Specific Antibody Production by Blood B Cells is Retained in Late Stage Drug-naı¨ve HIV-infected Africans

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Unseparated peripheral blood mononuclear cells (PBMCs) obtained from drug-naı¨ve African individuals living in a context of multi-infections and presenting with high viral load (VL), were cultured in vitro and tested for their ability to produce antibodies (Abs) reacting with HIV-1 antigens. Within these PBMCs, circulating B cells were differentiated in vitro and produced IgG Abs against not only ENV, but also GAG and POL proteins. Under similar experimental conditions, HAART treated patients produced Abs to ENV proteins only. The in vitro antibody production by drug-naı¨ve individuals’ PBMCs depended on exogenous cytokines (IL-2 and IL-10) but neither on the re-stimulation of reactive cells in cultures by purified HIV-1-gp160 antigen nor on the re-engagement of CD40 surface molecules. Further, it was not abrogated by the addition of various monoclonal Abs (mAbs) to co-stimulatory molecules. This suggests that the in vitro antibody production by drug-naı¨ve individuals’ PBMCs resulted from the maturation of already envelope and core antigen-primed, differentiated B cells, presumably pre-plasma cells, which are not known to circulate at homeostasy. As in vitro produced Abs retained the capacity of binding antigen and forming complexes, this study provides pre-clinical support for functional humoral responses despite major HIV- and other tropical pathogen-induced B cell perturbations.

Keywords: Anti-HIV Antibody; B cell; IVAP; PBMC

Abbreviations: CG B cells, centro germinative B cells; IVAP, in vitro antibody production; WB, Western blot; VL, viral load

INTRODUCTION

HIV-infection in Africa presents some unique features including diverse viral clades and clinical manifestations of disease. The limited access to HAART and also the presence of chronic tropical diseases induce profound lymph node and geriminal center disorganization. An important issue in order to take care of these patients is to determine if they are still capable of producing functional HIV-specific antibodies (Abs), and—at large—functional Abs to other pathogens.

HIV infection induces 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 accompanied by hypergammaglobulinemia yet lack specific neutralizing anti-HIV envelope glycoprotein Abs. It is commonly held that a profound B cell dysfunction is associated with HIV (Richard et al., 2002). Meanwhile, there is an increasing body of evidence that certain neutralizing Abs (NAbs) are instrumental in protection against HIV infection, as demonstrated both at the mucosal and the systemic level (Devito et al., 2000; Moja et al., 2000). NAbs may indeed achieve protection in the systemic compartment, at least in certain experimental hosts (Mascola et al., 1999; Baba et al., 2000).

We have previously shown that HIV-infected, drug-naı¨ve AIDS patients living in Africa, and exposed to multiple concurrent infections (Garin et al., 1997; Germani et al., 1998; Begaud et al., 2003), exhibited diminished levels of naïve and memory-blood B lymphocytes but augmented levels of centro-germinative (CG), post-germinative blood B cells, and pre-plasma cells which are aberrant cells in the periphery (Beniguel et al., 2004).

The present investigation aimed at examining whether peripheral blood mononuclear cells (PBMCs) obtained
from drug-naïve HIV-infected Africans presenting with AIDS and comprising of various B cells subsets—of which usually non-circulating B cells—could still produce HIV-1-specific Abs. The study also aimed at examining some characteristics of this in vitro Ab production (IVAP) to set up a rationale for immune-interventions when available.

MATERIALS AND METHODS

Patients

African donors were HIV-positive patients from the “Hôpital Communautaire” of Bangui, Central African Republic (CAR). The prevalence of HIV-infection in CAR is estimated to be 12.9% of the adult population (http://www.unaids.org; 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.

For comparison of biological parameters, European volunteer blood donors were also tested in this survey. They consisted of 10 HIV-positive patients (Table I) followed by the Department of Infectious Diseases at the University Hospital in St-Etienne. Informed consent was obtained from every patient according to the requirements of the National French Ethics Committee. They were all treated with antiretroviral drugs according to current protocols. Each European patient was also monitored for clinical and biological parameters predictive of disease progression. Matched controls consisted of HIV-negative African and European blood donors. Approximately 10 ml of heparinized blood was obtained for the present study.

