Immune activation in HIV-infected African individuals

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Objective: Immune activation induced by chronic infections, dietary limitations, and poor hygienic conditions is suggested to be present in African HIV infection and is at the basis of the hypothesis that HIV infection in Africa could be prevalently associated with immunopathogenetic mechanisms. Very limited data are nevertheless available supporting this theory, and in particular no data are reported on functional and phenotypic analyses performed on fresh peripheral blood mononuclear cells (PBMC) of African HIV-infected patients living in Africa.

Design: Immunological and virological parameters were analysed in fresh PBMC of HIV-infected African and Italian patients with advanced HIV disease and comparable CD4 and CD8 counts, sex, and age. Both functional (antigen- and mitogen-stimulated cytokine production) and phenotypic (activation markers; markers preferentially expressed by T helper (Th) type 2 cells or by memory and naive cells) analyses were performed. Results were compared with those of HIV-seronegative African and Italian controls. HIV plasma viraemia was analysed by competitive polymerase chain reaction (PCR) and branched DNA techniques.

Results: (1) The production of mitogen-stimulated IFN-γ and TNF-α as well as the production of env peptide-stimulated IFN-γ, TNF-α, and IL-10 are increased in African HIV infection; (2) the expression of activation and Th2-associated markers is augmented in African HIV infection as is the memory/naive ratio; (3) mitogen-stimulated IFN-γ and IL-10 production, as well as the expression of activation and Th2-associated markers and the memory/naive ratio, are augmented in African compared with Italian controls; and (4) plasma viraemia is reduced in African compared with Italian HIV-infected individuals.

Conclusions: These results, which are the first to be reported on fresh material from African HIV-infected patients living in Africa, indicate that HIV disease is associated with an abnormal immune hyperactivation and may be accompanied in these patients by lower loads of virus, and show that such activation is present even in HIV-seronegative controls.
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Introduction

The WHO estimates indicate that more than 30 million persons are infected with HIV worldwide [1]. The majority of HIV infections (> 90%) have occurred in developing countries, and in particular, more than 20 million cases were reported in sub-Saharan Africa (68% of the total) [1]. HIV transmission is probably facilitated in Africa, the progression of HIV infection to AIDS is accelerated, and the survival time after diagnosis of AIDS is shorter in African patients compared with patients in Europe or in the United States [2–10]. In addition, opportunistic infections, in particular tuberculosis (TB), are observed in African patients even in the presence of adequate CD4 counts [11–14]. Uganda is one of the countries in which the prevalence of HIV is overwhelming [15]. The first case of AIDS was described in Uganda in 1982. Since then the cumulative number of AIDS cases has continued on an upward trend, and by the middle of 1997 the data from that country showed the presence of 51 779 officially reported cases, whereas it is estimated that two million Ugandans (or 8% of the population) are HIV seropositive [15,16]. Recent data have shown that HIV has declined in young pregnant women in Kampala from 25% in the early 1990s to 15% in 1996 [17]. Although this is encouraging news, almost 50% of hospital beds in Kampala are occupied by people with HIV.

Abnormal activation of the immune system resulting from parasitic and non-parasitic infections, poor hygienic conditions, and nutritional deficiencies could contribute to the higher prevalence of HIV infection in Africa and the more rapid progression to AIDS in HIV-infected individuals [18–20]. That immune hyperactivation is present in HIV infection in African patients is suggested by studies of immune parameters of Ethiopian individuals [18,20]. Results showed the presence of immune hyperactivation in this population, but are biased by the fact that these studies were mostly performed after Ethiopians moved to Israel [18,20]. To investigate the contribution of viral and immunological factors to the progression of HIV infection in Africa we assessed a series of parameters on freshly drawn peripheral blood mononuclear cells (PBMC) from HIV-infected and uninfected individuals from a rural Ugandan population, thus evaluating immune parameters in African individuals living in Africa.

