

# Memory B-cell depletion is a feature of HIV-2 infection even in the absence of detectable viremia

Rita Tendeiro<sup>a</sup>, Sofia Fernandes<sup>a</sup>, Russell B. Foxall<sup>a</sup>,  
José M. Marcelino<sup>b,c</sup>, Nuno Taveira<sup>b,d</sup>, Rui S. Soares<sup>a</sup>,  
António P. Baptista<sup>a</sup>, Rita Cavaleiro<sup>a</sup>, Perpétua Gomes<sup>d,e,f</sup>,  
Rui M.M. Victorino<sup>a,g</sup> and Ana E. Sousa<sup>a</sup>

**Objective:** Memory B-cell loss has long been recognized as an important contributor to HIV immunodeficiency. HIV-2 infection, which is characterized by a slow rate of progression to AIDS and reduced to undetectable viremia, provides a unique model to investigate B-cell disturbances.

**Design and methods:** B-cell subsets were evaluated in 38 HIV-2-infected individuals, along with markers of T-cell activation and serum levels of immunoglobulins and a major B-cell homeostatic cytokine, B-cell activating factor (BAFF). Untreated HIV-1-infected and seronegative control individuals were studied in parallel. Statistical analysis was performed using Mann–Whitney tests and Spearman's correlations.

**Results:** We found that HIV-2 was associated with significant depletion of both unswitched (CD27<sup>+</sup>IgD<sup>+</sup>) and switched (CD27<sup>+</sup>IgD<sup>neg</sup>) memory B-cells that directly correlated with T-cell activation, even in individuals with undetectable plasma viral load. Nevertheless, the presence of detectable viremia, even at low levels, was associated with significant memory B-cell loss and higher BAFF levels. Moreover, these alterations were not recovered by antiretroviral-therapy, as treated HIV-2-infected patients showed more pronounced B-cell disturbances, possibly related to their extended length of infection.

**Conclusion:** These first data regarding B-cell imbalances during HIV-2 infection show that, irrespective of viremia, prolonged HIV infection leads to irreversible damage of memory B-cell homeostasis. © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

*AIDS* 2012, **26**:1607–1617

**Keywords:** AIDS, B-cell activating factor, B-cells, HIV-2, immune activation, memory B-cells

## Introduction

HIV-1 infection is associated with progressive impairment of specific humoral responses and loss of memory

B-cells that are only partly recovered by antiretroviral therapy (ART) [1–4]. These B-cell disturbances have been linked to the persistent heightened state of immune activation associated with HIV-1 infection [3–5]. In fact,

<sup>a</sup>Unidade de Imunologia Clínica, Instituto de Medicina Molecular, Faculdade de Medicina, <sup>b</sup>Unidade de Retrovírus e Infecções Associadas, Centro de Patogénese Molecular, Faculdade de Farmácia, Universidade de Lisboa, <sup>c</sup>Unidade de Microbiologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, <sup>d</sup>Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, <sup>e</sup>Laboratório de Biologia Molecular, Serviço de Medicina Transfusional, Centro Hospitalar Lisboa Ocidental, Hospital Egas Moniz, <sup>f</sup>Centro de Malária e Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, and <sup>g</sup>Clínica Universitária de Medicina 2, Centro Hospitalar Lisboa Norte, Hospital Universitário de Santa Maria, Lisboa, Portugal.

Correspondence to Ana E. Sousa, MD, PhD, Unidade de Imunologia Clínica, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Professor Egas Moniz, 1649-028 Lisboa, Portugal.

Tel: +351 21 799 95 25; fax: +351 21 799 95 27; e-mail: asousa@fm.ul.pt

Received: 23 February 2012; revised: 23 May 2012; accepted: 1 June 2012.

DOI:10.1097/QAD.0b013e3283568849

polyclonal B-cell activation with marked hypergammaglobulinemia is usually observed in acute HIV-1 infection [6], persisting throughout the chronic phase of the disease [3,5]. HIV-1 proteins, particularly gp120 [7,8] and Nef [9,10], have also been shown to induce intrinsic B-cell functional defects.

HIV-2 infection is characterized by a much slower rate of disease progression, with low-to-undetectable viremia, providing a unique naturally occurring model of attenuated disease to investigate B-cell disturbances in HIV pathogenesis. HIV-2 plasma viral load is usually low to undetectable throughout the entire disease course [11,12]. This low viremia is thought to account for the low transmission rates [13,14] contributing to the confinement of HIV-2 infection to west Africa and connected countries, such as Portugal [15,16]. Additionally, HIV-2 has limited impact on the mortality of infected adults, even in rural west African areas, where its prevalence has reached 8–10% [17–19]. In agreement, a prospective study of a French HIV-2 cohort has revealed that the rate of CD4 decline is, on average, 10 times lower than in HIV-1-infected individuals, leading to a median time of progression to AIDS of more than 20 years [20].

We have previously shown that as for HIV-1, CD4 depletion is directly linked to increased immune activation in HIV-2-infected individuals [12,21]. Moreover, HIV-2 infection also induces polyclonal B-cell activation, as demonstrated by the frequently observed hypergammaglobulinemia [22,23] and hyperplastic lymph nodes [24]. Of note, several studies have suggested a better ability of HIV-2-infected, in comparison with HIV-1-infected individuals, to generate and preserve significant levels of circulating HIV-neutralizing antibodies during the chronic phase of the disease [23,25–28]. This finding is thought to be mainly related to particular conformations of the HIV-2 envelope proteins that favour triggering of potent neutralizing antibody responses [23,25–30], although the possibility of a superior function of the B-cell

compartment in HIV-2-infected patients has not been formally evaluated.

Here, we report the first data on memory B-cell imbalances during HIV-2 infection. We found an unexpected major depletion of both switched and unswitched memory B-cells not recovered by ART in HIV-2-infected individuals, suggesting that, irrespective of viremia, prolonged HIV infection leads to irreversible damage of memory B-cell homeostasis.

## Methods

### Studied cohorts

The study involved 38 HIV-2-infected, 20 HIV-1-infected and 16 noninfected (seronegative) individuals. Table 1 details the cohort clinical and epidemiological data. HIV-infected patients were followed at the Hospital de Santa Maria, Lisbon, and had no ongoing opportunistic infections or tumours. All patients gave written informed consent for blood sample collection and processing. The study was approved by the Ethical Board of the Faculty of Medicine, University of Lisbon. All HIV-1-infected individuals were therapy naive. The HIV-2 cohort included 10 patients on ART that did not differ significantly from the untreated HIV-2-infected patients in respect to viremia and proviral DNA levels (Supplemental\_Digital\_Content, Table 1, <http://links.lww.com/QAD/A229>). HIV-2-infected individuals on ART showed significantly reduced CD4<sup>+</sup> T cells, both in relation to seronegatives and untreated HIV-2-infected patients (Supplemental\_Digital\_Content, Table 1, <http://links.lww.com/QAD/A229>), in agreement with previous reports on weak virologic and immunological responses to ART in HIV-2-infected individuals [20,31].