Serology and Viral Load (VL) Determination

HIV serology was routinely followed in Bangui using the Vironoctxia HIV Uniform II plus O test (Organon Teknika, Durham, NC). HIV serology in St-Etienne was followed by the Department of Infectious Diseases at the University Hospital in St-Etienne. Informed consent was obtained from every patient according to the requirements of the National French Ethics Committee. They were all treated with antiretroviral drugs according to current protocols. Each European patient was also monitored for clinical and biological parameters predictive of disease progression. Matched controls consisted of HIV-negative African and European blood donors. Approximately 10 ml of heparinized blood was obtained for the present study.

PBMC Preparation

Plasma was removed by centrifugation at 1,500 rpm/min and stored at −20°C. PBMCs were isolated by density gradient centrifugation (Lymphocyte Separation Medium, Eurobio, Les Ulis, France). As the B cell phenotyping was not performed in Bangui, 1 × 10^6 PBMC were stored at 4°C in Stabilcyte™ (BioErgonomics, St Paul, MN), for approximately 2 months prior to be shipped in France and labeled for flow cytometry analysis.

PBMC Cultures

PBMCs were cultured in Iscove’s Modified Dulbecco’s Medium (IMDM) culture medium (Biowhittaker, Verviers, Belgique) supplemented with 10% endotoxin-free heat-inactivated Fetal Calf Serum (FCS) (US14-901F; BioWhittaker), 1% MEM amino acids solution (Sigma-Aldrich, Dorset, UK), 1% MEM non-essential amino acids solution (Sigma), 1% Insulin–Transferin–Selenite medium (Sigma) and 1% antibiotics (Antibiotic–Antimyotytic solution; Sigma) as described (Garraud et al., 1995). This preparation will therefore be referred to as “complete medium”. In order to allow cultured PBMCs to shed membrane-bound Igs or immune complexes that could interfere with specific assays, PBMC were rested by an overnight incubation at 37°C as already described (Garraud et al., 2001). On the following day, PBMCs were readjusted at 10^6 cells/ml in fresh cultured medium.

| TABLE I Major characteristics of the HIV⁺ patients enrolled in the study. (A) African patients, (B) European patients |
|---------------------------|---------------------------|---------------------------|
| **Age** | **CD4** | **VL** |
| (A) African patients |
| 1 | F | 30 | nd | 5.15 |
| 2 | F | 28 | 246 | 5.88 |
| 3 | F | 18 | 1117 | nd |
| 4 | M | 45 | 133 | 5.51 |
| 5 | F | 43 | nd | nd |
| 6 | M | 33 | 65 | nd |
| 7 | F | 27 | 421 | nd |
| 8 | M | 33 | 23 | 5.88 |
| 9 | F | 30 | 445 | 5.4 |
| 10 | F | 22 | nd | 5.61 |
| 11 | M | 56 | nd | nd |
| 12 | F | 20 | 854 | nd |
| 13 | M | 32 | 378 | nd |
| 14 | F | 24 | 702 | nd |
| 15 | M | 32 | 9 | 4.2 |
| 16 | F | 38 | 268 | 5.2 |
| 17 | F | 16 | 992 | nd |
| 18 | M | 39 | 456 | 5.4 |
| 19 | F | 28 | 128 | nd |
| 20 | M | 33 | 68 | nd |
| 21 | F | 28 | 854 | nd |
| 22 | F | 25 | 956 | 4.5 |
| 23 | M | 43 | 1332 | 5.6 |
| 24 | F | 38 | 611 | 5.15 |
| (B) European patients |
| 1 | M | 64 | 499 | >1.7 |
| 2 | M | 44 | 303 | 3.81 |
| 3 | F | 49 | 759 | >1.7 |
| 4 | M | 39 | 383 | >1.7 |
| 5 | M | 31 | 650 | 4.52 |
| 6 | M | 46 | 439 | >1.7 |
| 7 | M | 41 | 415 | 3.63 |
| 8 | F | 66 | 442 | >1.7 |
| 9 | M | 45 | 322 | >1.7 |
| 10 | M | 32 | 465 | 3.85 |

Mean CD4⁺ = 502.9 ± 403.2 cells/μl; Mean VL = 5.24; Mean CD4⁺ = 468 ± 134 cells/μl; Mean VL = 2.6.

Note: M and F stands for Male and Female; Age is expressed in years; CD4 count is expressed in CD4⁺ cells per μl ± SD; plasma viral load is expressed in log (copies/ml).

nd: not determined.

Mean: ± SD; plasma viral load is expressed in log (copies/ml).
and 500 µl was added to each well of a 48-well culture plate (Costar, NY).

