cardiovascular disease, malignancy, and cognitive dysfunction (3, 4). Even in viremic individuals, it has long been recognized that viral replication is not the major cause of HIV disease but rather the high levels of immune activation that typically result from infection (5, 6).

The central role of immune activation rather than viral replication in HIV pathogenesis has been highlighted by studies of the natural hosts of simian immunodeficiency virus (SIV) infection, such as the sooty mangabey and the African green monkey (7). In these and some 40 species of nonhuman primates naturally infected with SIV (8, 9), high levels of viral replication are observed, typically with viral set points of ~10⁵ copies/ml, and yet these animals do not suffer from disease as a consequence. In adult HIV infection, immune activation is linked to viral load, whereas in the natural SIV hosts, immune activation remains low despite persistent high viremia. Understanding the mechanisms by which low systemic immune activation might arise in natural HIV infection independent of viral replication therefore is of major importance not only for vaccine development but also to address the growing burden of non-AIDS HIV-associated disease in individuals receiving long-term ART.

In pediatric HIV infection, disease progression in the absence of ART is typically more rapid than in adults, with the median time to AIDS being 1 year, compared to 10 years in untreated adult infection (10). It has long been recognized that progression in pediatric infection is biphasic (11, 12), with 60% mortality by 2.5 years (12); thereafter, progression to disease is much slower. A subset of ART-naïve, HIV-infected children who are clinically healthy and maintain normal-for-age CD4

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T cell counts throughout childhood exist (11, 13, 14). Previous reports of pediatric nonprogressors (PNPs) have not been plentiful, but it is clear that PNPs are much more common than their adult viremic nonprogressor (AVNP) counterparts (15, 16). Although no consistent CD4 and age criteria have been used to define PNPs (11, 14, 17), about 10% of ART-naïve, HIV-infected children reach middle childhood (ages 6 to 8 years) without disease, maintaining normal-for-age absolute CD4 T cell counts. Because, with very few exceptions, the PNP subjects described in the current study were only identified incidentally some years after birth, the precise percentage of these children becoming PNP is unknown, but a figure of 5 to 10% would be consistent with our own longitudinal studies in Durban (13). PNPs have been understudied because the pediatric HIV epidemic is so heavily concentrated outside North America and Europe. However, large nonprogressor pediatric cohorts similar to those presented here have been described from Uganda and Thailand (17, 18).

The mechanisms of nonprogression in children are not defined but differ from those in adult controller adults. Although “protective” human leukocyte antigen (HLA) class I alleles such as HLA-B*27 or HLA-B*57 are expressed in >50% of “elite controller” adults (19), by contrast, HLA class I variation does not influence progression rates significantly in pediatric infection (20). Furthermore, the high CD4 T cell counts observed in elite controller adults are associated with low viral loads beneath the level of detection, whereas in nonprogressor children, as described previously (17, 18) and in the current study, high CD4 T cell counts are typically associated with persistent high viral loads. To identify potential mechanisms of HIV nonpathogenesis, we first investigated whether nonprogression in pediatric infection is dependent on strong virus-specific immune responses, as in adult HIV elite controllers (21), or is independent of them, as in the natural hosts of SIV infection (22). We then tested the hypothesis that, as in sooty mangabeys (23), reduced infection of the long-lived central memory (Tcm) and stem cell memory (Tscm) CD4 T cells may contribute to the maintenance of high CD4 T cell counts despite persistent viremia in these nonprogressor children.

RESULTS
Normal CD4 T cell counts, low immune activation, and high viremia in PNPs
On the basis of normal absolute CD4 T cell counts in HIV-uninfected children (24, 25), we here defined PNPs as ART-naïve, HIV-infected children with CD4 T cell counts of >750 cells/mm³ at age ≥5 years, who have not met the CD4 or clinical criteria for ART initiation. An example of one such PNP followed from birth is shown in Fig. 1 (A to C). Characteristically, although absolute CD4 T cell count and percentage (CD4%), that is, percentage of peripheral blood lymphocytes expressing CD4) are close to the 50th centile for age-matched HIV-uninfected children, the viral loads reach a quasi–set point of 10⁴ to 10⁵ copies per milliliter of plasma. Pooling all the available longitudinal data from the 170 PNPs in the cohort of African children indicates a decrease in viral load over the first 5 years of life, to a viral set point of 20,000 to 30,000 copies/ml (Fig. 1, D and E). Viral loads of <100 copies/ml among PNPs are exceptionally rare (10). Furthermore, also in contrast with ART-naïve nonprogressor adults, in whom sustained high CD4 T cell counts are typically found in association with low viral loads (26), there is no association between absolute CD4 T cell count and viral load in PNPs [Fig. 1F; r = 0.06, P = not significant (ns)].