### Plasma viral load and proviral DNA assessment

HIV viremia was quantified by real time (RT)-PCR for both HIV-1-infected (detection threshold: 40 RNA

**Table 1. Clinical and epidemiological characteristics of the studied cohorts.**

	Seronegatives <sup>a</sup>	HIV-2 <sup>a</sup>	HIV-1 <sup>a</sup>
Number (men/women)	16 (6/10)	38 (14/24)	20 (15/5)
Age (years)	44 (27–57)	56 (19–78)*, #	38 (23–61)
White/black	15/1	21/17	15/5
CD4 <sup>+</sup> T-cells (%)	59 (40–77)	32 (4–66)***	30 (2–74)***
CD4 <sup>+</sup> T-cells/ $\mu$ l	818 (518–1312)	470 (52–1511)**	358 (18–1848)*
Viremia, HIV RNA copies/ml	–	200 (200–34 × 10 <sup>3</sup> )**	15 × 10 <sup>3</sup> (40–45 × 10 <sup>5</sup> )
Proviral DNA, copies/10 <sup>6</sup> PBMC	–	78 (5–1033)	66 (5–975)
B-cells (%)	6 (3–13)	7 (3–20)	6 (2–16)
B-cells/ $\mu$ l	120 (63–369)	133 (30–369)	118 (33–406)

PBMC, peripheral blood mononuclear cells. Data are expressed as median, with limits in brackets. Statistical analysis was performed with Mann–Whitney tests.

<sup>a</sup>A distinct cohort was used for serum B-cell activating factor quantification, as described in Fig. 3.

\* $P < 0.05$ .

\*\* $P < 0.01$ .

\*\*\* $P < 0.001$  in comparison with seronegatives.

# $P < 0.05$ .

\*\* $P < 0.01$  for comparisons between infected cohorts.

copies/ml, Roche, Basel, Switzerland) and HIV-2-infected (detection threshold: 200 RNA copies per millilitre, as described [31]) individuals. Viremia was, as expected [12,20,32], significantly lower in HIV-2-infected than in untreated HIV-1-infected patients (Table 1).

HIV-1 and HIV-2 total viral DNA (integrated and nonintegrated DNA species) were quantified, as previously described [33]. Briefly, DNA was extracted from  $5 \times 10^6$  peripheral blood mononuclear cell (PBMC) and total viral DNA was quantified using real-time PCR assays amplifying highly conserved regions in HIV-1 and HIV-2 *gag* (detection range: seven orders of magnitude; sensitivity: five copies). Cutoff values of the tests were considered for statistical analysis in cases in which detection was below these levels.

### Flow cytometry

PBMC were isolated immediately after venopuncture by Ficoll–Hypaque density gradient centrifugation (Sigma, St. Louis, Missouri, USA) and surface stained as previously described [34]. Briefly, after isolation PBMC were washed and surface stained for 20 min at room temperature with the following monoclonal antibodies (mAb) (clone specified in brackets): fluorescein isothiocyanate (FITC)-conjugated CD10 (CB-CALLA), phycoerythrin (PE)-Cy7-conjugated CD19 (HIB19) and allophycocyanin (APC)-conjugated CD27 (O323) from eBioscience (San Diego, California, USA), as well as with PE-conjugated immunoglobulin D (IgD) (IA6–2) and APC-Cy7-conjugated CD3 (SK7) from BD Biosciences (San Jose, California, USA). At least 150 000 events were acquired using a CANTO flow cytometer (BD Biosciences) and analyzed using FlowJo software (version 8.5.3, TreeStar, Inc., Ashland, Oregon, USA). Cells were successively gated on lymphocytes, according to forward/

side scatter characteristics and B-cells ( $CD19^+CD3^{neg}$ ), which were then analyzed in terms of CD27 and IgD expression, as illustrated in Fig. 1. T-cell activation was assessed as previously described [34] using the following mAbs (clone specified in brackets): FITC-conjugated HLA-DR (L243), PerCP-conjugated CD4 (SK3) and APC-Cy7-conjugated CD3 (SK7) from BD Biosciences; and PE-conjugated CD38 (HIT2) and APC-conjugated CD8 (RPA-T8), both from eBioscience.

### Interleukin-7, B-cell activating factor, total serum immunoglobulin, $\beta$ 2-microglobulin and specific antibodies quantifications

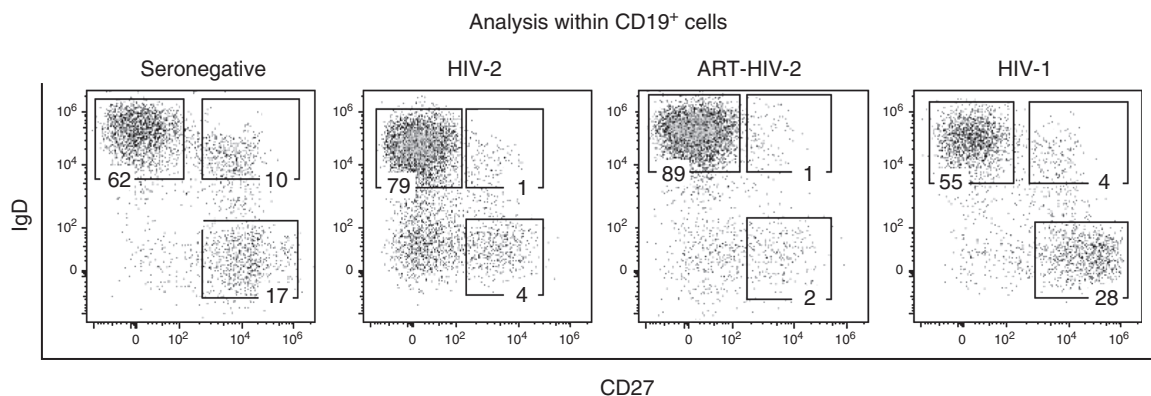
Serum interleukin (IL)-7 and B-cell activating factor (BAFF) were quantified by ELISA (R&D Systems, Minneapolis, Minnesota, USA), according to the manufacturer's specifications. Samples were assayed in duplicate.

Total immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were quantified by immunonephelometry (Beckman-Coulter, Brea, California, USA) and  $\beta$ 2-microglobulin by immunoturbidimetry (Roche Diagnostics, Indianapolis, Indiana, USA) at the clinical laboratory of the Hospital de Santa Maria.

Quantification of specific antibodies against HIV-2 *env* glycoproteins gp36 and gp125 (C2–C3 region) was performed using a dual-antigen ELISA, as previously described [29]. The results of the assay were expressed quantitatively as  $OD_{clinical\ sample}/OD_{cutoff}$  (S/CO) ratios. For ratio values of greater than 1, the samples were considered seroreactive.

### Statistical analysis

Statistical analysis was performed with Mann–Whitney tests and Spearman's correlations using GraphPad Prism



**Fig. 1. Flow-cytometric analysis of B-cell subsets.** Representative dot-plots of the flow cytometric analysis of circulating B cells ( $CD19^+CD3^{neg}$ ), according to the surface expression of IgD and CD27. Age-matched individuals are shown. Data refer to one seronegative (31 years old, 778  $CD4^+$  T cells/ $\mu$ l), one untreated HIV-2-infected (34 years old, 321  $CD4^+$  T cells/ $\mu$ l, undetectable viremia), one treated (ART) HIV-2-infected (34 years old, 326  $CD4^+$  T cells/ $\mu$ l, undetectable viremia) and one untreated HIV-1-infected (30 years old, 306  $CD4^+$  T cells/ $\mu$ l, 1814256 HIV-1 RNA copies/ml) individuals. Numbers inside quadrants represent the proportion of B cells expressing the respective markers. ART, antiretroviral therapy; IgD, Immunoglobulin D.

version 5.00 (GraphPad Software, San Diego, California, USA). Results were expressed as median and *P* values less than 0.05 were considered significant.

## Results

### HIV-2 disease was associated with a marked depletion of memory B-cells

We investigated here, for the first time, memory B-cell disturbances during HIV-2 infection. HIV-2-infected patients showed no significant alterations in either the total numbers of peripheral blood B-cells or the proportion of B-cells within total PBMC as compared with seronegative individuals (Table 1). Memory B-cell populations were assessed by flow cytometry within PBMC, as illustrated in Fig. 1.