PBMCs were then cultured in the presence or absence of additional stimuli to favor the terminal differentiation of B cells into Ig secreting cells. The following stimuli were used alone or in combinations at predetermined concentrations: (i) cytokines: IL-4 (Peprotech, Rocky Hill, NJ); IL-2 (Peprotech); IL-10 (R&D Systems, Minneapolis, MN) and TGF-β, all used at 20 ng/ml. (ii) Ag: HIV MN/LAI Ag gp160 (Aventis-Pasteur, Marcy-L’Étoile, France), used at 0.1 µg/ml; (iii) control cultures were performed in the presence of cycloheximide (CHX) (0.1 mg/ml; Sigma) to prevent protein synthesis. Some PBMC were also stimulated with 10 ng/ml of soluble CD40L molecule. Individual cultures were carried out for 3–12 days at 37°C in 5% CO₂ humidified atmosphere. Cell-free culture supernatants were stored at −20°C until used in Ab detection assays.

To test for the binding capacity of in vitro-produced anti-gp160 IgG Abs, individual 200 µl supernatant samples were incubated with purified recombinant gp160 (used at 100 ng as determined in a series of preliminary experiments), for 3 h at 37°C, prior to Western blotting (WB).

In an attempt to inhibit Ag presentation to B cells and/or direct cell adhesion, PBMCs were cultured in 96-well plates in the presence or absence of anti-CD54, anti-CD18, anti-CD11c and anti-HLA-DR mAbs, or isotype controls (Coulter-Immunotech, Marseille, France). These monoclonal Abs (mAbs) were used at a predetermined concentration of 1 µg/ml at the onset of the cultures.

Apoptosis Assays

In order to estimate ongoing apoptosis of cultured cells, culture supernatants (100 µl) were tested a posteriori for the presence of NMP 41/7 (Nuclear Matrix Protein, a protein released by cell death) in culture supernatants, using a commercial NMP ELISA (Calbiochem, San Diego, CA). It has been described that the presence of NMP41/7 marker, in cell culture supernatants, was correlated to cell DNA fragmentation and FAS-Ligand production (Baize et al., 1999).

Ab Production Analysis

For the broad detection of anti-HIV IgG Abs, plasma (dilution 1/90) or culture supernatants (dilution 1/9) were tested by WB (HIV BLOT 2.2, Abbott, Rungis, France) according to the manufacturer’s protocol. Briefly, samples were incubated overnight on specific nitrocellulose blots in the appropriate buffer. Positive and negative controls were high anti-HIV Ab titers serum and HIV-negative serum, respectively. After several washes, blots were incubated 30 min with a phosphatase alkaline conjugated anti-IgG and 15 min with the appropriate substrate.

Additionally, total IgG in culture supernatants were analyzed using ELISAs performed in 96-well MaxiSorp™ plates (NUNC, Roskilde, Denmark) as previously described (Garraud et al., 1995). IgG concentration was extrapolated from a reference curve generated by assaying dilutions of a pool of plasma specimens from blood donors whose IgG concentrations were determined by an immunonephelometric technique (Minineph™, The Binding Site, Oxford, UK).

PBMC Phenotyping

Standard T (CD4⁺) cell phenotyping was performed at the Institut Pasteur in Bangui using 100 µl of fresh whole blood labeled with a mAb (anti-CD4, Becton Dickinson Immunocytometry Systems, San Jose, CA) as described (Menard et al., 2003). Lymphocyte subsets were routinely analyzed on a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems).

As B cell phenotyping was not routinely performed in Bangui, PBMC were stored in Stabilecyte™ until the staining. After extensive washings in PBS with 10% FCS, PBMC (0.5 × 10⁶) were labeled with RPECy5 labeled anti-CD20 mAb (DAKO, Copenhagen, Denmark). Briefly, 0.5 × 10⁶ cells were stained with the mAb for 45 min on ice. Afterwards, the cells were washed in PBS/10% FCS and fixed in PBS containing 4% paraformaldehyde. Flow cytometry was performed on the following day using a FacsVantage-SF BD and the CellQuest-Pro™ software (Becton-Dickinson).

Statistical Analysis

Means ± standard deviations (SD) of total IgG in individual cultures were compared by means of the Wilcoxon test (Statview™, Cary, NJ).