These data suggest a similarity between PNP and the natural hosts of SIV infection, such as sooty mangabeys and African green monkeys, in whom persistent high viral loads are also associated with lack of disease progression and CD4 T cell preservation, and levels of immune activation are low (7). We observed levels of immune activation (CD38/HLA-DR coexpression on CD4⁴ and CD8⁸ T cells) that were strikingly low especially on CD4⁴ T cells in the PNPs and a strong negative correlation between levels of immune activation and absolute CD4 T cell count or CD4% across ART-naïve, HIV-infected children (Fig. 1, G and H). Notably, CD4 T cell activation was more strongly associated than CD8 T cell immune activation with CD4 T cell count among these infected children, in contrast with the stronger associations between immune activation of CD8 T cells and CD4 T cell count observed in adult infection (6). Furthermore, levels of soluble CD14 (sCD14) that are characteristically raised in pathogenic SIV or HIV infection, partly as a consequence of microbial translocation and chronic stimulation of monocytes/macrophages by lipopolysaccharide (27, 28), were similar in PNPs and uninfected children and significantly lower compared to age-matched progressor children (Fig. 1I). Pediatric “progressors” are defined here as ART-naïve children aged ≥5 years who met the prevailing CD4 T cell criterion for ART initiation, that is, an absolute CD4 T cell count of <500 cells/mm³. Levels of intestinal fatty acid–binding protein (iFABP), a biomarker of intestinal villous damage predictive of HIV-associated disease (29), were significantly lower in PNPs compared to progressors and also to pediatric uninfected controls (Fig. 1I). These data are consistent with a picture similar to that in the natural hosts of SIV infection, in which low immune activation in the face of persistent high viral loads is observed, associated with evidence of low-level microbial translocation (27).

Low-level CD4 T cell differentiation and maintained function in nonprogressing children
Nonpathogenesis despite high viremia, is independent of SIV-specific cellular immunity in sooty mangabeys (22). To determine whether virus-specific immune responses may play a role in nonprogressing pediatric HIV infection, we first evaluated CD4 T cell differentiation and function in PNPs. To compare CD4 T cell responses with age-matched uninfected children as well as with progressor children, we initially measured intracellular production by CD4⁴ T cells of T helper cell (TH1) cytokines interferon-γ (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor–α (TNF-α) in response to staphylococcal enterotoxin B (SEB). IL-2 production in PNP did not differ from that in infected controls but was significantly higher than that in progressors (Fig. 2, A and B). IFN-γ production was conversely significantly higher in progressors than in the other two groups. Similarly, in response to a pool of overlapping Gag peptides, intracellular IFN-γ production was lower and IL-2 responses in CD4 T cells were significantly higher in PNPs compared to progressors (P = 0.01; Fig. 2C). The same broad patterns were observed in CD4 T cell responses to the pools of Pol and Nef HIV proteins also tested (fig. S1, A and B).

These functional differences reflect the distinct patterns of CD4 T cell differentiation among PNP and progressor children. The levels of naïve CD4 T cells in PNPs are similar to those in age-matched uninfected children (median, 58%; 10th to 90th centiles, 42 to 74%; Fig. 2, D and E) (24, 25). In pediatric HIV infection, analyzing PNPs and progressors together, the percentage of naïve CD4 T cells is strongly associated with
high absolute CD4 T cell count, whereas an increasing percentage of effector memory CD4 T cells is associated with decreasing absolute CD4 T cell count (Fig. 2, D and E). Functionally, in response to SEB, IL-2 is principally produced by naïve and central memory CD4 T cells, whereas IFN-γ and TNF-α are mainly produced by effector memory CD4 T cells (Fig. 2, F and G). Expression of exhaustion markers such as PD1 and 2B4 is also higher on all CD4 T cell subsets in progressors compared to PNPs (Fig. 2, H and I, and fig. S1C). Together, these findings of increased CD4 T cell differentiation, exhaustion, and dysfunction in the pediatric progressors compared to PNPs are consistent with previous observations of the hierarchical exhaustion of the CD4 T cell response characteristic of chronic viral infection and persistent high viremia (30). In contrast, these features are largely absent in the PNPs, despite persistent high viral loads.

Notably, the high proportion of naïve T cells observed here among the PNPs (Fig. 2E) did not explain the low immune activation levels described above (Fig. 1, G and H) in these children. Comparing cells of the same differentiation phenotype by absolute CD4 T cell count, in each case, immune activation levels were correlated with absolute CD4 T cell count (Fig. 2, K and L).

### CD4 T cell immune activation is the major driver of progression in pediatric infection

Identification of multiple variables differentiating progressing versus nonprogressing pediatric infection, including level of immune activation, markers of microbial translocation, T cell differentiation, CD4+ and CD8+ T cell subset function, absolute CD4 T cell count, and viral load, prompts the question, what is the primary driver of progression in pediatric infection? To help visualize the nature of the associations between these parameters, we constructed a correlation matrix using data from a subset of 45 ART-naive children in whom measurement of all parameters had been undertaken at the same study visit (Fig. 3). Variables were grouped on the basis of principal components analysis using the R package “corgramm” (31). A clear dichotomy emerges between
Fig. 2. CD4 T cell differentiation and function in nonprogressing pediatric infection. (A to C) IFN-γ, IL-2, and TNF-α intracellular cytokine staining (ICS) response to SEB and Gag in PNPs. (A) Representative fluorescence-activated cell sorting (FACS) staining in a progressor and a non-progressor. (B) ICS responses to SEB in nonprogressors (n = 14), progressors (n = 32), and uninfected pediatric controls (n = 19), gating on bulk CD4+ T cells. (C) ICS responses to a Gag peptide pool in nonprogressors (n = 26) and progressors (n = 11), gating on bulk CD4+ T cells. (D and E) CD4 T cell differentiation in pediatric progressors and non-progressors (n = 161). (D) Representative FACS staining in a progressor and a non-progressor. (E) Percentage of CD4 T cells by absolute CD4 T cell count defined as naïve, central memory, effector memory, or terminally differentiated effector memory, by CCR7 and CD45RA expression as shown in (D). Correlation between absolute CD4 T cell count and percent CD4 T cells in each subset shown as Spearman’s rank correlation coefficient and P value. T_emra, effector memory RA. (F and G) Functional differences between distinct CD4 T cell subsets. (F) Representative FACS staining in response to SEB showing that IFN-γ-producing cells are predominantly effector memory, and IL-2–producing cells are predominantly naïve and central memory in phenotype. (G) Contribution of IL-2, IFN-γ, and TNF-α to the total CD4 T cell SEB response. The proportion of cells producing each cytokine to the total CD4 T cell SEB response was determined (data from 11 nonprogressors). (H to J) PD-1 expression on CD4 T cell subsets in pediatric progressors, nonprogressors, and uninfected controls (H). Increased PD-1 expression on CD4 T cells in pediatric progressors (n = 20) compared to nonprogressors (n = 32) and uninfected pediatric controls (n = 22). (I) Increased PD-1 expression on CD4 T cell subsets in pediatric progressors (n = 20) compared to nonprogressors (n = 30). (J) Representative FACS plots showing differential cytokine staining in relation to PD-1 expression. (K and L) T cell activation by memory subsets. Immune activation (CD38/HLA-DR expression) on CD4+ central memory T cells (T_cm) and other CD4+ and CD8+ T cell memory subsets (I) is inversely correlated with absolute CD4 T cell count in ART-naïve children aged >5 years (n = 97). Comparisons between groups were calculated by Mann-Whitney tests (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001). P and r values for bivariate associations were determined by Spearman’s rank correlation tests.
factors associated with disease nonprogression (upper left quadrant) and those associated with progression (lower right quadrant).