A marked reduction in the proportion of memory B-cells (CD27<sup>+</sup>) was found in HIV-2-infected individuals (Figures 1 and 2a), both in comparison with seronegative and HIV-1-infected individuals with similar degrees of CD4 depletion (Table 1). Of note, numbers of circulating memory B-cells were significantly lower in HIV-2 individuals than in seronegatives (Supplemental\_Digital\_Content, Figure 1, <http://links.lww.com/QAD/A229>).

Memory B-cell loss was strongly correlated with CD4 depletion and immune activation, assessed both in terms of T-cell activation and serum  $\beta$ 2-microglobulin, in HIV-2-infected individuals (Table 2 and Supplemental\_Digital\_Content, Table 2, <http://links.lww.com/QAD/A229> for absolute B-cell counts). In agreement, this loss was significantly more marked in advanced as compared to early stage HIV-2 disease (less or more than 350 CD4<sup>+</sup> T-cells; Figure 2b and Supplemental\_Digital\_Content, Figure 1B for absolute B-cell counts, <http://links.lww.com/QAD/A229>).

Additionally, an association was found between memory B-cell loss and viremia (Table 2 and Supplemental\_Digital\_Content, Table 2 for absolute B-cell counts, <http://links.lww.com/QAD/A229>). Accordingly, HIV-2-infected patients with measurable viremia featured significantly less memory B-cells than those with undetectable viremia (Fig. 2b and Supplemental\_Digital\_Content, Figure 1B, <http://links.lww.com/QAD/A229>), despite the highest measured level being only 34314 RNA copies per millilitre (Table 1).

No such correlations were found in the untreated HIV-1 cohort (Table 2 and Supplemental\_Digital\_Content, Table 2 for absolute B-cell counts, <http://links.lww.com/QAD/A229>), despite the viremia being, on average, 2-log higher than in the HIV-2 cohort (Table 1). The differences in memory B-cell loss between the two infections were particularly marked when groups of

infected individuals in advanced disease stage or with detectable viremia were compared (Supplemental\_Digital\_Content, Figure 2A and Tables 3 and 4, <http://links.lww.com/QAD/A229>).

In spite of the distinct viremia, comparable amounts of cell-associated viral load (proviral DNA) were observed in the two infections (Table 1; Supplemental\_Digital\_Content, Tables 3 and 4, <http://links.lww.com/QAD/A229>), as previously reported [31–33]. Notably, no significant correlations were found between levels of proviral DNA and frequency of memory B-cells in either infection (*P* > 0.05).

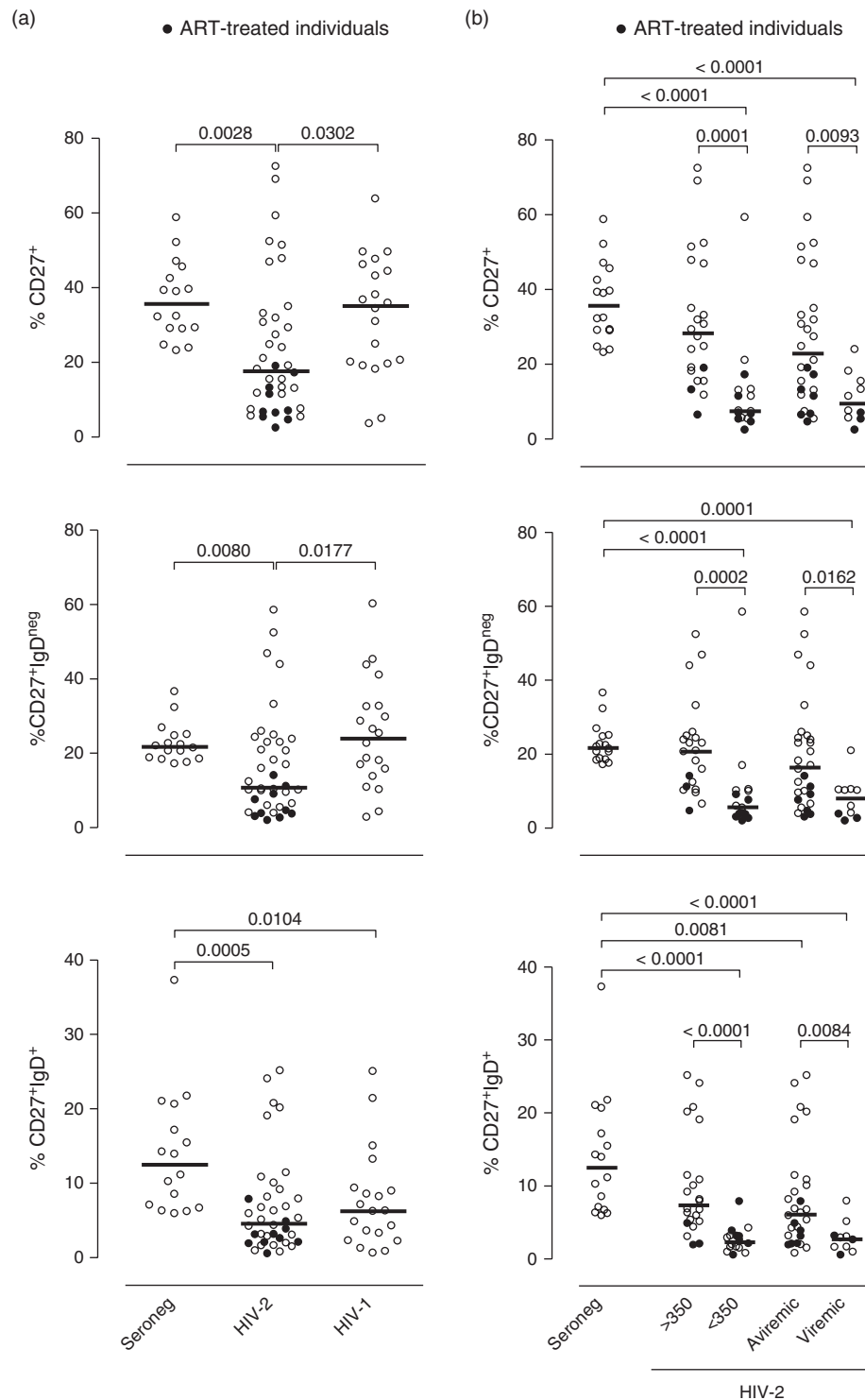
HIV-1 has also been associated with an expansion of peripheral blood immature (CD27<sup>neg</sup>CD10<sup>+</sup>) B-cells that was shown to be directly correlated with circulating IL-7 levels and suggested to arise from lymphopenia-induced IL-7-mediated homeostasis [53]. We have previously shown that serum IL-7 is significantly increased in HIV-2 infection in strong association with CD4<sup>+</sup> T-cell levels [39]. In fact, we also observed a significant expansion of CD27<sup>neg</sup>CD10<sup>+</sup> B-cells in HIV-2-infected patients (median: 1.09%, range: 0.09–7.95; *P* = 0.0007), as compared to seronegatives (median: 0.39%, range: 0.10–1.41). Nevertheless, we found no significant correlations between the frequency of CD27<sup>neg</sup>CD10<sup>+</sup> B-cells with serum IL-7 levels in the HIV-2 cohort (*n* = 35; serum IL-7 levels median: 12.21 pg/ml, range: 2.26–28.70; *r* = 0.1859, *P* = 0.2850) suggesting that other mechanisms are modulating the expansion of immature B-cells during HIV-2 infection.

