Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2012.04569.x

Altered frequency and phenotype of CD4$^+$ forkhead box protein 3$^+$ T cells and its association with autoantibody production in human immunodeficiency virus-infected paediatric patients

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Accepted for publication 19 January 2012
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Summary

The association between immune dysfunction and the development of autoimmune pathology in patients with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) is not clear. The frequency and phenotype of regulatory T cells, as well as the presence of autoantibodies, were evaluated in a paediatric cohort of HIV-infected patients without clinical evidence of autoimmune disease. Lower absolute counts but higher percentages of total CD4$^+$ forkhead box protein 3 (FoxP3)$^+$ T cells were recorded in children with severe immunosuppression than in those without evidence of immunosuppression. The frequencies of classical CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells were not altered, whereas CD4$^+$FoxP3$^+$CD25$^-$ T cells were found increased significantly in patients with severe immunosuppression. Like classical regulatory T cells, CD4$^+$FoxP3$^+$CD25$^-$ T cells display higher cytotoxic T-lymphocyte antigen 4 (CTLA-4) but lower CD127 expression compared with CD4$^+$FoxP3$^-$CD25$^+$ T cells. An improvement in CD4$^+$ T cell counts, along with a decrease in viral load, was associated with a decrease in CD4$^+$FoxP3$^+$CD25$^-$ T cells. The majority of the patients with severe immunosuppression were positive for at least one out of seven autoantibodies tested and displayed hypergammaglobulinaemia. Conversely, HIV-infected children without evidence of immunosuppression had lower levels of autoantibodies and total immunoglobulins. A decline in CD4$^+$FoxP3$^+$ T cell numbers or a variation in their phenotype may induce a raise in antigen exposure with polyclonal B cell activation, probably contributing to the generation of autoantibodies in the absence of clinical autoimmune disease.

Keywords: autoantibodies, paediatric HIV, T_{reg}

Introduction

Circulating CD4$^+$ regulatory T cells (T_{regs}) with constitutive immunosuppressive activity are one of the most important cellular subsets involved in controlling inappropriate or excessive immune activation [1–3]. Although the physiological function of T_{regs} is central for maintaining self-tolerance, the negative regulatory activity of these cells can also be counterproductive, as T_{regs} can suppress immune responses against tumours and viral infections [4–8].

Expression of forkhead box protein 3 (FoxP3), together with the interleukin (IL)-2 receptor α chain (CD25) by CD4$^+$ T cells, are used widely as markers to identify T_{regs}. However, it has been shown that both CD25 and FoxP3 expression could be induced in human naive CD4$^+$ T cells through cell activation, making the identification of FoxP3$^+$ T cells as pure T_{reg} cells difficult [9]. The lack of cell surface expression of CD127 (also known as IL-7 receptor α-chain) has been used to further distinguish T_{regs} [10,11]. Furthermore, cells with regulatory properties are also found in the subset of CD4$^+$FoxP3$^+$CD25$^-$ T cells [12], confirming the heterogeneity of T cell subsets with suppressive functions.

Chronic immune activation and progressive immune exhaustion are central features of human immunodeficiency virus (HIV) pathogenesis. The expansion of T_{regs} in HIV infection could, hypothetically, decrease the magnitude of T cell responses in viraemic patients and render them more susceptible to other pathogens [13]. Alternatively, T_{regs} may have a protective effect, restraining activation-induced immunopathology caused by persistent viraemia [6,14–17].

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Table 1. Summary of clinical characteristics of study participants.

	Group A ($CD4^+ < 15\%$)	Group B ($CD4^+ > 25\%$)	Uninfected ($CD4^+$ 35 ± 3%)
Number of patients	28	37	10
Year age median (range)	12·9 (3·1–17)	11·0 (3–17)	9·5 (2–12)
Co-morbidities (*n*)			No
Herpes zoster	1	1	
Oral candidiasis	1		
Acute otitis media	2		
Cryptococcus neoformans in CNS	2		
Varicella		1	
Pneumonia	5	1	
Tuberculosis	1		
Signs/symptoms of autoimmune disease	No	No	No
ART	*n* = 20 (NRTI, PI)	*n* = 27 (NRTI, PI, NNRTI)	–
Length of treatment	6·3 years	6·8 years	
Viral load mean ± SD (log_{10} IU/ml)	4·02 ± 1·27†	2·55 ± 1·18	–

†Significantly increased compared with group B ($P < 0{\cdot}001$, Fisher's exact test). NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; CNS, central nervous system; SD, standard deviation; ART, anti-retroviral therapy.

Several B cell defects have been reported in HIV-infected individuals, mainly in subjects with increased viral load, including polyclonal B cell activation, hypergammaglobulinaemia, induction of terminal differentiation of B cells and increased levels of autoantibodies [18]. In addition, HIV infection is associated with a loss of memory B cells and increased apoptosis in the B cell compartment [19,20]. The association between immune dysfunction and the development of autoimmune diseases in patients with HIV/acquired immune deficiency syndrome (AIDS) is intriguing [21]. The possible mechanisms for autoimmune manifestations include the direct effect of HIV on endothelial, synovial and haematopoietic cells, resulting in the destruction of $CD4^+$ T cells, increased cytotoxic cell activity and increased expression of autoantigens. Polyclonal B cell activation with increased levels of total immunoglubulins and autoantibodies have also been reported in HIV-infected children [22–27]. More recently, immune activation was associated with diminished frequencies of T_{regs} [28,29].

Because it is still not known whether or not immune deregulation in HIV infection influences the generation of autoimmune phenomena in paediatric HIV infection, we examined the association between the numbers or phenotype of T_{regs} and the presence of autoantibodies in a paediatric cohort of HIV-infected children with different degrees of immunosuppression.

Materials and methods

Study population

The group of children evaluated comprised 65 HIV-infected children (30 males and 35 females, aged between 3 and 17 years) treated at the authors' College Hospital. Vertical transmission of HIV infection was confirmed by enzyme-linked immunosorbent assay (ELISA) and Western blot analysis. Patients were selected on the basis of their clinical and immunological status according to the Centers for Disease Control and Prevention (CDC) 1994 paediatric classification [30]. HIV-infected children were divided into two groups: group A, subjects severely immunosuppressed (i.e. $CD4^+$ T cells < 15%, *n* = 28) and group B, subjects with no evidence of immunosuppression (i.e. $CD4^+$ T cells > 25%, *n* = 37) (Table 1). Control samples were obtained from 10 HIV-seronegative healthy children among the population coming to the hospital for vaccination. Informed consent was obtained from the parents of all children included in the study, which was approved by the Institution Ethical Committee.

