Identification of Germinal Center B Cells in Blood from HIV-infected Drug-naive Individuals in Central Africa

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To better understand the pathophysiology of B cell populations—the precursors of antibody secreting cells—during chronic human immunodeficiency virus (HIV) infection, we examined the phenotype of circulating B cells in newly diagnosed Africans. We found that all African individuals displayed low levels of naive B cells and of memory-type CD27+ B cells, and high levels of differentiated B cells. On the other hand, HIV-infected African patients had a population of germinal center B cells (i.e. CD20+, sIgM2, sIgD+, CD77+, CD138^), which are generally restricted to lymph nodes and do not circulate unless the lymph node architecture is altered. The first observations could be linked to the tropical environment whereas the presence of germinal center B cells may be attributable to chronic exposure to HIV as it is not observed in HIV-negative African controls and HAART treated HIV-infected Europeans. It may impact the management of HIV infection in countries with limited access to HIV drugs and urges consideration for implementation of therapeutic vaccines.

Keywords: HIV; B cells; Germinal center; Blood; Lymph nodes

Abbreviations: LN, lymph nodes; GC, germinal center; VL, viral load; CAR, Central African Republic

INTRODUCTION

The human immunodeficiency virus (HIV) induces profound disturbances in cellular and humoral immunity, both at the specific and non-specific levels (Fauci et al., 1996). Typically, untreated HIV-infected individuals experience disease progression and manifest hypergamma-globulinemia with antibodies (Abs) to core and surface components of HIV, but lack specific neutralizing anti-HIV envelop Abs and specific recall Abs to naturally encountered or vaccine delivered antigens (Ag) (Amadori and Chieco-Bianchi, 1990). A classic feature of HIV-disease progression is the consistent destruction of lymph nodes (LN) contributing to the progressive loss of the CD4+ T-cell compartment—the hallmark of HIV infection and AIDS (Richard et al., 2002).

Management of HIV-infected individuals in Africa—and particularly in poor countries—represents a real challenge. Few strategies are in place in tropical Africa, and while the introduction of antiretroviral drugs is planned, only vaccination strategies can truly represent a chance to limit infection spreading (Marcus et al., 2002). Whether these vaccines are expected to be preventive or therapeutic, their ultimate goal is to limit viral replication to favor the expansion of specific cytolytic T cells (CTLs) and induce B cells to produce neutralizing Abs (Peters, 2001). These strategies may be reinforced with the help of an intact innate immune system (Siegal and Spear, 2001).

In the present study, we characterized the peripheral B cell compartment of 15 drug-naive HIV-infected individuals. At homeostasis or in disease states that do not affect LN architecture or bone marrow production, blood B cells are comprised of naive and non-naive (mostly memory) B lymphocytes which recirculate, especially after antigenic exposure. These B cells represent a milestone of the specific infection they defend against as they differentiate into (neutralizing) Ab-secreting cells and, therefore are targets of vaccination strategies. Indeed, the presence of circulating cells assumed to be plasmocytes—capable of secreting anti-HIV Abs—has been reported in HIV+ patients; these studies examined treated Americans or untreated, Europeans with low viral load, respectively (Marcus et al., 2002; Richard et al., 2002). In the present work, we studied a group of HIV-infected individuals...
living in Central Africa, and unexpectedly observed
germinal center (GC) originating-B lymphocytes (not
plasmocytes) circulating in the periphery. This obser-
vation may impact future vaccination strategies.