Although, the HIV-2 cohort included 10 individuals under ART (Supplemental\_Digital\_Content, Table 1, <http://links.lww.com/QAD/A229>), the B-cell imbalances were also found when only untreated individuals were compared to seronegatives (Supplemental\_Digital\_Content, Figure 3A, <http://links.lww.com/QAD/A229>). Moreover, ART-treated patients showed a significantly lower frequency of memory B-cells than their untreated counterparts (Supplemental\_Digital\_Content, Figure 3A, <http://links.lww.com/QAD/A229>), which may reflect their prolonged infection, as estimated below, and the poor immunological recovery under ART typically observed in HIV-2 infection (Supplemental\_Digital\_Content, Table 1, <http://links.lww.com/QAD/A229>).

Overall, HIV-2 infection was associated with progressive loss of memory B-cells.

### Both switched and unswitched memory B-cells were lost during HIV-2 disease

The CD27<sup>+</sup> B-cell subset can be further subdivided in terms of class-switched and unswitched immunoglobulin production, assessed here by IgD surface expression [35].



**Fig. 2. Memory B-cell subsets frequency in HIV-2 infection.** (a) Relative proportions of total memory cells (CD27<sup>+</sup>, top graph), switched memory cells (CD27<sup>+</sup>IgD<sup>neg</sup>, middle graph) and unswitched memory cells (CD27<sup>+</sup>IgD<sup>+</sup>, bottom graph), within total B-cells, in the HIV-2 cohort, as well as seronegative (Seroneg) and HIV-1-infected individuals. (b) HIV-2-infected patients were further subdivided according to disease stage (early: >350 CD4<sup>+</sup> T-cells/ $\mu$ l; late: <350 CD4<sup>+</sup> T-cells/ $\mu$ l) and levels of plasma viral load (aviremic: undetectable plasma viral load; viremic: detectable). The frequencies of the B-cell subsets described in (a) were compared between seronegatives and all subgroups of HIV-2-infected individuals. Each dot represents one individual and bars indicate median. Filled circles refer to ART-treated individuals. Statistical analysis was performed using the Mann–Whitney test and the significant *P* values are shown. ART, antiretroviral therapy.

**Table 2. Relationship between B-cell subsets and markers of disease progression.**

	% B-cells	Analysis within B-cells			
		%CD27 <sup>neg</sup> IgD <sup>+</sup>	%CD27 <sup>+</sup>	%CD27 <sup>+</sup> IgD <sup>+</sup>	%CD27 <sup>+</sup> IgD <sup>neg</sup>
HIV-2 ( <i>n</i> = 38)					
CD4 <sup>+</sup> T-cells/ $\mu$ l	-0.0222; 0.8947	<b>-0.5551; 0.0003</b>	<b>0.6001; &lt;0.0001</b>	<b>0.7591; &lt;0.0001</b>	<b>0.5717; 0.0002</b>
Viremia, HIV RNA cp/ml	0.0167; 0.9209	<b>0.3697; 0.0223</b>	<b>-0.4214; 0.0084</b>	<b>-0.4250; 0.0078</b>	<b>-0.3981; 0.0133</b>
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD4 <sup>a</sup>	0.2132; 0.1988	<b>0.4842; 0.0021</b>	<b>-0.5541; 0.0003</b>	<b>-0.7152; &lt;0.0001</b>	<b>-0.5036; 0.0013</b>
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD8 <sup>a</sup>	0.1664; 0.3180	<b>0.4323; 0.0067</b>	<b>-0.4641; 0.0033</b>	<b>-0.6157; &lt;0.0001</b>	<b>-0.4224; 0.0082</b>
$\beta$ 2-microglobulin (mg/l) <sup>b</sup>	-0.0327; 0.8638	<b>0.5894; 0.0006</b>	<b>-0.6416; 0.0001</b>	<b>-0.7961; &lt;0.0001</b>	<b>-0.5877; 0.0006</b>
HIV-1 ( <i>n</i> = 20)					
CD4 <sup>+</sup> T-cells/ $\mu$ l	-0.0911; 0.7026	-0.2295; 0.3304	0.2528; 0.2822	<b>0.6566; 0.0017</b>	0.0391; 0.8700
Viremia, HIV RNA copies/ml	0.0866; 0.7165	-0.0934; 0.6953	0.1574; 0.5074	<b>-0.5467; 0.0126</b>	0.3614; 0.1174
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD4 <sup>a</sup>	-0.0730; 0.7598	0.1068; 0.6539	-0.0760; 0.7501	<b>-0.5709; 0.0086</b>	0.1873; 0.4291
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD8 <sup>a</sup>	0.0008; 0.9975	-0.2685; 0.2523	0.2941; 0.2082	-0.3820; 0.0965	<b>0.5759; 0.0079</b>
$\beta$ 2-microglobulin (mg/l) <sup>b</sup>	-0.4180; 0.1369	-0.0550; 0.8518	0.1342; 0.6474	-0.3934; 0.1640	0.2396; 0.4094

Spearman's correlation coefficient was used and results are expressed as *r*; *P*, with significant correlations in bold.

<sup>a</sup>Frequency of cells coexpressing the activation markers HLA-DR and CD38 within CD4<sup>+</sup> and CD8<sup>+</sup> T-cells (median, range). HIV-2: 3.4, 0.4–23.5 (CD4) and 15.1, 0.6–69.5% (CD8); untreated HIV-1: 5.5, 0.3–34.8 (CD4) and 23.7, 1.4–62.2% (CD8). No significant differences were found between infected cohorts, which exhibited significantly higher levels than seronegative controls (CD4: 1.1, 0.7–2.0%; CD8: 2.7, 1.3–22.7%).

<sup>b</sup> $\beta$ 2-microglobulin serum levels were assessed for 30 HIV-2 (median: 2.6 mg/l, range: 1.1–7.8 mg/l) and 14 HIV-1-infected (median: 2.4 mg/l, range: 1.3–6.0 mg/l) individuals, with no statistically significant differences being observed between cohorts.

Class-switched memory B-cells (CD27<sup>+</sup>IgD<sup>neg</sup>) are mainly generated in germinal centres and play a key role in adaptive immune responses, whereas unswitched CD27<sup>+</sup>IgD<sup>+</sup> B-cells are thought to include largely marginal zone B-cells, and are known to play a role in protection against encapsulated bacteria [35].

Memory B-cell loss observed during HIV-2 disease was related to a pronounced depletion of cells with a class-switched phenotype (Figs. 1 and 2a; Supplemental\_Digital\_Content, Figure 1 for absolute counts, <http://links.lww.com/QAD/A229>), which showed strong positive correlations with markers of disease progression (Table 2 and Supplemental\_Digital\_Content, Table 2 for absolute B-cell counts, <http://links.lww.com/QAD/A229>). No such correlations were observed in the HIV-1 cohort (Table 2 and Supplemental\_Digital\_Content, Table 2, <http://links.lww.com/QAD/A229>), and the switched memory B-cell depletion was much lower in HIV-1 than in HIV-2 infection (Figs. 1 and 2a; Supplemental\_Digital\_Content, Figure 1 for absolute counts, <http://links.lww.com/QAD/A229>). Moreover, in the HIV-2 cohort, switched memory B-cell frequency was significantly lower in patients with less than 350 CD4<sup>+</sup> T-cells per microlitre, as compared to early stage disease, as well as in patients with detectable viremia versus their aviremic counterparts (Figure 2B; Supplemental\_Digital\_Content, Figure 1B for absolute counts, <http://links.lww.com/QAD/A229>). This was also observed for untreated and ART-treated HIV-2-infected individuals, when analyzed separately (Supplemental\_Digital\_Content, Figure 3B, <http://links.lww.com/QAD/A229>), but was not documented for HIV-1 infection (Supplemental\_Digital\_Content, Figure 2B, <http://links.lww.com/QAD/A229>). Notably, despite both infections exhibiting similar levels of immune

activation (Table 2), as previously reported [12], the impairment in switched memory B-cell preservation seemed to be more strongly linked to the persistent immune activation in HIV-2 than in HIV-1 infection (Table 2 and Supplemental\_Digital\_Content, Table 2 for absolute B-cell counts, <http://links.lww.com/QAD/A229>).