Plasma HIV RNA viral load

Ethylenediamine tetraacetic acid (EDTA) anti-coagulated blood was centrifuged at 1000 ***g*** for 15 min for plasma collection and stored at −80°C until use. HIV RNA level was determined using Nuclisens EasyQ HIV version 1·2 (bioMérieux, Marcy l'Etoile, France). Results are expressed as log_{10} of number of copies/ml.

T_{reg} phenotyping

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by density gradient centrifugation with Ficoll-Hypaque. Staining of 2×10^6 PBMC with surface markers was performed using the following anti-human monoclonal antibodies: CD4 [peridinin chlorophyll (PerCP)], CD25 [fluorescein isothiocyanate (FITC)], CD127 (AlexaFluor 647), CD69 [allophycocyanin (APC)] and human leucocyte antigen D-related (HLA-DR) (APC) from BD Biosciences (San Jose, CA, USA). Intracellular markers were detected using FoxP3 [phycoerythrin (PE)], cytotoxic T lymphocyte antigen 4 (CTLA-4) (APC) and

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interferon (IFN)-γ (APC) from BD Biosciences, according to the manufacturer's instructions. Data were acquired on a fluorescence activated cell sorter (FACS) Calibur cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and analysed with CellQuest software (Becton Dickinson). At least 20 000 $CD4^+$ T lymphocytes were acquired for analysis of $FoxP3^+$ cells. An automatized blood cell count was recorded in all patients (Cell Dyn 3500, Abbott, Santa Clara, CA, USA) to determine the absolute levels of $CD4^+$ T cells, total $CD4^+FoxP3^+$ T cells or $CD25^+/CD25^-$ $CD4^+$ T_{reg} subsets.

Determination of autoantibodies

For determination of autoantibodies, immunoglobulin levels and C3 and C4 complement factors, blood to be used for serum analysis was allowed to coagulate at 37°C and centrifuged at 1000 ***g*** for 15 min for sera separation. Antinuclear factor (ANA), anti-DNA, anti-smooth muscle (ASMA), anti-mitochondrial (AMA) and anti-neutrophil cytoplasm (ANCAc, ANCAp) antibodies were assayed by indirect immunofluorescence (IMMCO, Diagnostic, Buffalo, NY, USA) while anti-cardiolipin (ACA) antibodies were measured by ELISA (Binding Site, Birmingham, UK). Rheumatoid factor (RF), total immunoglobulin as well as C3 and C4 levels were measured by nephelometry (Immage Beckman Coulter, Brea, CA, USA).

Statistical analysis

The Mann–Whitney *U*-test and Kruskal–Wallis with Dunn's correction test were performed to compare the levels of $CD4^+FoxP3^+$ and $CD4^+FoxP3^+CD25^+/CD25^-$ T cells among the different clinical groups evaluated. Fisher's exact test was applied to compare the frequency of subjects with positive tests in each clinical group. A correlation analysis was applied by Spearman's rank method. $P < 0{\cdot}05$ was considered statistically significant. A univariate analysis was performed to determine differences between children with or without signs of immunosuppression. Student's *t*-test or Wilcoxon's rank sum test were applied for continuous variables, while the χ^2 test was used for categorical variables. Only variables that were statistically different in the univariate analysis ($P < 0{\cdot}10$) were included in the multivariate analysis (logistic regression). Correlations between variables were explored with the Pearson test. Those variables that showed collinearity were analysed in different logistic regression models. All statistical analysis was carried out using Analytical Software Statistix version 8·0.

Results

Clinical characteristics and treatment of study population

The main characteristics of the entire study population are summarized in Table 1. All 65 paediatric patients acquired the infection by vertical transmission, but no prophylactic measures were applied in HIV-infected mothers, neither during pregnancy nor during delivery. Ages of diagnosis in children with severe immunosuppression (group A) were similar to those in children without evidence of immunosuppression (group B) (mean = 5·8 years and 4·5 years, respectively). Antiretroviral therapy (ART) included nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).

HIV infection was diagnosed recently in five of the eight children in group A who were not receiving ART, while treatment had been dropped by the remaining three patients. Similarly, HIV infection had been diagnosed recently in all children from group B who were not under treatment at initiation of the study. Eighteen children in group A and seven in group B showed virological failure, defined for all children as a $<1{\cdot}0$ $\log_{10}$ decrease in HIV RNA copy number from baseline after 8–12 weeks of therapy, HIV RNA >400 copies/ml after 6 months of therapy or repeated levels of HIV RNA copies above the limit of detection after 12 months of therapy. Eighteen of the 65 patients included were also evaluated at two different time-points with an average follow-up period of 12 months (Table 2). One of these 18 patients was diagnosed recently for HIV infection, and thus was naive of treatment at inclusion (i.e. subject 14 in Table 2), four children were already receiving the appropriate ART (i.e. patients 8, 12, 13 and 18 in Table 2), while the remaining 13 children showed virological failure due to deficient adherence to treatment.

Decreased numbers of $CD4^+FoxP3^+$ T cells in peripheral blood of HIV-infected children

The expression of FoxP3, one of the most specific markers for T_{regs} [31–34], was quantified to determine the levels of total T_{regs} in HIV-infected paediatric patients. Lower absolute levels of $CD4^+FoxP3^+$ T cells were recorded in children with severe immunosuppression (group A) than in children without evidence of immunosuppression (group B) and uninfected controls (Fig. 1a). Conversely, percentage levels of total $CD4^+FoxP3^+$ T cells were higher in group A compared with group B and the control group (Fig. 1b).

When co-expression between CD25 and FoxP3 was tested, no significant differences in $CD4^+FoxP3^+CD25^+$ T cells as percentage frequencies were found among patient groups (Fig. 1c), while $CD4^+Foxp3^+CD25^-$ T cells were increased significantly in HIV-infected children with severe immunosuppression in comparison with those without evidence of immunosuppression and healthy controls (Fig. 1c).

The absolute $CD4^+FoxP3^+$ T cell counts as well as the percentages of $CD4^+FoxP3^+CD25^-$ T cells correlated strongly with the degree of immunosuppression, as determined by multivariate analysis (Table 3). Similarly, viral load and the percentages of total $CD4^+FoxP3^+$ T cells were also statistically significant in the multivariate analysis (Table 3).

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Table 2. Clinical characteristics of 18 human immunodeficiency virus (HIV)-infected children at inclusion in the study and at follow-up.