RESULTS

Characteristics of the Blood Donors

The mean CD4⁺ T cell count ± SEM was 502.9 ± 403.2 cells/µl for HIV⁺ African donors and 913 ± 66 cells/µl for HIV⁻ African donors. The % mean of CD20⁺ B cell count ± SEM was 4.9 ± 0.7 for the HIV⁺ African donors and 6.7 ± 0.6 for HIV⁻ African donors. As expected, the plasma VL was high for drug-naïve African patients. It ranged from 10,000 to more than 800,000 copies/ml. For HAART-European patients, plasma VL was controlled by antiretrovirals and ranged from less than 50 to 50,000 copies/ml, with 6 patients having less than 50 copies/ml (Table I).

Ig Production In Vitro

PBMCs from HIV⁺ and HIV⁻ African donors produced equally large amounts of Ig in vitro (mean IgG production were 0.115 mg/l) as tested by ELISA. This IVAP was not statistically augmented by exogenous cytokines IL-2 and IL-10 and/or by recombinant HIV-gp160. As previously
shown, the Ig production in vitro in HIV+ individuals was detected as early as day 3 whereas maximum production was observed after a 12 day culture (Cognasse et al., 2003). This production was abrogated by CHX, indicating it was de novo protein synthesis.

**Specific Ab Production In Vitro by Peripheral Blood B Cells from HIV+ Individuals**

PBMCs from 14 drug-naive African HIV+ donors (Table I; (1–14) were cultured in vitro for 12 days in the absence of or presence of exogenous stimuli, IL-2 + IL-10 or HIV-1-gp160 Ag. In the vast majority (12/14) of individual cultures, there was a spontaneous production of anti-HIV IgG Abs, which was fairly augmented by addition of IL-2 and IL-10 at the onset of the cultures. For the two other individual cultures, a spontaneous Abs production could also be detected by WB but was not augmented by IL-2 and IL-10. In the 14 cultures, no specific anti-gp160 Ab production was detected when HIV-gp160 was added at the onset of the cultures. These data are summarized in Table II A. Thus the overall profile of IVAP in drug-naive HIV-infected Africans’ PBMC was a spontaneous Ab production which was augmented at least in terms of antigenic specificities of Ab by IL-2 and IL-10 (Fig. 1A). Of important note, soluble CD40L did not prove to augment IVAP under these conditions.

Characteristically, the specific in vitro induced Abs could recognize blotted Ags corresponding to gp160, gp41 (ENV-protein), p24, p17 (GAG-) and p66, p51 and p31 (POL-proteins). This broad distribution profile of Ab specificities was observed in all 14 productive cultures.

In contrast, when PBMCs from HAART-treated patients (Table IB) were cultured, three distinct profiles of IVAP could be observed. In 2/10 individual cultures there was no production of Ab. In the other 8/10 cultures, there was a detectable spontaneous Ab production against ENV Ag. This production could be augmented by IL-2 and IL-10 in 3/8 cases but could not in the other 5/8 cases, 4 of these 5 corresponding to individual cultures of PBMCs from donors with less than 50 mRNA copies/ml of plasma. These data are summarized in Table II B. The three individual cultures where IVAP was augmented by IL-2 and IL-10 were performed using PBMCs from donors with more than 2,500 mRNA copies/ml of plasma. Of important note, 8/8 productive individual cultures contained Abs which could recognize blotted Ags corresponding to gp160 only, and only 1/8 combined a mixture of anti-p31 (POL-protein) and anti-gp160 (ENV-protein) and only 1/8 combined a mixture of anti-p24 (GAG-protein) and anti-gp160 Abs (Fig. 1).

The kinetics of Ab production was next assayed in a novel series of cultures. This showed that Ab production was detectable in culture supernatants as early as day 3, in contrast to what is generally observed in PBMC cultures from HIV+, but treated, donors.

**Exogenous HIV-gp160 Extinguishes Spontaneous Ab Production in HIV+ Patients**

In the majority of the culture systems described so far, addition of a relevant stimulating Ag was required for PBMCs to produce Ag-specific Abs in vitro (Garraud et al., 1996). A stated above, “spontaneous” production of Ag-specific Abs was observed in the absence of stimulating Ag (e.g. recombinant gp160). Rather, in the presence of Ag, the Ab production was abolished, an observation which was made in every individual culture (Fig. 1). As Ag overstimulation can induce apoptosis in B cells, we tested a posteriori for the presence of NMP41/7 in every culture supernatant. No evidence of apoptosis or at least cell death was detected in any individual culture.