Conversely, the loss of unswitched (IgD<sup>+</sup>) memory B-cells in HIV-2 infection was comparable to that observed in HIV-1 infection (Fig. 2a and Supplemental\_Digital\_Content, Figure 1A for absolute counts, <http://links.lww.com/QAD/A229>). This loss was positively correlated with markers of disease progression in both HIV-1 and HIV-2 cohorts (Table 2 and Supplemental\_Digital\_Content, Table 2 for absolute B-cell counts, <http://links.lww.com/QAD/A229>), being significantly more pronounced in patients with CD4<sup>+</sup> T-cell counts below 350 cells/ $\mu$ l or detectable viremia (Fig. 2b; Supplemental\_Digital\_Content, Figure 1B and Figure 2C, <http://links.lww.com/QAD/A229>).

Of note, both switched and unswitched memory B-cells were markedly decreased in HIV-2-infected patients under ART (Supplemental\_Digital\_Content, Figure 3B and 3C, respectively, <http://links.lww.com/QAD/A229>), which may be, at least in part, related to the more prolonged length of disease. Given the slow rate of CD4 decline, it is expected that HIV-2-infected patients have been infected for much longer than untreated HIV-1-infected individuals. Our estimation of the length of follow-up, for those patients for whom these data were available, was in agreement with this possibility (median months for treated HIV-2: 97, range: 8–242, *n* = 10; untreated HIV-2: 71, range: 24–235, *n* = 16; untreated HIV-1: 75, range: 13–184, *n* = 9).

Notably, B-cell imbalances did not correlate with age in all the cohorts ( $P > 0.05$ ).

Altogether, progressive memory B-cell loss during HIV-2 infection was due to a depletion of both switched and unswitched memory B-cells.

### Memory B-cell imbalances did not translate into decreased immunoglobulin levels

Notwithstanding the generalized memory B-cell loss, most HIV-2-infected patients showed increased levels of IgG, as previously reported [22,23], while maintaining IgM and IgA titers within the normal range (Supplemental\_Digital\_Content, Figure 4A, <http://links.lww.com/QAD/A229>).

Additionally, we quantified serum levels of antibodies against HIV-2 *env* glycoproteins gp36 and gp125 (C2-C3 region) as previously described [29]. No significant differences were observed in specific antibody levels when HIV-2-infected patients were subdivided, either according to CD4<sup>+</sup> T-cell counts or viremia ( $P > 0.05$ ). Nevertheless, although IgG, IgM and IgA levels did not significantly differ between untreated and treated HIV-2 infection, HIV-2-infected individuals under ART had significantly lower levels of specific antibodies against the C2-C3 region of gp125 (Supplemental\_Digital\_Content, Figure 4B, <http://links.lww.com/QAD/A229>).

Overall, HIV-2-infected patients did not feature major impairments in total immunoglobulin levels or HIV-2-specific antibody production, despite the observed memory B-cell imbalances.

### B-cell activating factor levels increased during HIV-2 disease, particularly in the presence of detectable viremia, in direct association with memory B-cell disturbances

B-cell survival and differentiation rely on BAFF, a cytokine mainly produced by stromal cells, monocytes and T-cells, detectable in the serum of healthy individuals [36–38]. BAFF levels were significantly higher in the HIV-2 cohort, as compared with seronegatives (Fig. 3a). Notably, a direct correlation was found between serum BAFF levels and viremia in the HIV-2 cohort ( $r = 0.4939$ ,  $P = 0.0195$ ,  $n = 12$ ), and in agreement, significantly higher BAFF titres were observed in HIV-2-infected patients with detectable viremia, both in relation to aviremic individuals and seronegatives (Fig. 3B, Supplemental\_Digital\_Content, Figure 5A, <http://links.lww.com/QAD/A229>); this was observed in untreated as well as in ART-treated HIV-2 groups (Supplemental\_Digital\_Content, Figure 5B, <http://links.lww.com/QAD/A229>). A similar relationship between serum BAFF levels and viremia was observed in the HIV-1 cohort ( $r = 0.5916$ ,  $P = 0.0428$ ,  $n = 12$ ; and Supplemental\_Digital\_Content, Figure 5A, <http://links.lww.com/QAD/A229>).

Although no significant correlations were found between serum BAFF levels and the degree of CD4 depletion, advanced HIV-2 disease (less than 350 CD4<sup>+</sup> T-cells/ $\mu$ l) was associated with higher BAFF levels (Fig. 3b). Moreover, HIV-2-infected individuals under ART showed a significant increase in BAFF as compared to seronegatives irrespective of viremia possibly related to their reduced CD4 cell counts (Supplemental\_Digital\_Content, Figure 5B, <http://links.lww.com/QAD/A229>).

Of note, HIV-2-infected patients showed significant inverse correlations between serum BAFF levels and frequency of total ( $r = -0.5456$ ,  $P = 0.0086$ ,  $n = 22$ ) and switched memory B cells ( $r = -0.5247$ ,  $P = 0.0122$ ,  $n = 22$ ). Such correlations were not found in the HIV-1 cohort ( $P > 0.05$ ).

In conclusion, HIV-2 infection was associated with an increase in serum BAFF levels that correlated with memory B-cell loss.

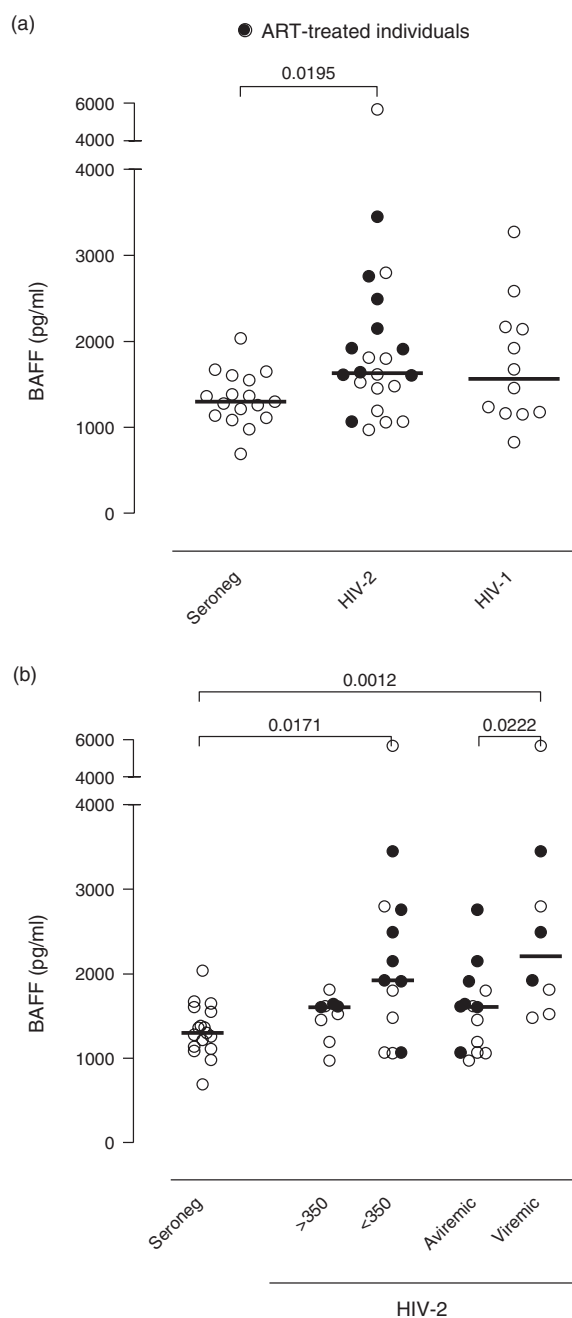
## Discussion

We report here, for the first time, that despite its relatively benign course and low viremia, HIV-2 disease progression was associated with major losses of both switched and unswitched memory B-cells and elevated serum BAFF levels. Additionally, we found that these disturbances were not recovered by ART.