Patient ID	CD4 count at initiation of study (cells/μl)	Viral load at initiation of study (log CV)	HIV-related illness	ARV at inclusion	Months of ARV at inclusion	CD4 count at follow-up (cells/μl)	Viral load at follow-up (log CV)
1	203	5·3†		d4T ABV FPV/r	60	175	4·8
2	85	5·25†	Otitis media	AZT 3TC LPV/r	24	140	3·74
3	86	5·43†	Cryptococcal meningitis	d4T ABV LPV/r	168	276	1·7
4	194	5·36†		AZT ABV LPV/r	120	155	4·23
5	184	3·71†		AZT DDI NFV	60	222	3·74
6	496	3·43†		d4T ABV FPV/r	156	426	4·07
7	185	2·84†		EFV ABV FPV/r	96	227	2·64
8	222	1·7		d4T ABV LPV/r	72	421	2·3
9	684	3·94†		d4T DDI DRV/r	156	470	4
10	853	4·88†		AZT DDI NFV	84	1123	2·1
11	879	3·11†		DDI NVP NFV	168	734	2·38
12	1073	1·7		AZT 3TC NFV	144	1325	3·99
13	687	1·7		d4T ABV IND/r	156	663	1·7
14	942	4·61		AZT 3TC NFV	0	556	3·07
15	546	3·56†		AZT 3TC NFV	120	556	3·31
16	487	5·75†	Pneumonia	AZT 3TC NFV	36	911	5·07
17	234	3·61†		AZT DDI LPV/r	16	497	1·7
18	669	1·7		AZT DDI NFV	60	1142	2·3
Mean	483·8	3·75				556·6	3·16
SD	321·7	1·42				363·6	109
Median	491·5	3·66				483	3·19

†Children with virological failure. d4T, stavudine; DDI, didanosine; AZT, zidovudine; ABV, abacavir; 3TC, lamivudine; NVP, nevirapine; NFV, nelfinavir; EFV, efavirenz; FPV/r, fosamprenavir + ritonavir; LPV/r, lopinavir + ritonavir; ARV, anti-retroviral therapy; SD, standard deviation; DRV, darunavir/rotanavir; IND, indinavir.

Phenotyping of CD4$^+$FoxP3$^+$CD25$^+$ and CD4$^+$FoxP3$^+$CD25$^-$ T cells

To confirm whether CD4$^+$FoxP3$^+$CD25$^-$ T cells were T_{regs} with altered CD25 expression, markers of suppressive (i.e. CTLA-4) and effector (i.e. IFN-γ production) functions, respectively, as well as activation (i.e. CD127, CD69 and HLA-DR), were measured on CD4$^+$FoxP3$^+$CD25$^+$, CD4$^+$FoxP3$^+$CD25$^-$ and CD4$^+$FoxP3$^-$CD25$^+$ T cells. No significant differences in CTLA-4 [Fig. 2a (upper panel), b],

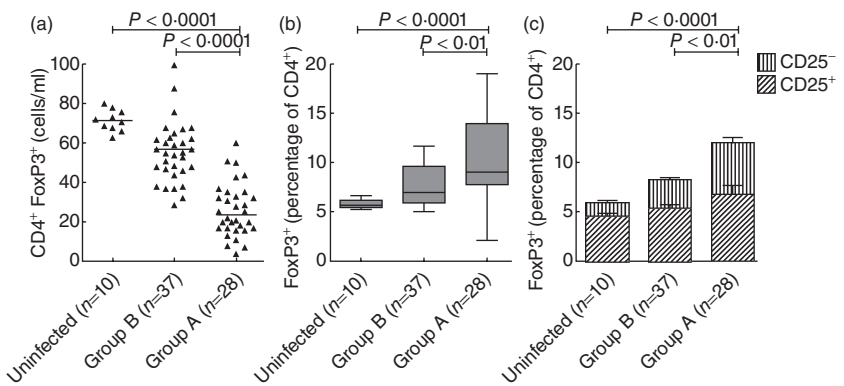

Fig. 1. The frequency and phenotype of CD4$^+$forkhead box P3 (FoxP3)$^+$ T cells are altered in human immunodeficiency virus (HIV)-paediatric patients. Peripheral blood mononuclear cells (PBMC) were collected and stained with anti-CD4, anti-CD25 and FoxP3 monoclonal antibodies. CD4$^+$ T cells were gated from a side-scatter (SSC) *versus* CD4 dot-plot and the expression of FoxP3 and CD25 was analysed with CellQuest Pro software. Blood cell count was recorded in all subjects. (a) Absolute numbers (shown as cells per millilitre of blood) of total CD4$^+$FoxP3$^+$ T cells. The horizontal lines denote the median values for each clinical group. (b) FoxP3$^+$ T cell levels (expressed as percentages of CD4$^+$ T cells) in the different clinical groups. Boxes represent values between the 25th and 75th percentiles and medians; bars indicate 10th and 90th percentiles. (c) Frequency of regulatory CD4$^+$ T cell subsets according to CD25 expression. CD4$^+$CD25$^-$FoxP3$^+$ T cells in group A were significantly higher compared with those in group B and uninfected controls.

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Table 3. Univariate and Multivariate analysis for immunosuppression in HIV-infected children.

	Univariate analysis			Multivariate analysis	
Study variables	Group A ($CD4^+ < 15\%$)	Group B ($CD4^+ > 25\%$)	*P*-value	Coefficient β	*P*-value
Age, years ± SD	10·97 ± 5·15	10·46 ± 4·6	0·67		
Anti-retroviral treatment† (%)	19/28 (68)	32/37 (86)	0·07	–0·45	0·68
Logarithm $_{10}$ of viral copies ± DS	3·97 ± 1·3	2·71 ± 1·22	0·0002	0·708	0·035
Co-morbidities† (%)	15/28 (54)	8/37 (22)	0·0076	1·1	0·20
Treatment adherence† (%)	7/24 (29)	23/33 (70)	0·0025	–0·50	0·67
Absolute count $CD4^+FoxP3^+$	28·7 ± 27·4	63·7 ± 27·7	<0·0001	–0·06	**0·0004***
$\%CD4^+FoxP3^+$	12·7 ± 8·4	7·7 ± 2·3	0·0043	0·22	**0·04***
$\%CD4^+FoxP3^+CD25^-$	5·98 ± 5·4	2·75 ± 1·2	0·0044	0·82	**0·002***
$\%CD4^+FoxP3^+CD25^+$	6·7 ± 4·59	4·84 ± 1·75	0·05	0·14	0·17*

*Variables with collinearity were analysed in different multivariate models. †No. of positive patients/total no. of subjects assayed. Bold type indicates significant differences. FoxP3, forkhead box protein 3; DS, double-stranded; SD, standard deviation.

CD127 (Fig. 2c), CD69 [Fig. 2a (bottom panel), e], HLA-DR (Fig. 2f) expression or IFN-γ production (Fig. 2d) were found between $CD4^+FoxP3^+CD25^+$ and $CD4^+FoxP3^+CD25^-$ T cells. Conversely, either $CD4^+FoxP3^+CD25^+$ or $CD4^+FoxP3^+CD25^-$ T cells exhibited higher CTLA-4 (Fig. 2b) but lower CD127 (Fig. 2c) expression than $CD4^+FoxP3^-CD25^+$ T cells.