The results confirmed the presence of immune activation in Ugandan HIV-infected and uninfected individuals compared with Italian patients and controls. Because an augmented production of inflammatory and cachexia-inducing cytokines and lower HIV plasma viraemia were observed in Ugandan HIV-infected individuals, these data confirm that AIDS in Africa is associated with an abnormal and potentially deleterious immune activation.

Materials and methods

The Ugandan–Italian AIDS project for HIV/AIDS

In 1994 a research project on HIV/AIDS was initiated by Ugandan and Italian institutions. This project supports virological, immunological, and epidemiological research in HIV infection in Uganda. The research project is linked to a public health programme addressing the need for diagnosis, treatment, and health education. The two components are integrated in the programme 'Global Support to the National Plan for HIV/AIDS Control in Uganda', which is run on the Italian side by a coordinated effort of the Italian Istituto Superiore di Sanità, the Italian Ministry of Foreign Affairs, the First Infectious Disease Unit, L. Sacco Hospital, Milan, and the Chair of Immunology of the University of Milan, and on the Ugandan side by the Lacor St Mary's Hospital, Gulu, the Ugandan Ministry of Health, and the Ugandan Virus Research Institute.

Blood drawing, shipping and patient selection

Whole blood was collected by venopuncture in vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) (Becton Dickinson and Co., Rutherford, NJ, USA). An aliquot of blood was processed to obtain plasma samples by two-step centrifugation at 400 and 800***g*** for 20′ at 4°C, The samples were aliquoted, frozen and kept in liquid nitrogen. A second aliquot of blood was hand-carried from Gulu, Uganda to Milan, Italy. Upon arrival, PBMC were separated on lymphocyte separation medium (Organon Teknika Corp., Durham, NC, USA), washed twice in phosphate-buffered saline (PBS), and the number of viable leukocytes was determined by trypan blue exclusion. The entire procedure was carried out within 30 h of drawing; all specimens were coded and laboratory personnel were blinded throughout the experimental procedures. Frozen plasma aliquots were shipped to Italy in temperature-controlled (liquid NO_2) thermal containers. Blood specimens were obtained from Ugandan HIV-infected patients followed at St Mary's Lacor Hospital, Gulu, Uganda, and from Italian HIV-infected patients followed by the First Infectious Disease Unit, L. Sacco Hospital in Milan, Italy (to minimize differences between the two cohorts, blood from these patients was processed after a minimum of 24 h from drawing). Forty-three Ugandan and 33 Italian HIV-infected patients with advanced HIV disease were analysed. Eighteen healthy individuals from Uganda and 18 age- and sex-matched healthy Italian individuals were also included in the study. Healthy controls were hospital workers selected and screened for the presence of viral or parasitic infections.

In vitro cytokine production

The production of IFN-γ, IL-2, IL-10, and TNF-α by PBMC was determined by culturing 3×10^6/well PBMC in 24-well LINBRO plates (Flow Laboratories,

Inc., McLean, VA, USA) at 37°C in a moist, 7% CO_2 atmosphere. PBMC were either unstimulated or were stimulated with: (1) phytohemaglutinin (PHA; M form, Grand Island, NY, USA) diluted 1:100 for 48 h; (2) lipopolysaccharide (LPS) (Sigma, St Louis, MO, USA) (1 μg/ml) for 48 h; (3) a pool of synthetic HIV-1 peptides (Env) (2.5 μg), which have been previously described [21], for 5 days. These conditions were chosen because of our previous experience in measuring cytokine production. The cultures were supplemented with 5% pooled AB^+ human serum (Sigma). Supernatants were harvested, frozen and stored at –20°C until assayed for cytokine production. Cytokine production was evaluated with commercially available enzyme-linked immunosorbent assays (ELISA; Predicta; Genzyme, Cambridge, MA, USA). Values for all the cytokines were calculated from a standard curve of the corresponding recombinant human cytokine.