MATERIALS AND METHODS

Patients

African donors were consultants at the “Hôpital Commu-
nautaire” of Bangui, Central African Republic (CAR) as
spouses of hospitalized patients with AIDS. The
prevalence of HIV-infection in CAR is estimated to be
12.9% of the adult population (UNAIDS/WHO 2002). All
donors enrolled in this study were informed and consented
to donate blood for research purposes, according to the
rules of the ad hoc Ethics Committee set up by the Institut
Pasteur in Bangui, in the absence of a National Ethics
Committee. None of the blood donors were previously
diagnosed with HIV infection, and all were indeed
antiretroviral drug-naïve. Patients were selected on the
basis of African AIDS symptoms defined by the WHO at
Bangui in 1985 (Diagnosing symptomatic HIV infection
and AIDS in adults, 1993). Approximately 10 ml of
heparinized blood was obtained from a series of
individuals and 15 were diagnosed HIV+. HIV serology
was routinely followed in Bangui using the Vironostika
HIV-Uniform II plus O test (Organon Teknika, Durham,
NC). Plasma viral load (VL), determined in Saint-Etienne
using a nested PCR technique as previously described
(Peters, 2001), ranged from 10,000 to 80,000 copies/ml.
HIV-1 clades A predominate in Bangui (Muller-Trutwin
et al., 1999). Matched controls consisted of 9 HIV-
negative patients African PBMC samples.

In order to compare certain biological parameters, the
phenotype of blood B cells from European volunteer blood
donors was also tested in this survey. Informed consent
was obtained from the European patients according to the
requirements of the National French Ethics. There
were 30 HIV+ European blood donors followed by the
Department of Infectious Diseases at the University
Hospital in Saint Etienne, France. Approximately 10 ml of
heparinized blood was obtained specifically for the
present study. All patients enrolled in this study were
treated with antiretroviral drugs according to current
protocols and were monitored for clinical and biological
parameters for disease progression. Plasma VL was
routinely monitored in patients over time. At the time of
sampling for the present work, 28/30 patients had VL of
less than 50 copies/ml. Matched controls consisted of 9
HIV-negative patients European PBMC samples.

T and B Cell Phenotyping

Standard T (CD4+) cell phenotyping was performed
extemporarily at the Institut Pasteur in Bangui. Lympho-
cyte subsets were analyzed on a FACSCalibur flow


cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) with two monoclonal antibodies
(MAbs) (aCD4–aCD8, Becton Dickinson Immunocyto-
meter Systems).

The extended B cell phenotyping was performed in
Saint-Etienne. PBMCs, stored in Stabilcyte™ (BioErgo-
nomics, St Paul, MN) in Africa, were stained with the
following mAbs conjugated with fluorescein isothio-
cyanate (FITC), phycoerythrin (PE) or RPE-Cy5
suitable for three-color flow cytometry. The following
markers were used: CD20-RPECy5, IgD-FITC, IgM-PE,
CD38-FITC, CD38-PE, CD27-PE (all from DAKO,
Copenhagen, Denmark); CD77-FITC (BD-Pharmingen,
San Diego, CA); CD138-PE (Coulter-Immunotech,
Marseille, France). Ig isotype-matched FITC-, PE- and
RPECy5-conjugated mouse Ab (DAKO) were used as
negative controls to test for non-specific staining. Briefly,
0.5 × 10^6 cells were stained with the appropriate mAb for
45 min on ice. Afterwards, the cells were washed in PBS
with 10% fetal calf serum and fixed in PBS containing 4%
parafomaldehyde. Flow cytometry was performed on
the following day using a FacsVantage-SF BD and
the CellQuest-Pro™ software (Becton-Dickinson,
San-Jose, CA).

Statistics

Means ± SEM of data between the blood donor groups
were compared using the Mann–Whitney test. Statistical
analysis was performed using the Statview™ software
(SAS Institute INC, Cary, NJ).

RESULTS

African Blood Donor Group

The design of the present survey, i.e. the study of blood B
cell of volunteer, HIV-infected African individuals
unaware of their infection, living with AIDS-spouses,
has considerably limited the size of this cohort. The initial
serological test performed on blood samples for each
volunteer defined two groups (HIV+ and HIV−). Newly
recognized HIV+ individuals were informed of their
serological status, counseled by the physician at the
hospital and entered into the specific HIV program at the
clinics. The mean CD4+ T cell count ± SEM was
316.6 ± 65.6 cells/µl for the 15 HIV+ individuals and
913 ± 66 cells/µl for the 11 HIV− individuals; P = 0.0007) (Table I). As very few of the recently
diagnosed patients were hospitalized (except #6 and #8)
for various reasons (including economical), we were
unable to perform CDC staging.