Levels of autoantibodies and total immunoglobulins in HIV-infected children

The decreased absolute $CD4^+FoxP3^+$ T cell counts found in subjects with severe immunosuppression prompted us to investigate the presence of autoantibodies in relation to the levels of T_{regs} in a cohort of 30 HIV-infected children and 10 uninfected healthy controls. Demographic and clinical features of this patient group are shown in Table S1.

Thirteen of 15 (87%) patients in group A were positive for at least one of seven autoantibodies tested (range 1–3 positive antibodies/patient), whereas only four of 15 (27%) patients in group B showed detectable autoantibodies (range 1–2 positive autoantibodies/patient) (Table 4). Conversely, autoantibodies were not found in uninfected children (Table 4).

RF and ASMA were the most frequently observed autoantibodies in both clinical groups of infected children

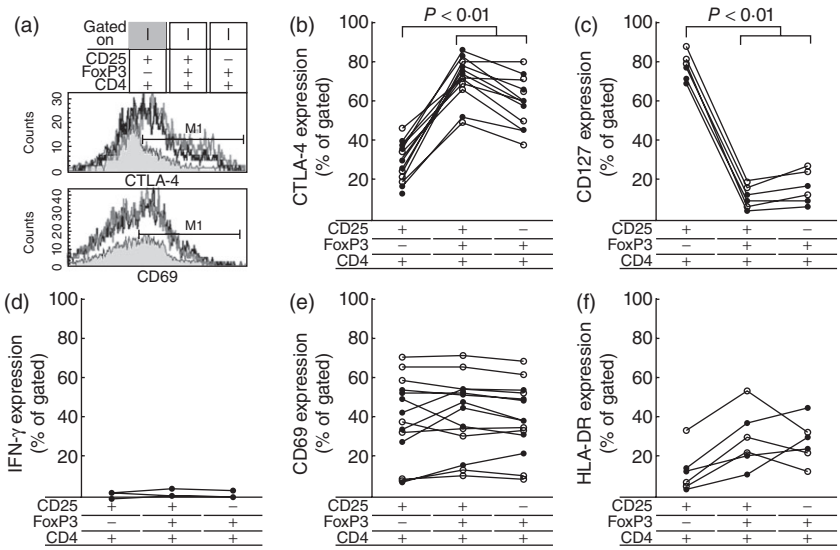

Fig. 2. Phenotypic analysis of forkhead box P3 (FoxP3)-expressing $CD4^+$ T cells. Peripheral blood mononuclear cells (PBMC) were collected and stained with anti-CD4, anti-CD25 and anti-FoxP3 in combination with anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) ($n = 14$), CD69 ($n = 14$), CD127 ($n = 6$), interferon (IFN)-γ ($n = 4$) or human leucocyte antigen D-related (HLA-DR) ($n = 6$). Lymphocytes were identified by forward- and side-scatter properties. From this population, a CD4 *versus* FoxP3 dot-plot was set. Subsequently, $CD4^+FoxP3^+$ or $CD4^+FoxP3^-$ T cell populations were gated and analysed for CD25 *versus* CTLA-4, CD127, IFN-γ, CD69 or HLA-DR. (a) Representative histogram plot from a single human immunodeficiency virus (HIV)-infected child showing CTLA-4 (upper panel) and CD69 (bottom panel) expression on $CD4^+FoxP3^-CD25^+$, $CD4^+FoxP3^+CD25^+$ and $CD4^+FoxP3^+CD25^-$ T cells. Proportions of $CD4^+FoxP3^-CD25^+$, $CD4^+FoxP3^+CD25^+$ and $CD4^+FoxP3^+CD25^-$ T cells co-expressing CTLA-4 (b), CD127 (c), IFN-γ (d), CD69 (e) or HLA-DR (f) were measured in HIV-infected children exhibiting immunosuppression (closed circle) or without signed of immunosuppression (open circle).

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Table 4. Levels of $CD4^+FoxP3^+$ T cells, autoantibodies and total immunoglobulins in human immunodeficiency virus (HIV)-infected and uninfected children.

		Group A ($n = 15$)	Group B ($n = 15$)	Uninfected ($n = 10$)
$CD4^+FoxP3^+$ T cells (cells/mm^3 ± SD)		16·5 ± 14·2[a]	70·1 ± 24·1	72 ± 9·1
No. of positive tests (median IU/ml, range)[b]	RF	10	8	0
		37·6 (20–223)[c]	21 (20–42)	<20[d]
	ACA	10	3	0
		29 (18–69)[e]	19 (18–29)	<15[d]
	ANA	1	0	0
	DNA	0	0	0
	AMA	2	0	0
	ASMA	7	2	0
	ANCA	3	0	0
No. of patients with positive findings for at least one autoantibody out of the total evaluated (%)		13 (87)[f]	4 (27)	0 (0)
Total immunoglobulins (median mg/dl, range)	IgA	302 (107–838)[g]	205·3 (43–459)	124 (64–184)
	IgM	297 (132–344)[h]	136 (58–386)	79 (56–102)
	IgG	2190 (823–3500)[i]	1580 (900–3390)	946 (822–1070)

[a]$P = 0·001$ *versus* group B and uninfected subjects, Kruskal–Wallis test. [b]Applicable for quantitative tests. [c]$P = 0·049$ *versus* group B, Mann–Whitney *U*-test. [d]Detection limit. [e]$P = 0·009$ *versus* group B, Mann–Whitney *U*-test. [f]$P = 0·022$ *versus* group B; $P = 0·008$ *versus* uninfected controls, Fisher's exact test. [g]$P = 0·001$ *versus* group B and uninfected controls, Kruskal–Wallis test. [h]$P < 0·0001$ *versus* group B and uninfected controls, Kruskal–Wallis test. [i]$P < 0·0001$ *versus* uninfected controls, Kruskal–Wallis test. Ig, immunoglobulin; ANCA, anti-neutrophil cytoplasm; ASMA, anti-DNA, anti-smooth muscle; AMA, anti-mitochondrial; ANA, anti-nuclear factor; FoxP3, forkhead box protein 3; RF, rheumatoid factor; SD, standard deviation.

(Table 4). Conversely, ACA were found mainly in children with severe immunosuppression (Table 4). Although differences in autoantibody levels based on the degree of immunosuppression were not significant, ASMA were detected frequently more among patients with severe disease (Table 4).