To better determine the major driver of these effects that are linked by multicollinearity, we assessed the influence of each parameter using a generalized linear model (GLM), applying the least absolute shrinkage and selection operator (LASSO) principle on scaled covariates \((32, 33)\).

All three of the LASSO fitting routines in R provide very similar results \((34, 35)\), with a consensus of 6 of the 17 covariates selected by all of the methods as making an independent contribution to CD4 T cell count in pediatric HIV infection (Table 1). Notably, viral load made no independent contribution to pediatric progression according to these analyses, consistent with the lack of relationship between absolute CD4 T cell count and viral load in the nonprogressor children described above (Fig. 1F).

Finally, an unregularized GLM analysis \((36)\) was then run on the selected set of six covariates to determine statistical significance and the \(\beta\)-coefficient estimates (Table 1). The covariates most strongly linked to nonprogression were low immune activation of CD4 T cells, followed by low percentage of proinflammatory effector memory CD4 T cells. This analysis confirms the significance of all of these six influences on nonprogressing pediatric infection, driven principally by low immune activation on CD4 T cells.

### Potent, broadly neutralizing antibodies in pediatric compared with adult infection

Although high-titer, broadly neutralizing antibodies (bnAbs) are not a feature of AIDS resistance in sooty mangabeyes \((37–39)\), persistent high viremia in the context of healthy CD4 T cell activity might be expected to provide the optimal setting for the generation of bnAb responses in nonprogressing HIV-infected children \((40–42)\). We initially compared neutralization of a panel of 16 tier2 and tier3 clade A, B, and C viruses by plasma from PNPs and progressors with plasma from a South African cohort of adults \((41)\) 5 years after infection \{viral load median, 31,200 copies/ml \(\pm\) interquartile range (IQR), 3104 to 73,347 copies/ml; absolute CD4 T cell count median, 449 cells/mm\(^3\) \(\pm\) IQR, 308 to 568 cells/mm\(^3\)\}). Overall, 75% \((64 of 85)\) of the pediatric subjects studied were able to neutralize \(\geq 50\%\) of the panel of 16 viruses, compared to 19% of the adults \((P < 0.0001;\) Fig. 4A). The ability of nonprogressing children \{median: age, 6.6 years; absolute CD4 T cell count, 1050 cells/mm\(^3\); viral load, 14,000 copies/ml\} to neutralize this panel of viruses was somewhat less than that of the progressing children \{median: age, 8.2 years; absolute CD4 T cell count, 225 cells/mm\(^3\); viral load, 71,803 copies/ml; Fig. 4B\}. Although these differences between the pediatric groups approached statistical significance \((P = 0.13;\) Fig. 4A), this was likely influenced by the fact that progressors were older and had higher viral loads than...
CD8+ T cell breadth associates with lower viral load but not than in the adult samples (titers of more than 1:1000 was fivefold higher in the pediatric samples all 16 viruses in the panel, the frequency of responses with neutralization also higher (Fig. 4, DoF). For example, comparing nAb activity against observed in the pediatric samples, the magnitude of these responses was \( P = 0.006 \); Fig. 4C). In addition to the greater breadth of virus neutralization pediatric subjects was associated with neutralization breadth (Fig. 4, DoF). The main contributions in the pediatric cohort to decreasing CD4 T cell count in association with increasing CD8+ T cell breadth were the Pol-specific responses (\( r = -0.34 \), \( P = 0.0009 \); Fig. 5E). A GLM of data from the pediatric subjects in whom immune activation had been measured contemporaneously with the ELISPOT assays demonstrated that CD8+ HLA-DR/CD38 expression is significantly and independently correlated with both viral load and CD8+ T cell breadth (Fig. 5F and table S1). These data further support the conclusion that control of viral replication is less important than avoidance of raised immune activation in the maintenance of pediatric HIV nonprogression, because generation of a broad HIV-specific CD8+ T cell response may actually drive disease progression through immune activation.