We next tested for the possibility that specific anti-gp120/160 Abs were released in culture supernatants earlier than usual when naïve B cells undergo the complete pathway of cell differentiation in vitro. An indirect approach consisted of testing for the capacity of in vitro-produced Abs to make immune complexes with Ag. Culture supernatants clearly showing spontaneous Ab production were incubated a posteriori with recombinant gp160 for 3h. This operation led to the complete adsorption of anti-gp120/160 Abs in every culture supernatant tested as can be seen in Fig. 1B.

| Antigen reactivity | IVAP in individual PBMC cultures in drug-naive Africans | IVAP in individual PBMC cultures in HAART-Europeans |
|-------------------|------------------------------------------------------|---------------------------------------------------|
|                   | CHX ns IL-2 IL-10 HIV-1 gp160                         | CHX ns IL-2 IL-10 HIV-1 gp160                      |
| gp160             | 0/14 14/14 14/14 0/14                                  | 0/10 8/10 8/10 0/10                               |
| p66               | 0/14 1/14 7/14 1/14                                   | 0/10 0/10 0/10 0/10                               |
| p51               | 0/14 1/14 5/14 1/14                                   | 0/10 0/10 0/10 0/10                               |
| gp41              | 0/14 0/14 3/14 0/14                                   | 0/10 0/10 1/10 0/10                               |
| p31               | 0/14 0/14 6/14 0/14                                   | 0/10 0/10 1/10 0/10                               |
| p24               | 0/14 2/14 3/14 2/14                                   | 0/10 0/10 0/10 0/10                               |
| p17               | 0/14 0/14 1/14 0/14                                   | 0/10 0/10 0/10 0/10                               |

Note: The Ab production in three culture conditions was estimated according to WB in the two groups of patients. The number of patients producing the specific Ab are indicated. ns stands for non-stimulated PBMC.
In contrast, anti-GAG and anti-POL proteins remained unchanged.

**Differential Intercellular Cooperation Affecting Spontaneous Specific Ab Production In Vitro**

The above reported data showed that: (i) HIV\(^+\) African individuals’ PBMCs produced anti-HIV Ab, against ENV proteins but also against GAG and POL proteins; (ii) such Abs, characterized as IgG, were functional since they bound exogenous gp160; (iii) in the majority of individual cultures, this Ab production was augmented by exogenous IL-2 and IL-10. We next examined the role of intercellular molecular events between lymphocytes and other cell types in PBMCs from HIV\(^+\) patients. PBMCs from 10 Africans (Table I, 14–24) were incubated with a cocktail of anti-leukocyte adhesion molecules (i.e. anti-CD54, anti-CD18, anti-CD11c, and anti-HLA-DR), in the presence or absence of IL-2 and IL-10, and in the presence or absence of gp160. The mAb cocktail was added at the onset of the 12-day PBMC culture. Spontaneous anti-ENV, as well as anti-POL and anti-GAG, proteins remained unaffected in every individual culture. Although it cannot be excluded that mAbs were insufficient in amounts, or that repetitive addition of this cocktail over time would have been necessary, or that other intercellular Ag specificities would have been of greater effect, those experiments suggest that spontaneous Ab production *in vitro* by HIV\(^+\) individuals’ PBMCs is relatively independent of Ag presentation (at least by non B-cells), but is dependent on certain accessory cell-derived soluble factors such as cytokines. In a limited number of experiments, similar results were obtained when monocytes were depleted from PBMCs by adhesion to plastic prior to the culture steps (data not shown).

**DISCUSSION**

Early in the AIDS epidemic, one commonly reported biological disturbance was hypergammaglobulinemia. Spontaneous production of Ig of diverse, but often irrelevant, specificities, has been recorded when PBMCs of HIV-infected individuals were examined in short- or longer-term cultures (Amadori *et al.*, 1988; Vendrell *et al.*, 1991). The African environment of concurrent infections may impact humoral responses at large, as shown by total Ig production in HIV\(^+\) and HIV\(^-\) donors. This is related to observations of immune activation in a tropical environment (Lukwiya *et al.*, 2001).

Observations over time have also been challenged with the considerable progress achieved in managing the patients, such as HAART and consistent reduction of VL (Opravil *et al.*, 2002). The machinery involved in such Ab production dysfunction, however, most probably resides in the LNs.