In the 30 patients evaluated, high levels of ACA (Fig. 3a) and RF (Fig. 3b) were associated with low $CD4^+$ T cell counts. While ACA levels were not related to either the absolute number of total $CD4^+FoxP3^+$ T cells ($CD4^+FoxP3^+CD25^+$ and $CD4^+FoxP3^+$ $CD25^-$ T cells) or any of the $CD25^+/CD25^-$ regulatory T cell subsets (data not shown), a negative correlation was found between RF and absolute total $CD4^+FoxP3^+$ T cell counts (Fig. 3c). Conversely, when $CD4^+FoxP3^+CD25^+$ and $CD4^+FoxP3^+CD25^-$ T cells were analysed separately, no

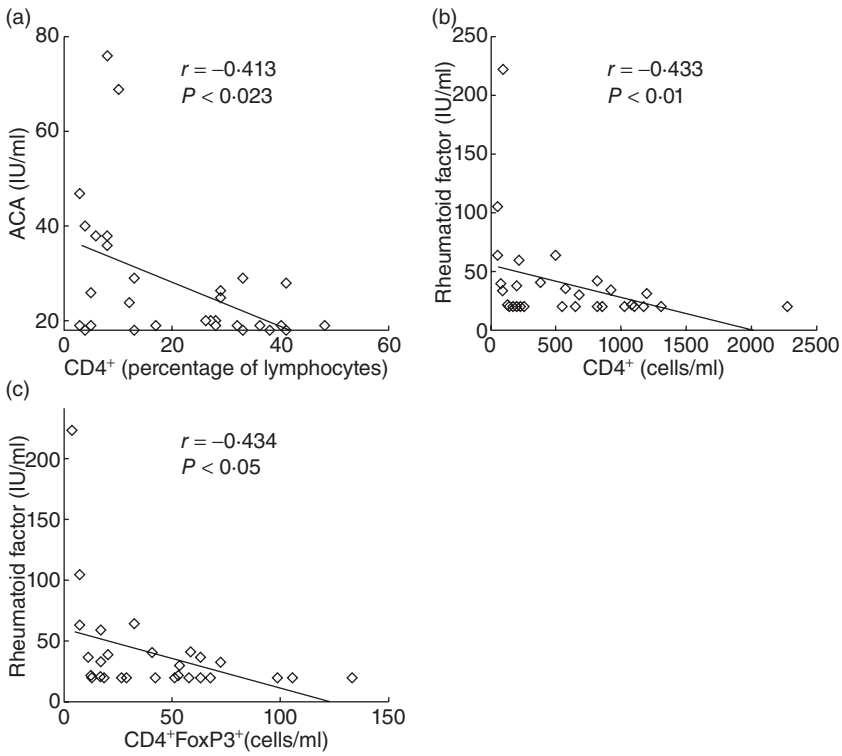

Fig. 3. Relationship among total regulatory T cells (T_{reg}), anti-cardiolipin (ACA), rheumatoid factor (RF) and CD4 count. Correlation analysis was performed by Spearman's rank method. (a) ACA *versus* percentage of $CD4^+$ T cells. (b) RF *versus* $CD4^+$ T cell counts (cells/ml). (c) RF *versus* total $CD4^+FoxP3^+$ T cell counts (cells/ml).

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association was observed between T_{reg} and RF levels. In the present study, the 30 patients evaluated did not present any clinical evidence of autoimmune disease with normal serum concentrations of C3 and C4 (data not shown), even in those patients with a five- to 10-fold increase in RF levels (reference range <20 IU/ml). The higher prevalence of autoantibodies observed in patients with severe immunosuppression compared with those without evidence of immunosuppression was linked to increased levels of total immunoglobulins (Table 4).

Taken together, these results support that depletion of T_{regs} in HIV-infected children is linked to the appearance of RF autoantibodies, while the reduction in $CD4^+$ T cells appeared to be related mainly to the presence of ACA and RF autoantibodies.

Monitoring of $CD4^+FoxP3^+$ T cells in HIV-infected children

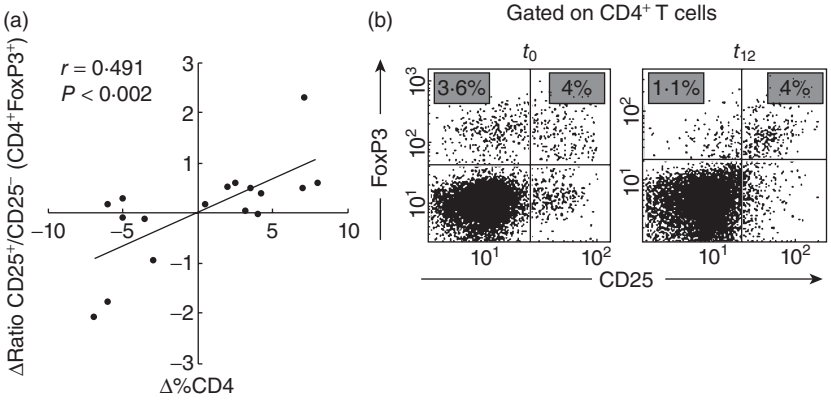
We next analysed whether changes in CD4 T cell count were linked with phenotypical alterations in T_{regs} in an average 12-month follow-up period in 18 HIV-infected children (Table 2). Although substantial changes in T_{reg} numbers were generally not observed, a positive correlation between changes in immunological status during the follow-up period ($\Delta\%CD4 = \%CD4_{t12} - \%CD4_{t0}$) and changes in $FoxP3^+$ T cells phenotype ($\Delta ratio = \%CD25^+_{t12}/\%CD25^-_{t12} - \%CD25^+_{t0}/\%CD25^-_{t0}$) was recorded (Fig. 4a). As observed in Fig. 4b,c, an improvement in $CD4^+$ T cell counts along with a decrease in viral load was associated with a decline in the frequency of $CD4^+FoxP3^+CD25^-$ T cells to levels comparable to those observed in healthy controls.

Even though a substantial alteration in RF levels was not found during follow-up, the two patients (i.e. patients 3 and 17 in Table 2) who showed the higher decrease in RF also had a diminution in $CD4^+FoxP3^+CD25^-$ T cells (Fig. 4b,c). It is noteworthy that both patients showed improved immune and virological status, as determined by a rise in $CD4^+$ T cell count and a decrease in viral load.

Discussion

HIV infection is characterized by a progressive loss of $CD4^+$ T cells, chronic immune activation and an increasing array of immune dysfunctions [35,36]. Polyclonal hypergammaglobulinaemia, depletion and/or dysfunction of $CD4^+$ T_{regs} probably contribute to the production of autoantibodies observed in a proportion of HIV-infected children in the absence of clinical autoimmune disease [22–27]. This study reports for the first time that diminished absolute levels of T_{regs} are associated with a higher prevalence of autoantibodies and hypergammaglobulinaemia in HIV-infected children with severe immunosuppression.