### Table 1. Association of clinical and immunological parameters with CD4 T cell count

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<td>CD4 Tem</td>
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The main contributions in the pediatric cohort to decreasing CD4 T cell count in association with increasing CD8+ T cell breadth were the Pol-specific responses (\( r = -0.34 \), \( P = 0.0009 \); Fig. 5E). A GLM of data from the pediatric subjects in whom immune activation had been measured contemporaneously with the ELISPOT assays demonstrated that CD8+ HLA-DR/CD38 expression is significantly and independently correlated with both viral load and CD8+ T cell breadth (Fig. 5F and table S1). These data further support the conclusion that control of viral replication is less important than avoidance of raised immune activation in the maintenance of pediatric HIV nonprogression, because generation of a broad HIV-specific CD8+ T cell response may actually drive disease progression through immune activation.

**CD8+ T cell breadth associates with lower viral load but not nonprogression in children**

We next investigated whether HIV-specific CD8+ T cell responses might influence progression in pediatric infection. In sooty mangabeys, SIV-specific CD8+ T cell breadth is unrelated to CD4 T cell count or viral load (22), but in adult HIV infection, increasing breadth of the virus-specific Gag-specific CD8+ T cell response is related to decreasing viral load and increasing CD4 T cell count (4#). For each subject, recognition of a panel of 410 overlapping peptides spanning the HIV-1 C clade proteome was determined in enzyme-linked immunospot (ELISPOT) assays, initially using pools of 12 peptides and then confirming recognition of individual peptides in a second assay. Exactly as previously described in adults (44), there is a discordant protein-specific relationship between the breadth of the CD8+ T cell response and viral load, with increasing Gag breadth associated with decreasing viral set point, and increasing Env breadth associated with increasing viral set point (Fig. 5, A to C). However, in contrast to adults, the breadth and magnitude of the HIV-specific CD8+ T cell response were associated with decreasing absolute CD4+ T cell count, an indicator of disease progression (Fig. 5D). The main contributions in the pediatric cohort to decreasing CD4 T cell count in association with increasing CD8+ T cell breadth were the Pol-specific responses (\( r = -0.34 \), \( P = 0.0009 \); Fig. 5E). A GLM of data from the pediatric subjects in whom immune activation had been measured contemporaneously with the ELISPOT assays demonstrated that CD8+ HLA-DR/CD38 expression is significantly and independently correlated with both viral load and CD8+ T cell breadth (Fig. 5F and table S1). These data further support the conclusion that control of viral replication is less important than avoidance of raised immune activation in the maintenance of pediatric HIV nonprogression, because generation of a broad HIV-specific CD8+ T cell response may actually drive disease progression through immune activation.

**Decreased HIV infection in long-lived memory CD4 T cells (Tem and Tscm) in PNPs**

To further investigate mechanisms to explain the maintenance of normal-for-age CD4 T cell counts in nonprogressing children despite persistent high viral loads, we next addressed the hypothesis that the CD4 T cells predominantly infected with HIV are the short-lived effector memory subsets in these children, in contrast to progressive HIV infection in which the long-lived central memory CD4 T cells are the main T cell subset infected with HIV. Similar observations have been made in the natural hosts of SIV—sooty mangabeys and African green monkeys—compared with pathogenic SIV infection in the rhesus macaque (23). We first determined expression of CCR5 on different CD4 T cell subsets and noted strikingly lower CCR5 expression on central...
Fig. 4. Neutralization of a panel of 16 tier 2 and tier 3 subtype C, B, and A viruses by pediatric and adult plasma samples. (A) Neutralization breadth in PNP (n = 66) and progressors (n = 19) compared with adults (n = 21). (B) The frequency of bnAbs among PNP (median: age, 6.6 years; absolute CD4 T cell count, 1050 cells/mm²; viral load, 14,000 copies/ml), pediatric progressors (median: age, 8.2 years; absolute CD4 T cell count, 225 cells/mm²; viral load, 71,803 copies/ml), and adults (5 years after infection; median: absolute CD4 T cell count, 449 cells/mm²; viral load, 31,200 copies/ml). (C) Correlation between viral load and percent viruses neutralized among pediatric subjects. P and r values were calculated by Spearman’s rank correlation tests. (D to F) Geometric means of neutralization titers for pediatric and adult samples against subtype C (n = 6), B (n = 6), and A (n = 4) viruses. Comparisons between groups were calculated by Mann-Whitney tests.