Our studied population comprised of African blood donors unaware of their HIV\(^+\) status although they displayed characteristics of AIDS infection as they were recruited on the basis of the WHO classification for adult AIDS in Africa (Greenberg *et al.*, 1997).

We previously shown—in the same African population—that HIV\(^+\) patients displayed an abnormal circulating blood B cell phenotype. Within the circulating
B cell pool there was an aberrant contingent of (CD20<sup>+</sup>/CD77<sup>−</sup>, sIgM<sup>−</sup> sIgG<sup>−</sup> sIgA<sup>−</sup> sIgD<sup>−</sup>) CG and (CD20<sup>+</sup>/CD38<sup>−</sup> CD138<sup>−</sup>) post-CG B cells usually restricted to lymph nodes or equivalent secondary lymphoid organs (Beniguel et al., 2004). In general, at homeostasis, CG B cells are prone to apoptosis unless they (i) encounter specific Ag; (ii) receive a CD40/CD40L signal; (iii) receive activated T cell-mediated signals; and/or (iv) are in contact with follicular dendritic cells which provide Ag stimulation and other cognate and secretory signals (Defrance et al., 2002).

In the present study, we questioned whether untreated HIV<sup>+</sup> African patients’ peripheral B cells were able to differentiate in vitro and produce anti-HIV Abs, as those B cells comprised of numerous CG and post-CG cells which are usually prone to apoptosis, along with naïve and memory B cells (which can be rather easily differentiated in vitro). As there was no evidence of cell death in the cultures, it cannot be excluded that most of those cells, which were prone to apoptosis, died earlier on at the time of the 24h resting period preceding the culture. Cultured cells could produce fair levels of anti-HIV Abs in the presence of survival signals such as IL-2 and IL-10 but did not necessitate the re-engagement of CD40 and the re-exposure to virus-derived Ags. This strongly suggest that at least one major sub-population of B cells prone to differentiate terminally were in fact already differentiated and matured in such a manner that they produced not only anti-ENV but also anti-GAG and POL-Abs. This hypothesis was supported by the blocking studies which did not express CD40, and would thus be insensitive to exogeneously supplied soluble CD40L signals.

Interestingly, our results indicate that B cell priming of populations able to differentiate in vitro has occurred in vivo. Indeed, these B cells have been likely primed by surface Ag—as expected—but also by core Ag—which means that at least some subsets of B cells in cultures have met their epitope processed by an APC in vivo. Ags other than ENV, i.e. GAG and POL are internal Ags and must be processed or exposed (cell/virus lysis) for subsequent presentation to the relevant Ag-reactive B cell. This hypothesis was supported by the blocking studies which aimed at interrupting intercellular cooperation. Our results do not exclude the possibility that specific Ab production can be affected by exogenous- or accessory-cell derived-stimuli such as cytokines [24] along with IL-2 and IL-10 are two cytokines which affect almost all the activation and differentiation steps of B lymphocytes especially their maturation (Banchereau and Rousset, 1992), and rescue germinal center-B cells from apoptosis (Levy and Brouet, 1994). The role of accessory- or B cells derived-IL-6 and -TNF-α was found to be critically important; we did not test for the addition of NAbs to IL-6 or TNF-α in individual cultures since it is well established by others and ourselves that this affects all Ab production in vitro in most individual culture conditions (Garraud et al., 1996; Fournier et al., 2002).

Of note, it is assumed that most Ag-reactive B cells recognize the native form of the envelope gp120/160 Ag, exposed by a cell-free or a cell-bound virus. We show here that patients’ blood B lymphocytes in uncontrolled HIV infection (no-anti viral therapy) were capable of producing in vitro Abs against various HIV Ags unlike the majority of patients with controlled viremia. Our data is consistent with other published studies (Zamarchi et al., 2002) since there is not stricto sensu a defined correlation between the initial VL and the capacity to produce Abs. We propose instead that this parameter parallels the length of exposure to the virus and/or the absence of therapeutic control.

We report here that HIV-infected individuals—even AIDS patients with disorganized lymph node machinery (Legendre et al., 1998)—can produce Abs reactive to HIV envelope Ags which—at least in an in vitro model—bind their cognate Ags, and possibly also against Ags from concurrent infections (ongoing experiments). Patients seem thus retain the capacity of developing certain Ab responses. This property could be appropriately targeted by immuno-interventions against HIV but also apportunistic and/or concurrent infections.

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