In agreement with the findings in HIV-infected adults, RF, ACA and ASMA were the most prevalent autoantibodies [37–41]. The highest levels of RF and ACA as well as total immunoglobulins were recorded in patients with the lowest $CD4^+FoxP3^+$T cell count, supporting the notion that T_{regs} play a main role in the control of immune activation and autoantibody production. Moreover, correlation analysis supports that depletion of T_{regs} in HIV-infected children is linked to the appearance of RF autoantibodies, while the reduction in $CD4^+$ T cells appeared to be related mainly to the presence of ACA and RF autoantibodies.



(c)

	t_0	t_{12}
Viral load (log_{10} copies/ml)	5·4	1·7
CD4 (cells/ml)	86	276
RF (IU/ml)	223	118
Ratio $CD25^+/CD25^-$ ($CD4^+FoxP3^+$)	1·1	3·6

Fig. 4. Monitoring of $CD4^+$ forkhead box P3 (FoxP3)$^+CD25^+$ T cells and $CD4^+$ T cell counts in human immunodeficiency virus (HIV)-infected children. The frequency of $CD4^+FoxP3^+CD25^+$ and $CD4^+$ T cells were determined at two different time-points during an average of 12-month follow-up period. (a) Correlation analysis between changes in the levels of $CD4^+$ T cells (Δ% CD4) and changes in regulatory T cell (T_{reg}) phenotype (Δratio $\%CD4^+FoxP3^+CD25^+/\%CD4^+FoxP3^+CD25^-$). (b) Representative dot-plot of CD25 and FoxP3 expression in $CD4^+$ T cells during follow-up (t_0 and t_{12} patient 3 in Table 2). $CD4^+$ T cells were gated from a side-scatter (SSC) *versus* CD4 dot-plot and the expression of CD25 and FoxP3 was calculated. The figures in the upper left quadrants show the percentages of $CD4^+FoxP3^+CD25^-$ T cells, while the percentages of $CD4^+FoxP3^+CD25^+$ T cells are shown in the upper right quadrants. (c) Summary of immune/virological status of the same patient showed in (b).

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In contrast to the diminished absolute T_{reg} numbers, the percentages of total $CD4^+FoxP3^+$ T cells are raised in children with signs of severe immunosuppression. Previous reports have shown low levels of classical T_{reg} $CD4^+FoxP3^+CD25^+$, along with an increased activation status in an HIV^+ paediatric cohort [29]. In the present study, the rise in total $CD4^+FoxP3^+$ T cells is due to an increase in a putative T_{reg} population $CD4^+FoxP3^+CD25^-$ but not to an increase in classical $CD4^+FoxP3^+CD25^+$ T_{regs}. The regulatory T cell subset $CD4^+FoxP3^+CD25^-$ might arise from activated $CD4^+$ T cells that express FoxP3, or constitutes atypical T_{reg} [42–44]. Other studies have demonstrated that $CD4^+FoxP3^+CD25^-$ T cells represent a peripheral reservoir of committed regulatory T cells, recruited to the $CD25^+$ T cell pool upon homeostatic expansion or activation [45]. The relative increase of this subset in the group with lower $CD4^+$ T cell counts would be a consequence of the extended HIV-associated chronic immune activation, or higher induction of atypical T_{regs} which, in turn, can differentiate into conventional T_{regs} [45]. The diminution in the absolute levels of $CD4^+$ T cells and the simultaneous percentage increase in $CD4^+FoxP3^+CD25^-$ T cells might also be due to a slower decline of the latter T cell subset [46]. In our study, the absolute total $CD4^+FoxP3^+$ T cell counts and the percentages of $CD4^+FoxP3^+CD25^-$ T cells appeared to be associated strongly with immunosuppression.

This particular subset of $CD4^+FoxP3^+CD25^-$ T cells was also reported in patients with systemic lupus erythematosus [47–49] and displayed a suppressive capacity for T cell proliferation but not for IFN-γ production [49], supporting that $CD4^+FoxP3^+$ T cells were dysfunctional T_{regs}. In this study, $CD4^+FoxP3^+CD25^-$ T cells showed similar phenotypical features to $CD4^+FoxP3^+CD25^+$ T cells, including CTLA-4 expression, a typical marker of suppressive capacity in T_{regs}; nor was the activation status of these T_{reg} subsets different, as determined by the expression of CD69, CD127 and HLA-DR. Moreover, IFN-γ production, distinctive of effector T cells but not T_{regs}, was not detected. Therefore, $CD4^+FoxP3^+CD25^-$ in HIV-infected children appeared to be T_{regs} with abnormal CD25 expression. However, we cannot rule out that this T_{reg} subpopulation displays some kind of dysfunction.

In our cohort of patients, we observed a strong inverse association between the presence of autoantibodies and absolute numbers of $CD4^+$ or $CD4^+FoxP3^+$ T cells, respectively, in the absence of signs of rheumatic disease, altered blood-clotting test, thromboembolic phenomena or liver involvement. As the level of CD25 expression correlates with the suppressive capacity of conventional T_{regs} [50,51], we can hypothesize that the rise in $CD4^+FoxP3^+CD25^-$ T cells might enhance the immune deregulation observed in children with severe immunosuppression, allowing autoreactive B cells to differentiate into antibody-producing plasma cells [17]. Supporting this notion, two children with deficient adherence to treatment who restarted an anti-retroviral regimen showed a marked decrease in RF levels along with a decline in the proportion of $CD4^+FoxP3^+CD25^-$ T cells (i.e. patients 3 and 17 in Table 2). Moreover, diminished levels of $CD4^+FoxP3^+$ T cells or changes in their phenotype, together with a scarce T cell surveillance, might lead to the appearance of recurrent or persistent infections. This may, in turn, induce a rise in antigen exposure with polyclonal B cell activation that probably contributes to the generation of autoantibodies in the absence of clinical autoimmune disease. However, we cannot rule out that hyperactivation of B cells observed during HIV infection could be responsible for the presence of autoantibodies in HIV-infected children. Aberrant immune activation of B cells is also supported by the increased expression of activation, proliferation and terminal differentiation markers on circulating B cells [18], as well as by the production of a large amount of immunoglobulins when PBMC obtained from HIV-1-infected subjects are cultured *in vitro* [52,53]. Furthermore, HIV-infected subjects who developed hypergammaglobulinaemia, thrombocytopenia or other autoimmune manifestations associated with HIV infection had increased frequencies of $CD5^+$ B cells, a subset of the natural immune system related to the generation of naturally occurring autoantibodies [54]. Conversely, other studies have shown that $CD5^+$ B cells do not contribute to hypergammaglobulinaemia or autoantibody production in HIV-infected subjects [55,56].