We showed that memory B-cell levels negatively correlated with T-cell activation in HIV-2-infected individuals, suggesting a close association between B-cell disturbances and hyperimmune activation, even in the context of low viremia. This is in agreement with previous reports associating B-cell dysregulation during HIV-1 disease with hyperimmune activation [3,4].

In order to account for the possible impact of CD4 lymphopenia on B-cell disturbances, we compared HIV-1-infected and HIV-2-infected individuals with similar degrees of CD4 depletion, despite differences in length of disease and viremia. Importantly, memory B-cell loss, particularly of the switched memory pool, was much more marked in HIV-2 than HIV-1 infection, despite the clearly better prognosis and the much lower amounts of circulating virus in HIV-2 infection.

A recent report associated the loss of switched memory B-cells in HIV-1 infection with impairment in the ability of B-cells to respond to IL-2 or other  $\gamma$ c cytokines leading to an increase in their susceptibility to apoptosis [4]. Although this possibility was not excluded in the current study of HIV-2-infected patients, our previous data support a relatively better preserved ability to produce



**Fig. 3. Serum B-cell activating factor levels in HIV-2 infection.** (a) Serum B-cell activating factor (BAFF) levels in HIV-2-infected, HIV-1-infected, and seronegative (Seroneg) individuals. Due to sample availability, a distinct seronegative cohort was used [11 women/six men; 39 (26–61) median years of age; 63.85 (40.10–76.90) median CD4<sup>+</sup> T-cell frequency; 908 (537–1312) median CD4<sup>+</sup> T-cells/ $\mu$ l; 8.15 (2.56–18.19) median B-cell frequency; 163 (63–383) median B-cells/ $\mu$ l], as well as representative subgroups of the infected cohorts [HIV-2, including the 10 treated individuals: 13 women/nine men; 56 (19–72) median years of age; 28.02 (4.51–63.20) median CD4<sup>+</sup> T-cell frequency; 337 (52–1336) median CD4<sup>+</sup> T-cells/ $\mu$ l; 200 (200–34314) median RNA copies per millilitre; 108 (5–1002) median proviral DNA copies per 10<sup>6</sup> PBMC; 7.41 (2.89–19.70) median B-cell frequency; 132 (30–369) median B-cells per microlitre; and untreated HIV-1: three women/nine men; 42 (23–61) median years of age; 32.85 (7.71–74.4) median CD4<sup>+</sup> T-cell frequency; 431 (77–1425) median CD4<sup>+</sup> T-cells/ $\mu$ l;  $1.4 \times 10^4$  (40–4.5  $\times 10^6$ ) RNA copies per millilitre; 88 (5–573) median proviral DNA copies per 10<sup>6</sup> PBMC; 6.64 (2.08–15.60) median B-cell frequency; 112 (56–324) median B-cells per microlitre]. (b) The HIV-2 cohort was further stratified according to disease stage (early: >350 CD4<sup>+</sup> T-cells/ $\mu$ l; late: <350 CD4<sup>+</sup> T-cells/ $\mu$ l) and levels of plasma viral load (aviremic: undetectable; viremic: detectable), and the BAFF levels were compared between seronegatives and all groups of HIV-2-infected individuals. Each dot represents one individual and bars indicate median. Filled circles refer to antiretroviral therapy (ART)-treated individuals. Statistical analysis was performed using the Mann–Whitney test and the significant *P* values are shown.

and respond to  $\gamma\text{C}$  cytokines in HIV-2, as compared to HIV-1 infection [39–41].

The marked levels of switched memory B-cell depletion in HIV-2 infection were unlikely to be solely attributable to loss of the small subset of IgM-only ( $\text{CD27}^+\text{IgD}^{\text{neg}}\text{IgM}^+$ ) memory B-cells [42,43]. Nevertheless, it would be of interest to address in future studies the detailed phenotype of memory B-cells, including levels of expression of IgM and CD21, whose loss has been associated with ongoing HIV replication and disease progression in HIV-1-infected individuals [35].

It is also possible that cell redistribution contributed, at least in part, to the reduction of circulating memory B-cells, emphasizing the relevance of tissue studies. Moreover, given the expected prolonged length of HIV-2 disease, it is plausible that the marked loss of switched memory B-cells may be related to cumulative lymphoid tissue damage, and consequent disruption of the generation of germinal centres where these cells are produced, and/or of the microenvironment required for their survival, ultimately reaching an irreversible level. Accordingly, a more marked switched memory B-cell loss was found in HIV-2-infected patients under long-term antiretroviral treatment, who were estimated to have been infected for a longer period of time.

To our knowledge, there are no data on HIV-2-infected secondary lymphoid organs (SLO). The persistent immune activation observed during HIV-2 infection [12] may be associated with SLO inflammation and collagen deposition with progressive disruption of their architecture, as has been described during HIV-1 infection [44]. HIV-1 has been shown to mainly reside in lymphoid tissues, trapped in the follicular dendritic cell (FDC) network, potentially contributing to progressive disruption of germinal centre responses and limiting the survival signals required for memory B-cell maintenance [45,46]. It is also possible that low levels of HIV-2 replication occur in SLO, contributing to the hyper-activated immune state and the putative progressive tissue damage. In fact, the cytopathicity documented upon HIV-2 infection of human lymphoid tissue explant cultures was shown to be comparable to that observed for HIV-1 [47].

Importantly, we and others have reported similar levels of cell-associated viral burden in HIV-1-infected and HIV-2-infected patients, as assessed by the levels of proviral DNA within PBMC [31–33]. Thus, the ability to disseminate and establish a reservoir of infected cells is apparently similar in both infections. Furthermore, we have recently provided evidence of a significant degree of ongoing viral replication in HIV-2-infected individuals based on the levels of viral *gag* and *tat* gene transcripts within PBMC [31]. These data raised questions about the control of HIV-2 latency. It is plausible that HIV-2

continuously replicates in SLO at low levels, possibly by cell-to-cell mediated transmission. In spite of this putative HIV-2 replication being locally contained, given the reduced viremia, it may have a long-term impact, contributing to accelerated immune senescence. Additionally, it may contribute to the poor immunological recovery that is usually observed in HIV-2-infected patients receiving ART [20,31,48,49], highlighting the importance of rethinking the guidelines on when to start ART in HIV-infected patients [50].

Our findings are particularly relevant in view of the cumulative data suggesting that HIV-1-infected patients under apparently effective ART have significant degrees of replication in SLO [51,52]. These low levels of viral replication were suggested to contribute to a proinflammatory state and to the related complications found in long-term treated HIV-1-infected patients [52].

Notably, in spite of the reduced amounts of plasma HIV-2 load, irrespective of disease stage, we found that the presence of detectable viremia was associated with significantly lower levels of memory B-cells. These results suggest a direct role of the free virions in B-cell disturbances. Alternatively, the presence of detectable virus may itself represent indirect evidence for the disruption of the FDC network, which eventually becomes unable to contain the produced virions.