Major Characteristic of Circulating B Cells in HIV-
infected African Blood Donors

The HIV+ African blood donors presented lower B cell
counts than the HIV− Africans. The % mean of CD20+ B
cell count ± SEM was 4.9 ± 0.7 for the 15 HIV+ individuals and 6.7 ± 0.6 for the 9 HIV- individuals; \( P = 0.1 \).

A careful examination of the B cell subpopulation phenotype was then carried out by 3 color-flow cytometry. We first determined the relative proportions of naive and non-naive circulating blood B cells on the basis of their reactivity with anti-CD20 and anti-IgM ± anti-IgD fluorescent-dye labeled Abs. As shown in Fig. 1, CD20+, surface-(s)IgM+/sIgD+ cells assumed to be naive B cells (Banchereau and Rousset, 1992) were nearly equivalent in HIV+ than in HIV- blood donors (28 ± 4.6 vs. 20.4 ± 4.3%, respectively). The HIV+ blood donors also displayed approximately equal CD20+, sIgM+, sIgD- differentiated B cells (61.1 ± 4.6 vs. 67.1 ± 7.9%, respectively). In contrast, CD20+ sIgD+ sIgM- germinatal center (GC)-type B cells were significantly higher in HIV+ compared to HIV- patients (11.9 ± 2 vs. 5.7 ± 0.6%; \( P = 0.03 \)). In total, 8/15 (53%) African HIV+ donors displayed ≥10% of GC-type CD20+ B cells.

To further document these findings, and to ascribe potential functions to the non-naive blood B cells in HIV+ donors, we tested the CD20+ B cells for reactivity with mAbs to CD27 (characterizing circulating memory-type blood B cells), CD77 (characterizing GC-type B cells), CD138 (characterizing post-GC B cells) and anti-CD38 (characterizing pre-plasmocytes and plasmocytes, and certain activated B cells). African HIV+ blood donors displayed similar levels of CD20+ CD27+ memory-type blood B cells compared with their non-infected counterparts (Fig. 2A). However, there were significantly more CD20+ CD77+ GC-type B cells (9.7 ± 1.8 vs. 3.2 ± 0.7%; \( P = 0.006 \)) compared to HIV- Africans. Of note, although this cannot be compared\textit{ stricto sensu}, unexpected proportions of CD20+ CD138+ post-GC B cells were found in HIV+ and HIV- Africans compared to as in Europeans (Fig. 2A,B). Moreover, HIV+ and HIV- African donors had fewer CD20+, sIgD+, sIgM+ naive B cells (28.1 ± 4.6 and 20.4 ± 4.3%, respectively) compared to HIV+ and HIV- European individuals (72.2 ± 3 and 73.2 ± 2.2%, respectively). African donors also had more CD20+, sIgM+, sIgD- differentiated B cells (61.1 ± 4.6 vs. 23.5 ± 1.9%) and considerably more CD20+ sIgD+ sIgM- CG-type B cells compared to European patients (HIV+: 11.9 ± 2 vs. 3.2 ± 0.7; HIV-: 5.2 ± 0.6 vs. 3.2 ± 0.7, respectively). Moreover, HIV-infected or uninfected, all African individuals

![FIGURE 1](image1.png)
displayed at least five fold fewer memory-type CD27+ B cells (4.9–5 vs. 24.1–38.4%) than Europeans while HIV+ infected Europeans had significantly less CD20+ CD27+ memory-type B blood B cells than their non-infected counterparts (P = 0.01; Fig. 2B).

**DISCUSSION**

In this study, we attempted to integrate observations on circulating B lymphocytes in HIV-infected drug-naive individuals. We tried to understand to what extent, and how, the B cell compartment, is affected by HIV-infection, especially in drug-naive patients which represent the majority of infected individuals worldwide. The next step would be to try to circumvent the B cell disorders and in so doing protect the B cell compartment to allow the development of protective-type Abs, awaiting an anti-disease vaccine.