Fig. 5. HIV-specific CD8⁺ T cell responses in pediatric infection in relation to viral load and absolute CD4 count. (A to C) Breadth of IFN-γ ELISPOT responses correlates with viral load in a protein-specific fashion. (A and B) Total breadth and Gag-specific breadth of IFN-γ ELISPOT response (n = 90). (C) Env-specific breadth of IFN-γ ELISPOT response (n = 90). (D and E) Total breadth and Pol-specific breadth of IFN-γ ELISPOT response in ART-naïve pediatric subjects are inversely correlated with absolute CD4 count (n = 90). (F) Total breadth of IFN-γ ELISPOT response directly correlates with CD8⁺ T cell activation in pediatric infection (n = 30, Spearman’s rank correlation tests).
memory (Tcm) CD4 T cells in the nonprogressor children both compared to progressor children (P < 0.0001) and compared to CCR5 expression in nonprogressors on effector memory cells (Tem) (Fig. 6, A and B). To compare HIV DNA levels in nonprogressor children with those in control subjects in whom data were not confounded by the effects of CD4 T cell depletion, we identified a group of progressing HIV-infected adults, termed here “future progressors,” from whom cryopreserved samples were available before progression, at a time when absolute CD4 T cell counts (median, 1106 CD4 T cells/mm^3; IQR, 811 to 1284 cells/mm^3; CD4%, 36%; IQR, 28 to 40%) were well within the normal range for uninfected individuals. Viral loads in this control group (median, 31,500; IQR, 16,750 to 119,500) were also matched with the PNPs (Fig. 6C). Consistent with the CCR5 expression data described above, in the PNPs, lower HIV DNA levels were observed in the long-lived stem cell memory (Tscm) and central memory (Tcm) CD4 T cell subsets compared to those in effector memory cells (Tem) (P < 0.0001 in each case; Fig. 6D). As previously shown in pathogenic SIV infection in rhesus macaques (23) and also in studies of HIV-infected adult progressors (15), in the future progressors, HIV infection of Tscm and Tcm was higher than that of Tem, whereas the reverse was the case in the nonprogressing children (Fig. 6, C and D). These data therefore are consistent with the hypothesis that, in nonprogressing children, reduced HIV infection of the long-lived CD4 T cell subsets, Tscm and Tcm, in association with relatively low expression of CCR5 on these cells, enables absolute CD4 T cell counts to be maintained despite persistent high viremia.

**DISCUSSION**

The mechanisms by which disease-free infection is achieved in HIV-infected elite controllers and in the natural hosts of SIV infection, such as the sooty mangabey and the African green monkey, are diametrically opposed. Elite controllers suppress HIV replication to undetectable levels via immune responses mediated principally by protective HLA class I molecules such as HLA-B^*27 and HLA-B^*57. In contrast, nonpathogenic SIV infection is independent of virus-specific immunity, and viral replication continues unabated but without ill consequences. These current studies of nonprogressing pediatric HIV infection demonstrate that, although—in contrast to the sooty mangabeys—strong virus-specific immune activity is present, it is not the major contributor to the maintenance of normal CD4 T cell counts and absence of disease, consistent with observations in the natural hosts of SIV (22, 37–39). The normal-for-age CD4 T cell counts and low immune activation despite persistent high viral loads seen in these nonprogressing children are also characteristic of SIV infection in African nonhuman primates (7). In both cases, CD4 T cell count is unrelated to viral load. A further feature of nonprogressing pediatric HIV infection in common with nonpathogenic SIV
infection in the sooty mangabey natural hosts is decreased CCR5 expression on long-lived central memory CD4 T cells and the associated low level of virus infection in these T_{cm} and T_{em} subsets (23). Together, these data suggest that the mechanisms that operate to achieve disease-free pediatric HIV infection may have closer similarities to those that have evolved over thousands of years (45) in African nonhuman primates as a consequence of SIV infection than to the well-described HLA-mediated immunosuppression of HIV observed in adult elite controllers.

However, clear differences are apparent between nonpathogenic SIV infection and nonprogressing pediatric HIV infection, particularly in relation to virus-specific immune responses observed. SIV-specific CD4^{+} T cell responses in sooty mangabeys are relatively low in magnitude and breadth (22), whereas the same cytokine responses—IFN-γ, IL-2, and TNF-α—measured here, in response to HIV peptides in the nonprogressing HIV-infected children, were of relatively high magnitude and breadth. Similarly, nAb responses in sooty mangabeys are detectable but are relatively low level (37–39), whereas remarkably potent and bnAb responses are observed in the PNPs, albeit somewhat lower than those in the progressors. Nonetheless, in common with the sooty mangabey, the virus-specific cellular and humoral responses detected in the nonprogressing children appear not to be primarily responsible for lack of immunodeficiency virus disease, as evidenced by the GLM and the observation of equivalent or potentially broader nAb responses in the nonprogressing children compared to the nonprogressors.

The second of the two key immunological features of nonpathogenic SIV infection shared by PNPs, in addition to low immune activation despite high viral load, is low CCR5 expression on long-lived central memory CD4 T cells. This finding would help explain how normal absolute numbers of long-lived central memory T cells, despite high viral load, are maintained by modestly increased thymic output while maintaining normal naïve CD4 T cell numbers. Low CCR5 expression has also been proposed to play a role in the surprisingly low mother-to-child transmission rates (<7%) observed in sooty mangabeys, given their high viral loads (46), compared to HIV-infected humans (~40% in breast-fed infants) (10).

Comparable studies of cellular subset localization of virus have been undertaken in the rare group of HIV-infected adults—so-called AVNPs—who express a similar phenotype to the PNP children of normal CD4 T cell counts despite persistent high viral loads (15). In a comparison of cellular localization of HIV infection in AVNPs with putative progressor adults who were matched for CD4 T cell count, viral load, and immune activation levels but studied before progression to minimize possible confounding effects of CD4 T cell decline, reduced HIV infection in T_{cm} and T_{em} was observed in the AVNPs in association with increased numbers of these long-lived memory cells (15). The AVNPs showed increased proliferation of CD4^{+} memory T cells compared to the putative progressors, as did SIV-infected sooty mangabeys compared to either uninfected sooty mangabeys or SIV-infected rhesus macaques with progressive disease (47), consistent with the rapid turnover of T_{cm} being maintained by modestly increased thymic output in nonpathogenic infection. In progressive HIV and SIV infections, the viral reservoir is principally localized in the central memory CD4^{+} T cells (23, 48), and preferential infection of T_{cm} is a feature of progressive HIV infections (49).