BAFF has been shown to be critical for B-cell maturation and survival upon bone-marrow egress [36]. Of note, we found that serum BAFF levels were increased in HIV-2-infected patients, particularly in association with viremia, in agreement with previous reports in HIV-1 infection [37,38]. Moreover, HIV-2-infected individuals under ART, who had longer follow-up, featured significant increases in BAFF levels irrespective of viremia, which may contribute to their heightened inflammatory state and B-cell imbalances, as has been suggested in HIV-1 infection [8,37,38,54].

Overall, we showed, for the first time, that despite the reduced amount of circulating virus, HIV-2 infection is associated with a marked depletion of both switched and unswitched memory B-cells, in association with an increase in serum BAFF levels. Our data provide evidence that prolonged HIV disease, even in the absence of detectable viremia, leads to an irreversible damage of memory B-cell homeostasis.

## Acknowledgements

We gratefully acknowledge the collaboration of the following colleagues: E. Valadas, F. Antunes, M. Doroana, M. Lucas, and S. Sousa.

R.T. designed and performed experiments, analyzed data and wrote the article; S.F. analyzed data; R.B.F., J.M.M., N.T., R.S.S., A.P.B., R.C. and P.G. performed experiments; R.M.M.V. contributed to the design of the study and data interpretation; A.E.S. designed the research and wrote the article.

This work was supported by grant (PIC/IC/82712/2007) from 'Fundação para a Ciência e a Tecnologia' (FCT) and by 'Programa Operacional Ciência e Inovação 2010' (POCI2010), as well as by Fundação Calouste Gulbenkian to AES. RT, RBF, RSS, and RC received scholarships from FCT.

## Conflicts of interest

The authors have no conflicting financial interests.

## References

- Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS. **Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome.** *N Engl J Med* 1983; **309**:453–458.
- De Milito A, Morch C, Sonnerborg A, Chiodi F. **Loss of memory (CD27) B lymphocytes in HIV-1 infection.** *AIDS* 2001; **15**:957–964.
- Moir S, Malaspina A, Ogwaro KM, Donoghue ET, Hallahan CW, Ehler LA, et al. **HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals.** *Proc Natl Acad Sci U S A* 2001; **98**:10362–10367.
- van Grevenynghe J, Cubas RA, Noto A, DaFonseca S, He Z, Peretz Y, et al. **Loss of memory B cells during chronic HIV infection is driven by Foxo3a- and TRAIL-mediated apoptosis.** *J Clin Invest* 2011; **121**:3877–3888.
- De Milito A, Nilsson A, Titanji K, Thorstensson R, Reizenstein E, Narita M, et al. **Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection.** *Blood* 2004; **103**:2180–2186.
- Titanji K, Chiodi F, Bellocchio R, Schepis D, Osorio L, Tassandin C, et al. **Primary HIV-1 infection sets the stage for important B lymphocyte dysfunctions.** *AIDS* 2005; **19**:1947–1955.
- Rieckmann P, Poli G, Fox CH, Kehr JH, Fauci AS. **Recombinant gp120 specifically enhances tumor necrosis factor-alpha production and Ig secretion in B lymphocytes from HIV-infected individuals but not from seronegative donors.** *J Immunol* 1991; **147**:2922–2927.
- He B, Qiao X, Klasse PJ, Chiu A, Chadburn A, Knowles DM, et al. **HIV-1 envelope triggers polyclonal Ig class switch recombination through a CD40-independent mechanism involving BAFF and C-type lectin receptors.** *J Immunol* 2006; **176**:3931–3941.
- Qiao X, He B, Chiu A, Knowles DM, Chadburn A, Cerutti A. **Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells.** *Nat Immunol* 2006; **7**:302–310.
- Swingler S, Zhou J, Swingler C, Dauphin A, Greenough T, Jolicœur P, Stevenson M. **Evidence for a pathogenic determinant in HIV-1 Nef involved in B cell dysfunction in HIV/AIDS.** *Cell Host Microbe* 2008; **4**:63–76.
- Popper SJ, Sarr AD, Travers KU, Gueye-Ndiaye A, Mboup S, Essex ME, Kanki PJ. **Lower human immunodeficiency virus (HIV) type 2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2.** *J Infect Dis* 1999; **180**:1116–1121.
- Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM. **CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load.** *J Immunol* 2002; **169**:3400–3406.
- Kanki PJ, Travers KU, Mboup S, Hsieh CC, Marlink RG, Gueye NA, et al. **Slower heterosexual spread of HIV-2 than HIV-1.** *Lancet* 1994; **343**:943–946.
- O'Donovan D, Ariyoshi K, Milligan P, Ota M, Yamuah L, Sarge-Njie R, Whittle H. **Maternal plasma viral RNA levels determine marked differences in mother-to-child transmission rates of HIV-1 and HIV-2 in The Gambia.** *MRC/Gambia Government/University College London Medical School working group on mother-child transmission of HIV.* *AIDS* 2000; **14**:441–448.
- Valadas E, Franca L, Sousa S, Antunes F. **20 years of HIV-2 infection in Portugal: trends and changes in epidemiology.** *Clin Infect Dis* 2009; **48**:1166–1167.
- Carvalho A, Valadas E, Franca L, Carvalho C, Aleixo M, Mendez J, et al. **Population mobility and the changing epidemics of HIV-2 in Portugal.** *HIV Med* 2011.
- Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, et al. **Reduced rate of disease development after HIV-2 infection as compared to HIV-1.** *Science* 1994; **265**:1587–1590.
- van der Loeff MF, Larke N, Kaye S, Berry N, Ariyoshi K, Alabi A, et al. **Undetectable plasma viral load predicts normal survival in HIV-2-infected people in a West African village.** *Retrovirology* 2010; **7**:46.
- Poulsen AG, Aaby P, Larsen O, Jensen H, Naucner A, Lisse IM, et al. **9-year HIV-2-associated mortality in an urban community in Bissau, west Africa.** *Lancet* 1997; **349**:911–914.
- Drylewicz J, Matheron S, Lazaro E, Damond F, Bonnet F, Simon F, et al. **Comparison of viro-immunological marker changes between HIV-1 and HIV-2-infected patients in France.** *AIDS* 2008; **22**:457–468.
- Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM, Paul WE. **CD4<sup>+</sup> T-cell depletion in HIV infection: are we closer to understanding the cause?** *Nat Med* 2002; **8**:319–323.
- Kestens L, Brattegaard K, Adjorlolo G, Ekpin E, Sibailly T, Diallo K, et al. **Immunological comparison of HIV-1-, HIV-2- and dually-reactive women delivering in Abidjan, Cote d'Ivoire.** *AIDS* 1992; **6**:803–807.
- Marcelino JM, Nilsson C, Barroso H, Gomes P, Borrego P, Maltez F, et al. **Envelope-specific antibody response in HIV-2 infection: C2V3C3-specific IgG response is associated with disease progression.** *AIDS* 2008; **22**:2257–2265.
- Brun-Vezinet F, Rey MA, Katlama C, Girard PM, Roulot D, Yeni P, et al. **Lymphadenopathy-associated virus type 2 in AIDS and AIDS-related complex. Clinical and virological features in four patients.** *Lancet* 1987; **1**:128–132.
- Bjorling E, Scarlatti G, von Gegerfelt A, Albert J, Biberfeld G, Chiodi F, et al. **Autologous neutralizing antibodies prevail in HIV-2 but not in HIV-1 infection.** *Virology* 1993; **193**:528–530.
- Ozkaya Sahin G, Holmgren B, da Silva Z, Nielsen J, Nowroozizadeh S, Esbjornsson J, et al. **Potent intratype neutralizing activity distinguishes human immunodeficiency virus type 2 (HIV-2) from HIV-1.** *J Virol* 2012; **86**:961–971.
- de Silva TI, Aasa-Chapman M, Cotten M, Hue S, Robinson J, Bibollet-Ruche F, et al. **Potent autologous and heterologous neutralizing antibody responses occur in HIV-2 infection across a broad range of infection outcomes.** *J Virol* 2012; **86**:930–946.
- Kong R, Li H, Bibollet-Ruche F, Decker JM, Zheng NN, Gottlieb GS, et al. **Broad and potent neutralizing antibody responses elicited in natural HIV-2 infection.** *J Virol* 2012; **86**:947–960.
- Marcelino JM, Barroso H, Goncalves F, Silva SM, Novo C, Gomes P, et al. **Use of a new dual-antigen enzyme-linked immunosorbent assay to detect and characterize the human antibody response to the human immunodeficiency virus type 2 envelope gp125 and gp36 glycoproteins.** *J Clin Microbiol* 2006; **44**:607–611.
- Barroso H, Borrego P, Bartolo I, Marcelino JM, Familia C, Quintas A, Taveira N. **Evolutionary and structural features of the C2, V3 and C3 envelope regions underlying the differences in HIV-1 and HIV-2 biology and infection.** *PLoS One* 2011; **6**:e14548.
- Soares RS, Tendeiro R, Foxall RB, Baptista AP, Cavaleiro R, Gomes P, et al. **Cell-associated viral burden provides evidence of ongoing viral replication in aviremic HIV-2-infected patients.** *J Virol* 2011; **85**:2429–2438.
- Popper SJ, Sarr AD, Gueye-Ndiaye A, Mboup S, Essex ME, Kanki PJ. **Low plasma human immunodeficiency virus type 2 viral load is independent of proviral load: low virus production in vivo.** *J Virol* 2000; **74**:1554–1557.
- Soares R, Foxall R, Albuquerque A, Cortesao C, Garcia M, Victorino RM, Sousa AE. **Increased frequency of circulating CCR5<sup>+</sup> CD4<sup>+</sup> T cells in human immunodeficiency virus type 2 infection.** *J Virol* 2006; **80**:12425–12429.