Although the role of these autoantibodies is not clear at present, a long-term infection that leads the host immune system to chronic activation and exhaustion [57] may provide an appropriate environment to trigger an autoimmune disease. We have shown previously that chronic immune activation is a major feature in HIV-infected children [58,59]. The present work points out that not only the frequencies but also the phenotype of $CD4^+FoxP3^+$ T cells are important parameters to characterize the immunoregulatory status during paediatric HIV infection. Atypical T_{regs} seem to represent an additional marker of immune dysfunction in children with HIV infection.

The identification of additional markers for monitoring treatment efficacy and disease progression is especially needed in perinatally acquired HIV infection, in which the development of a naive immune system in the presence of a persistent pathogen might manifest clinical and immunological features different from those observed in HIV-infected adults. Furthermore, the findings obtained in children might be subjected to certain variability due to virological and/or immunological failure related to possible suboptimal adherence or regimen discontinuations.

In summary, this study shows that low absolute numbers of $CD4^+FoxP3^+$ T cells were associated with an increased prevalence of autoantibodies and increased levels of total immunoglobulins in HIV-infected children with severe immunosuppression. This work also reports for the first time enhanced frequencies of a particular $CD4^+FoxP3^+CD25^-$ T_{reg} subset. Longitudinal follow-up of HIV-infected children without clinical symptoms of autoimmune disease would shed light on the prognostic significance of these findings.

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Acknowledgements

We thank Professor Verónica Gatto Bellora for technical assistance and Dr Rodolfo Viotti for statistical analysis. This study was supported by the Health Department, Government of the City of Buenos Aires, Argentina. Susana Laucella is member of the Scientific Career of The National Council for Scientific and Technical Research (CONICET), Argentina.

Disclosure

The authors declare no financial or commercial conflicts of interest.

References

1 Adeegbe D, Bayer AL, Levy RB, Malek TR. Cutting edge: allogeneic CD4+CD25+Foxp3+ T regulatory cells suppress autoimmunity while establishing transplantation tolerance. J Immunol 2006; **176**:7149–53.
2 Chang X, Zheng P, Liu Y. Homeostatic proliferation in the mice with germline Foxp3 mutation and its contribution to fatal autoimmunity. J Immunol 2008; **181**:2399–406.
3 Piccirillo CA, d'Hennezel E, Sgouroudis E, Yurchenko E. CD4+Foxp3+ regulatory T cells in the control of autoimmunity: *in vivo veritas*. Curr Opin Immunol 2008; **20**:655–62.
4 Kotsianidis I, Bouchliou I, Nakou E *et al.* Kinetics, function and bone marrow trafficking of CD4+CD25+FOXP3+ regulatory T cells in myelodysplastic syndromes (MDS). Leukemia 2009; **23**:510–18.
5 Card CM, McLaren PJ, Wachihi C, Kimani J, Plummer FA, Fowke KR. Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+) FOXP3(+) regulatory T cells. J Infect Dis 2009; **199**:1318–22.
6 Manigold T, Shin EC, Mizukoshi E *et al.*Foxp3+CD4+CD25+ T cells control virus-specific memory T cells in chimpanzees that recovered from hepatitis C. Blood 2006; **107**:4424–32.
7 Bi X, Suzuki Y, Gatanaga H, Oka S. High frequency and proliferation of CD4+Foxp3+ Treg in HIV-1-infected patients with low CD4 counts. Eur J Immunol 2009; **39**:301–9.
8 Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol 2004; **78**:2454–9.
9 Gavin MA, Torgerson TR, Houston E *et al.* Single-cell analysis of normal and Foxp3-mutant human T cells:Foxp3 expression without regulatory T cell development. Proc Natl Acad Sci USA 2006; **103**:6659–64.
10 Liu W, Putnam AL, Xu-Yu Z *et al.* CD127 expression inversely correlates with Foxp3 and suppressive function of human $CD4^+$ Treg cells. J Exp Med 2006; **203**:1701–11.
11 Seddiki N, Santner-Nanan B, Martinson J *et al.* Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med 2006; **203**:1693–700.
12 Zheng Y, Manzotti CN, Burke F *et al.* Acquisition of suppressive function by activated human CD4+ CD25− T cells is associated with the expression of CTLA-4 not Foxp3. J Immunol 2008; **181**:1683–91.
13 Mozos A, Garrido M, Carreras J *et al.* Redistribution of Foxp3-positive regulatory T cells from lymphoid tissues to peripheral blood in HIV-infected patients. J Acquir Immune Defic Syndr 2007; **46**:529–37.
14 Bandera A, Ferrario G, Saresella M *et al.* CD4+ T cell depletion, immune activation and increased production of regulatory T cells in the thymus of HIV-infected individuals. PLoS ONE 2010; **5**:e10788.
15 Gudo ES, Bhatt NB, Bila DR *et al.* Co-infection by human immunodeficiency virus type 1 (HIV-1) and human T cell leukemia virus type 1 (HTLV-1): does immune activation lead to a faster progression to AIDS? BMC Infect Dis 2009; **9**:211–23.
16 Leligdowicz A, Feldmann J, Jaye A *et al.* Direct relationship between virus load and systemic immune activation in HIV-2 infection. J Infect Dis 2010; **201**:114–22.
17 Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells. J Immunol 2005; **175**:4180–3.
18 Moir S, Fauci AS. Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease. J Allergy Clin Immunol 2008; **122**:12–19.
19 De Milito A, Mörch C, Sönnerborg A, Chiodi F. Loss of memory (CD27) B lymphocytes in HIV-1 infection. AIDS 2001; **15**:957–64.
20 Titanji K, Nilsson A, Mörch C *et al.* Low frequency of plasma nerve-growth factor detection is associated with death of memory B lymphocytes in HIV-1 infection. Clin Exp Immunol 2003; **132**:297–303.
21 Zandman-Goddard G, Shoenfeld Y. HIV and autoimmunity. Autoimmun Rev 2002; **1**:329–37.
22 Rodriguez C, Stiehm ER, Plaeger-Marshall S. Peripheral B-cell activation and immaturity in HIV-infected children. Ann NY Acad Sci 1993; **693**:291–4.
23 Jarvis JN, Taylor H, Iobidze M, Dejonge J, Chang S, Cohen F. Rheumatoid factor expression and complement activation in children congenitally infected with human immunodeficiency virus. Clin Immunol Immunopathol 1993; **67**:50–4.
24 González CM, López-Longo FJ, Samson J *et al.* Antiribonucleoprotein antibodies in children with HIV infection: a comparative study with childhood-onset systemic lupus erythematosus. AIDS Patient Care STDS 1998; **12**:21–8.
25 Stricker K, Knipping E, Böhler T, Benner A, Krammer PH, Debatin KM. Anti-CD95 (APO-1/Fas) autoantibodies and T cell depletion in human immunodeficiency virus type 1 (HIV-1)-infected children. Cell Death Differ 1998; **5**:222–30.
26 Eley BS, Hughes J, Potgieter S, Keraan M, Burgess J, Hussey GD. Immunological manifestations of HIV-infected children. Ann Trop Paediatr 1999; **19**:3–7.
27 Shah I, Chudgar P. Antiphospholipid syndrome in a human immunodeficiency virus 1-infected child. Pediatr Infect Dis J 2006; **25**:185–6.
28 Legrand FA, Nixon DF, Loo CP *et al.* Strong HIV-1-specific T cell responses in HIV-1-exposed uninfected infants and neonates revealed after regulatory T cell removal. PLoS ONE 2006; **1**:e102.
29 Prendergast A, O'Callaghan M, Menson E *et al.* Factors influencing T cell activation and programmed death 1 expression in HIV-infected children. AIDS Res Hum Retroviruses 2011. [Epub ahead of print].
30 CDC Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. Morb Mortal Wkly Rep 1994; **43**:1–10.
31 Yagi H, Nomura T, Nakamura K *et al.* Crucial role of Foxp3 in the