Although the cellular distribution of HIV in PNPs is substantially lower in long-lived central memory versus effector memory CD4 T cells and therefore is similar to the cellular localization of virus observed in SIV infected sooty mangabeys, the precise contribution of low CCR5 expression, independent of immune activation, remains to be fully evaluated in these nonprogressor children. In the measurements of CCR5 expression undertaken here, resting and activated cells were not differentiated as immune activation markers were in a separate antibody panel. In part, therefore, CCR5 expression differences between PNPs and progressors could theoretically have resulted from differences in immune activation. However, the current data can accurately be used to compare different subsets within the same patient where levels of activation will be consistent. These data show that, in PNPs, there are substantially lower levels of CCR5 in central memory cells compared to effector memory cells. However, in the progressors, levels of CCR5 in central and effector memory cells are similar. These data indicate that substantially lower levels of CCR5 in longer-lived subsets only exist in PNPs and not in progressors, consistent with lower levels of CCR5 in longer-lived subsets as a mechanism explaining the PNP phenotype.

A further point to note with respect to the measurements of viral DNA in different T cell subsets presented both here and in the sooty mangabey and AVNP studies described above (15, 23) is the fact that total viral DNA is being measured, as distinct from replication-competent virus (50). Unfortunately, there is no assay currently available that accurately quantifies replication-competent virus. Polymerase chain reaction (PCR) approaches substantially overestimate, and viral outgrowth assays substantially underestimate true levels of replication-competent virus (51). However, it has been proposed that total HIV DNA measured here is the most meaningful of all the assays available, because this measure correlates with time to viral rebound after coming off ART (32). Additionally, total HIV DNA has been recently shown to correlate with viral outgrowth measures (53).

The contrast between nonpathogenic HIV infection achieved via suppression of viral replication in adult elite controllers and that achieved via mechanisms independent of HIV-specific immune responses in nonprogressing children deserves further comment. Previous studies have shown that, although more than 50% of adult elite controllers express one of the main protective HLA class I alleles, HLA-B*27 or HLA-B*57 in Caucasians (19), or HLA-B*57/58:01/81:01 in African populations (54), HLA class I differences do not significantly influence disease progression in pediatric infection (20). Although suppression of viremia as a result of strong CD8^{+} T cell responses presented by these protective class I molecules can interrupt and terminate the vicious cycle of immune activation and CD4 T cell decline in adult infection, in pediatric infection, reduction in viremia via increasing potency of the HIV-specific CD8^{+} T cell response coincides with increased immune activation and therefore comes at the cost of CD4 T cell decline (Fig. 5F). The fact that no elite controllers of HIV infection have been described in ART-naïve pediatric infection is itself evidence of the dangers of an immune strategy designed to suppress viremia if at the same time this is associated with increased immune activation. Consistent with this are the reports of a subset of ART-naïve elite controller adults who succeed in suppressing viral replication to undetectable levels but have progressed to AIDS because of increased immune activation (2).

The potency and high frequency of the bnAb responses in nonprogressing children are an additional feature that is striking. As well as described previously, bnAbs typically do not neutralize contemporaneous autologous virus (55) and therefore do not protect against disease
progression (56); bnAbs are characteristically seen in HIV-infected individuals with high viral loads progressing to AIDS (41). Similarly, the bnAbs observed in the PNP s do not appear to protect against progression, because levels are, if anything, higher in the progressors. Nonetheless, bnAbs—defined by neutralization of 50% of a panel of tier 2 and tier 3 clade A, B, and C viruses—were observed in 75% of ART-naive pediatric subjects studied, compared to only 19% of adults infected with the same HIV subtype (clade C). This figure of about one in five HIV-infected adults having bnAbs is highly consistent with other studies (40, 41, 56). In part, this high frequency of bnAbs in the pediatric subjects is driven by persistent high viremia, although the effect here was not strong. However, high viral loads in typical adult infection lead to progression, whereas in the PNP s, it does not. Thus, exposure to high viral loads for a longer duration is more common in pediatric infection.

Previous studies have also shown early generation of bnAbs in HIV-infected children in the first 2 years of life (42). Additional factors contributing to this observation may therefore include the T112 bias of the immune response in early life, which potentiates humoral immunity and therefore might be expected to favor bnAb development (57), and the hypothetical possibility that a proportion of the mothers of PNP s may share the same phenotype and therefore transmit a virus already molded by bnAbs in the mother. Passive immunization of bnAbs in macaques has been shown to result in enhanced autologous neutralizing responses in infants (58).

Nonetheless, irrespective of the apparent lack of a role of the potent bnAb responses in maintaining disease nonprogression in these infected children, access to pediatric cohorts such as those described here represents a precious resource from which there is the potential to isolate novel monoclonal bnAbs that have a key role in the combination bnAb treatment and cure strategies that are being developed against HIV (59). It will also be important in future work to determine whether the bnAbs generated by HIV-infected children differ in specificity from those generated in adult infection, as has been suggested (42).