34. Cavaleiro R, Baptista AP, Soares RS, Tendeiro R, Foxall RB, Gomes P, *et al.* **Major depletion of plasmacytoid dendritic cells in HIV-2 infection, an attenuated form of HIV disease.** *PLoS Pathog* 2009; **5**:e1000667.
35. Moir S, Fauci AS. **Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease.** *J Allergy Clin Immunol* 2008; **122**:12–19; quiz 20–11.
36. Mackay F, Browning JL. **BAFF: a fundamental survival factor for B cells.** *Nat Rev Immunol* 2002; **2**:465–475.
37. Fontaine J, Chagnon-Choquet J, Valcke HS, Poudrier J, Roger M. **High expression levels of B lymphocyte stimulator (BLyS) by dendritic cells correlate with HIV-related B-cell disease progression in humans.** *Blood* 2011; **117**:145–155.
38. Rodriguez B, Valdez H, Freimuth W, Butler T, Asaad R, Lederman MM. **Plasma levels of B-lymphocyte stimulator increase with HIV disease progression.** *AIDS* 2003; **17**:1983–1985.
39. Albuquerque AS, Cortesao CS, Foxall RB, Soares RS, Victorino RM, Sousa AE. **Rate of increase in circulating IL-7 and loss of IL-7/Ralpha expression differ in HIV-1 and HIV-2 infections: two lymphopenic diseases with similar hyperimmune activation but distinct outcomes.** *J Immunol* 2007; **178**:3252–3259.
40. Foxall RB, Soares RS, Albuquerque AS, Cortesao CS, Victorino RM, Sousa AE. **Increased frequency of CD25dimCD4<sup>+</sup> T-cells in HIV-2 infection, a naturally occurring attenuated form of HIV-1.** *Clin Immunol* 2008; **127**:158–167.
41. Sousa AE, Chaves AF, Loureiro A, Victorino RM. **Comparison of the frequency of interleukin (IL)-2-, interferon-gamma-, and IL-4-producing T cells in 2 diseases, human immunodeficiency virus types 1 and 2, with distinct clinical outcomes.** *J Infect Dis* 2001; **184**:552–559.
42. Klein U, Kuppers R, Rajewsky K. **Human IgM<sup>+</sup>IgD<sup>+</sup> B cells, the major B cell subset in the peripheral blood, express V kappa genes with no or little somatic mutation throughout life.** *Eur J Immunol* 1993; **23**:3272–3277.
43. Klein U, Kuppers R, Rajewsky K. **Variable region gene analysis of B cell subsets derived from a 4-year-old child: somatically mutated memory B cells accumulate in the peripheral blood already at young age.** *J Exp Med* 1994; **180**:1383–1393.
44. Zeng M, Smith AJ, Wietgreffe SW, Southern PJ, Schacker TW, Reilly CS, *et al.* **Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections.** *J Clin Invest* 2011; **121**:998–1008.
45. Rademakers LH, Schuurman HJ, de Frankrijker JF, Van Ooyen A. **Cellular composition of germinal centers in lymph nodes after HIV-1 infection: evidence for an inadequate support of germinal center B lymphocytes by follicular dendritic cells.** *Clin Immunol Immunopathol* 1992; **62**:148–159.
46. Pantaleo G, Cohen OJ, Schacker T, Vaccarezza M, Graziosi C, Rizzardi GP, *et al.* **Evolutionary pattern of human immunodeficiency virus (HIV) replication and distribution in lymph nodes following primary infection: implications for antiviral therapy.** *Nat Med* 1998; **4**:341–345.
47. Schramm B, Penn ML, Palacios EH, Grant RM, Kirchhoff F, Goldsmith MA. **Cytopathicity of human immunodeficiency virus type 2 (HIV-2) in human lymphoid tissue is coreceptor dependent and comparable to that of HIV-1.** *J Virol* 2000; **74**:9594–9600.
48. Chiara M, Rony Z, Homa M, Bhanumati V, Ladomirskaya J, Manzi M, *et al.* **Characteristics, immunological response and treatment outcomes of HIV-2 compared with HIV-1 & dual infections (HIV 1/2) in Mumbai.** *Indian J Med Res* 2010; **132**:683–689.
49. van der Ende ME, Prins JM, Brinkman K, Keuter M, Veenstra J, Danner SA, *et al.* **Clinical, immunological and virological response to different antiretroviral regimens in a cohort of HIV-2-infected patients.** *AIDS* 2003; **17** (Suppl 3):S55–61.
50. Moir S, Buckner CM, Ho J, Wang W, Chen J, Waldner AJ, *et al.* **B cells in early and chronic HIV infection: evidence for preservation of immune function associated with early initiation of antiretroviral therapy.** *Blood* 2010; **116**:5571–5579.
51. Buzon MJ, Codoner FM, Frost SD, Pou C, Puertas MC, Massanella M, *et al.* **Deep molecular characterization of HIV-1 dynamics under suppressive HAART.** *PLoS Pathog* 2011; **7**:e1002314.
52. Deeks SG. **HIV infection, inflammation, immunosenescence, and aging.** *Annu Rev Med* 2011; **62**:141–155.
53. Malaspina A, Moir S, Ho J, Wang W, Howell ML, O'Shea MA, *et al.* **Appearance of immature/transitional B cells in HIV-infected individuals with advanced disease: correlation with increased IL-7.** *Proc Natl Acad Sci USA* 2006; **103**:2262–2267.
54. Moir S, Malaspina A, Pickeral OK, Donoghue ET, Vasquez J, Miller NJ, *et al.* **Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF superfamily.** *J Exp Med* 2004; **200**:587–599.