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development and function of human CD25+CD4+ regulatory T cells. Int Immunol 2004; **16**:1643–56.
32 Xing S, Fu J, Zhang Z *et al*. Increased turnover of Foxp3high regulatory T cells is associated with hyperactivation and disease progression of chronic HIV-1 infection. J Acquir Immune Defic Syndr 2010; **54**:455–62.
33 Gaardbo JC, Nielsen SD, Vedel SJ *et al*. Regulatory T cells in human immunodeficiency virus-infected patients are elevated and independent of immunological and virological status, as well as initiation of highly active anti-retroviral therapy. Clin Exp Immunol 2008; **154**:80–6.
34 Montes M, Lewis DE, Sanchez C *et al*.Foxp3+ regulatory T cells in antiretroviral-naive HIV patients. AIDS 2006; **20**:1669–71.
35 Stevenson M. HIV-1 pathogenesis. Nat Med 2003; **9**:853–60.
36 Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol 2008; **214**:231–41.
37 Avcin T, Toplak N. Antiphospholipid antibodies in response to infection. Curr Rheumatol Rep 2007; **9**:212–18.
38 Metlas R, Srdic T, Veljkovic V. Anti-IgG antibodies from sera of healthy individuals neutralize HIV-1 primary isolates. Curr HIV Res 2007; **5**:261–5.
39 Haynes BF, Nicely NI, Alam SM. HIV-1 autoreactive antibodies: are they good or bad for HIV-1 prevention? Nat Struct Mol Biol 2010; **17**:543–5.
40 Hernandez GT, Critchfield JM, Rodriguez RA. Interpretation of serologic tests in an HIV-infected patient with kidney disease. Nat Clin Pract Nephrol 2006; **2**:708–12.
41 Shoenfeld Y, Blank M, Abu-Shakra M *et al*. The mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune diseases. Isr Med Assoc J 2008; **10**:13–19.
42 Zelenay S, Lopes-Carvalho T, Caramalho J, Moraes-Fontes MF, Rebelo M, Demengeot J. Foxp3+ CD25− CD4 T cells constitute a reservoir of committed regulatory cells that regain CD25 expression upon homeostatic expansion. Proc Natl Acad Sci USA 2005; **102**:4091–6.
43 Horwitz DA. Identity of mysterious CD4+CD25–Foxp3+ cells in SLE. Arthritis Res Ther 2010; **12**:101–3.
44 Nishioka T, Shimizu J, Iida R, Yamazaki S, Sakaguchi S. CD4+CD25+Foxp3+ T cells and CD4+CD25–Foxp3+ T cells in aged mice. J Immunol 2006; **176**:6586–93.
45 Sodora D, Silvestri G. Immune activation and AIDS pathogenesis. AIDS 2008; **22**:439–46.
46 Eggena MP, Barugahare B, Jones N *et al*. Depletion of regulatory T cells in HIV infection is associated with immune activation. J Immunol 2005; **174**:4407–14.
47 Suen JL, Li HT, Jong YJ, Chiang BL, Yen JH. Altered homeostasis of CD4(+)Foxp3(+) regulatory T-cell subpopulations in systemic lupus erythematosus. Immunology 2009; **127**:196–205.
48 Yang J, Chu Y, Yang X *et al*. Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. Arthritis Rheum 2009; **60**:1472–83.
49 Bonelli M, Savitskaya A, Steiner CW, Rath E, Smolen JS, Scheinecker C. Phenotypic and functional analysis of CD4+ CD25– Foxp3$^+$ T cells in patients with systemic lupus erythematosus. J Immunol 2009; **182**:1689–95.
50 Moes N, Rieux-Laucat F, Begue B *et al*. Reduced Expression of Foxp3 and regulatory T-cell function in severe forms of early-onset autoimmune enteropathy. Gastroenterology 2010; **139**:770–8.
51 Fazekas de St Groth B, Landay AL. Regulatory T cells in HIV infection: pathogenic or protective participants in the immune response? AIDS 2008; **22**:671–83.
52 De Milito A, Nilsson A, Titanji K *et al*. Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. Blood 2004; **103**:2180–6.
53 Titanji K, De Milito A, Cagigi A *et al*. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. Blood 2006; **108**:1580–7.
54 Sampalo A, López-Gómez M, Jiménez-Alonso J, Ortiz F, Samaniego F, Garrido F. CD5+ B lymphocytes in HIV infection: relationship to immunological progression of disease. Clin Immunol Immunopathol 1993; **66**:260–8.
55 Grünewald T, Burmester GR, Schüler-Maué W, Hiepe F, Buttgereit F. Anti-phospholipid antibodies and CD5$^+$ B cells in HIV infection. Clin Exp Immunol 1999; **115**:464–71.
56 Redgrave BE, Stone SF, French MA, Krueger R, James IR, Price P. The effect of combination antiretroviral therapy on CD5 B-cells, B-cell activation and hypergammaglobulinaemia in HIV-1-infected patients. HIV Med 2005; **6**:307–12.
57 Herbert W, Virgin E, Wherry J, Ahmed R. Redefining chronic viral infection. Cell 2009; **138**:30–50.
58 Gaddi E, Laucella S, Balbaryski J *et al*. Prognostic value of soluble intercellular adhesion molecule-1 (s-ICAM-1) in HIV-infected children. Scand J Immunol 2000; **52**:628–33.
59 Gaddi E, Quiroz H, Balbaryski J *et al*. L-selectin expression on T lymphocytes and neutrophils in HIV infected children. Medicina (Buenos Aires) 2005; **65**:131–7.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical features of human immunodeficiency virus (HIV)-infected children evaluated for the presence of autoantibodies and regulatory T cell (T_{reg}) levels.

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