The study of PNP s described here has focused primarily on HIV-specific cellular immunity, limited to CD8+ T cell and T111 CD4 T cell activity, and the nAb response. Further work is necessary to investigate the potential role of other CD4 T cell subsets, including T112, T117, and regulatory CD4+ T cells, in contributing to nonprogression in these children. The observation of potent bnAb responses among PNP s, although not contributing to slow progression, prompts the question of the mechanism by which these responses are generated and the hypothesis that aspects of follicular helper CD4 T cell activity (60) specific to early life may be key. If sufficient study subject numbers can be recruited to make genetic studies feasible, then it would be important to determine the genetic determinants of the immune strategy adopted by PNP s and the impact of such an immune strategy on responses to vaccines and pathogens other than HIV. Previous studies have addressed the role of HLA class I variation of replicative capacity of the transmitted virus in pediatric nonprogression (20). Finally, the ability of the innate immune system to sense HIV-1 and the resulting activation of innate immune pathways clearly differ across distinct patient groups (61). Immune activation is a strong independent predictor for the speed of HIV-1 disease progression, and low levels of innate immune activation might contribute to the PNP phenotype.

Further studies are therefore needed to determine the specific mechanisms underlying low immune activation and nonprogression in HIV-infected children, as well as in the abovementioned AVNPs. AVNPs, in contrast to their pediatric viremic nonprogressor counterparts described here, appear to be extremely rare. Of the four studies published to date, the largest cohort of AVNPs comprised only nine subjects (15). Although their rarity has restricted the possible analyses, there are also indications in AVNPs of mechanisms in common with the natural SIV hosts, including localization of viral infection predominantly in Tcm, CD4+ T cell subsets as described above (15), as well as high expression of certain regulatory genes such as SOCS1 and EEFID (16). Of note, even among the natural hosts of SIV infection, notably the sooty mangabeys (23) and African green monkeys (62), distinct specific mechanisms have evolved to arrive at the similar disease-free phenotype characterized by high SIV viral loads, low immune activation, and preservation of long-lived memory CD4 T cells. Although Darwinian selection has had sufficient time in the natural hosts of SIV to eliminate the less well-adapted animals, HIV in humans is a very recent infection in the evolutionary time scale, and as a result, there is the opportunity here to study both the well-adapted nonprogressors and the ill-adapted progressors. Further defining these mechanisms is relevant not only to reaching a better understanding of HIV pathogenesis and therefore the development of new immunotherapeutic approaches to prevent HIV disease but also to the HIV cure field, where blocking infection of long-lived memory CD4 T cell subsets such as Tcm may be a prerequisite to elimination of HIV infection (48).

MATERIALS AND METHODS

Study design

The aim of this observational cohort study was to describe the clinical and immunological phenotype of nonprogressing HIV-infected children. More detailed information on conception of individual experiments is provided in the specific sections below.

Study subjects

A total of 275 ART-naive, HIV-1 C clade–infected children from southern Africa were studied from clinics at Kimberley Hospital (Kimberley, South Africa), Ithembalabantu Clinic (Durban, South Africa), and Great Ormond Street Hospital (London, U.K.). PNP s (n = 170) were defined as ART-naive children infected via mother-to-child transmission, aged >5.0 years, whose absolute CD4 T cell count was >750 cells/mm^3. PNP s had a median age of 8.3 years (IQR, 6.6 to 10.5 years), a median absolute CD4 T cell count of 885 cells/mm^3 (IQR, 815 to 1019), and a median viral load of 25,957 copies/ml (IQR, 5338 to 75,500). With very few exceptions, these PNP s were not followed from birth and were identified incidentally some years later. Pediatric progressors were defined as ART-naive children infected via mother-to-child transmission, aged >5.0 years, whose absolute CD4 T cell count was <500 cells/mm^3. For these progressors, the median age was 10.6 years (IQR, 8.35 to 13.0), the median CD4 T cell count was 336 cells/mm^3 (IQR, 197 to 432), and the median viral load was 57,500 copies/ml (IQR, 19,000 to 159,424). The median age of South African HIV-uninfected control children (n = 21) was 13.1 years (IQR, 10.1 to 15.5).

For the corrgram (Fig. 3) and the LASSO model (Table 1), a subset of n = 45 HIV-infected, ART-naive children with available data for all regarded parameters from the same study visit were included in the analysis. This subset comprised ART-naive, HIV-infected children and included both progressors and nonprogressors: median age, 11.5 years (IQR, 8.9 to 13.9 years); median absolute CD4 T cell count,
425 (IQR, 327 to 717 cells/mm³); and median viral load, 39,815 (IQR, 7950 to 158,500).

The adult subjects in whom neutralization assays were undertaken were from the South African CAPRISA 002 cohort, as previously described (41). Five years after infection in these adults, median viral loads were 31,200 copies/ml (IQR, 3104 to 73,347 copies/ml), and median absolute CD4 T cell counts were 449 cells/mm³ (IQR, 308 to 568 cells/mm³).

The subjects termed future progressors (Fig. 5) were infected young adults from Durban, South Africa (median: age, 24 years; absolute CD4 T cell count, 1106 cells/mm³; CD4%, 36%; viral load, 31,500) selected to match the PNPs for CD4 T cell count and viral load in order that measurements of HIV infection in distinct CD4 T cell subsets would not be confounded by CD4 T cell decline. These adults were termed “future” progressors because these individuals were young and therefore identified relatively early in infection and likely to be progressors; in 7 of 12 of the future progressors, later samples with accompanying clinical data were available, showing that in all 7 cases, CD4 T cell count had declined subsequent to the initial time point by a median of 347 CD4 T cells/mm³ within a 6-month period (P = 0.05, paired t test), whereas viral loads were not significantly altered (P = 0.38, Wilcoxon matched-pairs signed-rank test).