The study population consisted of African individuals unique in four areas: (i) origin and genetic background; (ii) hyperactivation of the immune system by numerous infectious pathogens leading to the so-called tropical AIDS (Germani et al., 1998; Bentwich et al., 2000; Begaud et al., 2003); (iii) no access to antiretroviral treatment and insufficient access to health care and (iv) virus clade.

This and other studies have shown that HIV infection leads to profound alterations at the level of B cell homeostasis (Richard et al., 2002). Although exacerbated plasmacytosis in bone marrow and in peripheral tissues have been consistently reported in HIV infection (Calenda and Chermain, 1992; Nagase et al., 2001), no consensus has been reached on whether plasmocytes can circulate. We did not find circulating plasmocytes in our close examination of blood smears (data not shown). However, we found a fraction of circulating B lymphocytes phenotypically modified when compared to HIV-uninfected individuals, such as the reduction in CD27+ memory-type B cells (De Milito et al., 2001). Certain studies aimed at characterizing the Ab secreting cell precursors found various phenotypic changes such as the low expression of CD21 (Moir et al., 2001), CD23 (Rodriguez et al., 1996), CD38, CD39, CD20, CD37, CD71 (Fournier et al., 2002) and modified expression of CD22, slgM and slgG molecules (Dawood et al., 1998) etc, suggesting that these cells could be early plasma cells originating from GCs (Forster et al., 1997). Our study showed that African individuals had fewer naive (CD20+/slgM+/slgD+) and memory-type (CD20+/ CD27+ ± slgD− slgM2) B cells compared to European individuals. This could be linked to the tropical environment where, for example, chronic parasite infections are known to affect the immune system. Multiparasitisme is indeed frequently observed in CAR (Begaud et al., 2003). On the other hand, we found significantly more CD20+/CD77+ B cells (CD77 being a phenotypic characteristic of centroblasts (Wiels, 2000)) in HIV+ Africans compared to HIV-uninfected individuals or European patients. This second particularity seems to be directly associated with the evolution of HIV infection in African individuals and with the presence of the virus (high VL) in the absence of treatment. We also found elevated numbers of B cells expressing surface CD138 (a feature of post-GC B cells) (Wijdenes et al., 1996). However, CD138 marker is imprecisely known, rendering this staining difficult to interpret. Taken together, our findings strongly suggest that the unexpected circulating B cells share the features of GC- (Bm3σ and Bm3) and post-GC-B cells (Liu et al., 1996). In vivo, such lymphocytes may have received progression and differentiation signals and/or may be pre-switched. They may differentiate terminally in bone marrow and in tissue-associated lymphoid organs, and some may possibly be aberrantly located as a result of HIV-infection (Forster et al., 1997). It is not clear, however, that these GC cell subsets differentiate in vivo and in vitro (our unpublished observations) as we have not yet specifically identified a population ascribed to a centrocytic phenotype.
Our data agrees with several studies on the architectural disturbance of the LN in HIV-infection, AIDS and HIV-associated Hodgkin and non-Hodgkin lymphomas (Clarke and Glaser, 2001). Further, it has been reported that disruption of GCs blocks the formation of B cell memory (Roy et al., 2002) which fits with the present observation. Our study may also suggest that the hypergammaglobulinemia observed during HIV-infection at least partly reflects the disorganization of LNs and particularly GCs, which may prematurely release into the periphery GC- and post-GC-B lymphocytes poised to differentiate terminally. Our study differs from other studies in that we have enrolled African patients who were heavily infected with HIV. Our observations focused on the direct relationship between peripheral B cell subset imbalances and LN disorganization, and the absence of treatment (and the absence of viral control). The architectural disorganization of the LN—along with the aberrant release of early plamocytes by the bone marrow—seems largely responsible for the B cell dysregulation observed during HIV-infection progression in African countries. This strongly argues for the establishment of therapeutic vaccines trials as we await the implementation of drug treatment programs.

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