Viral load measurement was undertaken using the Roche Amplicor version 1.5 assay until 2010 (range, 400 to 750,000 copies/ml). Thereafter, the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2.0 by Roche (CAP/CTM v2.0) (range, 20 to 10 million copies/ml) was used except at the Ithembalabantu Clinic where the BioMérieux NucliSens Version 2.0 Easy Q/Easy Mag (NucliSens v2.0) assay (range, 20 to 10 million copies/ml) was used from 2010.

Informed consent was obtained from all adult study participants, and for underage children, informed consent was obtained from their caregivers. Additionally, assent to participate in the study was given directly by children in the appropriate age groups. Studies were approved by the University of the Free State Ethics Committee, Bloemfontein; Biomedical Research Ethics Committee, University of KwaZulu-Natal, Durban; and Research Ethics Committee, University of Oxford.

Flow cytometry and ICS assays

Isolated peripheral blood mononuclear cells (PBMCs) were used for staining with fluorescent monoclonal antibodies against markers of immune activation (HLA-DR and CD38), immune exhaustion (PD-1 and 2B4), memory differentiation (CD45RA and CCR7), and CCR5 expression. Briefly, cells were rested in R10 medium for 1 hour at 37°C in 5% CO₂ and then washed with phosphate-buffered saline (PBS) and stained in the dark for 20 min on a 96-well plate in a volume of 50-μl staining solution with titrated concentrations of fluorochrome-conjugated monoclonal antibodies against cell surface markers and the Near-IR Dead Cell solution with titrated concentrations of fluorochrome-conjugated antibodies against cell surfaces. The ability of plasma from infected children and adults to neutralize HIV was measured against a panel of 16 tier 2 and tier 3 clade A, B, and C viruses (listed in table S2) as a reduction in luciferase gene expression after a single round of infection of JCS3bl-13 cells, also known as TZM-bl cells (National Institutes of Health AIDS Research and Reference Reagent Program), with Env-pseudotyped viruses (65). Titer was calculated as the reciprocal plasma/serum dilution causing a 50% reduction of relative light units [median infective dose (ID₅₀)].

Cell sorting and cell-associated virus quantification

CD4⁺ T cells were sorted onto a MoFlo XDP (Beckman Coulter) into T cell subsets Tₙ (naïve), Tₘ (stem cell memory), Tₖ (central memory), and Tₑ (effector memory) on the basis of expression of CD27, CD45RO, and CD28.
8E5 cells containing one integrated copy of HIV-1 per cell were used in a single round of 40 cycles. Template DNA samples were diluted to contain 10^8, 5 × 10^5, and 2.5 × 10^5 cells per well for analysis in triplicate wells of a 96-well plate. Template copies were calculated using ABI software.

**IFN-γ ELISPOT assays**

Responses were determined to a set of 410 overlapping peptides, whose sequence was based on the 2001 C-clade consensus, arranged in a matrix system with 11 to 12 peptides in each pool. Responses to matrix pools were deconvoluted by subsequent testing with the individual 18-mer peptides within each pool, and the identity of the individual 18-mers recognized was thus confirmed, as previously described (54).

**Statistical analysis**

The influence of several predictor variables on the absolute number of CD4^+ T cells was assessed by a GLM (32). In a GLM, the linear predictor has the form η_i = β_0 + x_i β_1 + ... + x_i β_p, and is connected to the (conditional) mean of the response variable, that is, μ_i := E[y_i | x_i] = h(η_i), where g(·) and h(·) denote suitable link and response functions, respectively. x_i := (x_i0, ..., x_ip) is the vector of covariates, and β := (β_0, β_1, ..., β_p)', represents the vector of covariate effects. Additionally, given x_i, the responses y_i are assumed to be (conditionally) independent vectors of a simple exponen- tial family. In the present situation, a Poisson regression model with log-link function is chosen. Approaches to variable selection are used by applying the so-called LASSO principle on scaled covariates (33). All computations have been carried out in the statistical software R. For GLMs, there exist three different LASSO fitting routines in R that allow one to fit the underlying model: the glmnet (34), the penalized (35), and the gplasso (36) packages. For all three methods, the optimal tuning parameter λ, which reflects the amount of penalization and hence controls variable selection, is determined via 10-fold cross-validation on the basis of the model’s deviance. A final unregularized conventional GLM is fitted on the selected set of covariates using the R-function glm. Another statistical analysis was undertaken using Pearson’s chi-square test regarding binary variables, and an ANOVA test for the analysis of variance between groups.

**SUPPLEMENTARY MATERIALS**

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Fig. S2. nAb potency in pediatric and adult subjects.

Table S1. CD8^+ T cell activation is independently associated with total breadth of HIV-specific cytotoxic T lymphocyte responses and viral load.

Table S2. List of pseudoviruses tested for neutralizing sera activity by HIV-1 subtype.

Source data

**REFERENCES AND NOTES**


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RESEARCH ARTICLE


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Nonprogressing HIV-infected children share fundamental immunological features of nonpathogenic SIV infection

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HIV progression at a standstill

Although most people that get infected with HIV develop AIDS, rare individuals maintain immune function in the presence of virus, a phenomenon also seen in natural hosts of the closely related SIV. Muenchhoff et al. describe a cohort of pediatric HIV patients who have normal CD4 T cell counts, despite high viremia and lack of antiviral treatment. These children have low immune activation, including less chemokine receptor CCR5 expression on central memory CD4 T cells, similar to sooty mangabeys infected with SIV. The immune mechanisms described in these patients shed light on HIV pathogenesis, which may help develop future treatments.