Immunophenotypic analyses

Lymphocyte subsets were evaluated using an Epics XL flow-cytometer (Coulter Electronics Inc., Miami Lakes, FL, USA) using 100 ml of EDTA peripheral blood incubated 30′ at 4°C with fluorochrome labelled monoclonal antibodies (mAb). The following mAbs were used: anti CD3-phycoerythrin-cyanine 5 (PC5/PE-CY-5), anti-CD4 phycoerythrin-Texas-Red (ECD), anti CD8 phycoerythrin-Texas-Red (ECD), anti CD8 fluorescein-isothiocyanate (FITC), anti human leukocyte antigen (HLA)-DR phycoerythrin (PE/RD1), anti CD16 FITC, anti CD45RA PE/RD1, anti CD45RO FITC, anti CD11a FITC, anti CD7 FITC, anti CD25 PE, and the isotype controls Ms IgG1, PE-RD/Ms IgG1, PE-Cy-5/MsIgG1, ECD/MsIgG1 FITC and MsIgG2a FITC/PE-RD1 (Cyto-stat Coulter clone; Coulter Instrumentation Laboratory, FL, USA). Erythrocyte lysis was obtained, after incubation with the Immuno-Prep Epics Kit (Coulter Electronics) and Q-prep Work Station (Coulter Electronics). Lymphocytes were selectively analysed using forward scatters (FSC) and side scatters (SSC) properties. For each sample, multiparametric data were acquired for 5000 events.

Quantitative polymerase chain reaction

Analyses were performed with a competitive polymerase chain reaction (PCR; Shuttle-Biotech, Milan, Italy) as suggested in the instructions. Briefly, RNA was extracted from 100 μl of plasma employing the Shuttle Pure H-RNA kit. Cell-free RNA was quantified in a competitive assay using the SK38/39 set of primers, which amplifies a 233 bp fragment of HIV gag. Each RNA sample (10 μl) was co-transcribed with 2 μl of an increasing copy number RNA competitor (10, 100, 1000, 10 000 copies). The cycle of retrotranscription and amplification were done using a Gen AMP PCR System 2400 (Perkin–Elmer Cetus, Norwalk, MA, USA) as suggested in the instructions. The amplified product was visualized by gel electrophoresis (polyacrylamide minigel, 10%) and ethidium bromide staining (Shuttle Easy-gel). Densitometric analyses were performed using the Lynx system. All the samples showing a plasma viraemia lower than 1000 copies/ml were subjected to a qualitative reverse transcriptase (RT)–PCR using a primer set amplifying a 233 bp fragment of HIV gag (Shuttle), different from the one employed for the quantitative detection of the virions. Branched DNA analyses were performed on 20 Ugandan and 20 Italian patients using a commercially available branched DNA assay (Chiron Diagnostics Ltd., Halstead, UK).

Statistical analysis

The values of cytokine production that were not precisely detected because they were too small, were randomly assigned in the interval between 0 and half of the minimum level detected for each cytokine. In order to obtain a better approximation to a normal distribution, data referred to cytokine production and plasma viraemia were transformed in their natural and common logarithms, respectively, before running statistical procedures. The geometric means of all cytokines and plasma viraemia and the arithmetic means of phenotypic markers for each population are reported with the 95% confidence interval. The significance of the differences between the mean values of cytokines, plasma viraemia, and phenotypic markers in Africans and Europeans was evaluated using multiple linear regression models taking into account sex, age, CD4 and CD8 as potential cofounders. The statistical analysis was performed using the SPSS-PC statistical package (SPSS Inc., Chicago, IL, USA).

Results

Clinical characterization of patients

Forty-three Ugandan and 33 Italian HIV-infected patients were studied. All patients were diagnosed as being affected by advanced HIV disease (B2 and C2 clinical categories) as established by the 1993 CDC classification criteria [22]. Sex (Ugandan: 19 males; 24 females; Italian: 17 males; 16 females), median age (Ugandan: 31.5 years; Italian: 30.2 years) and CD4 and CD8 counts were comparable between the two groups of patients; all patients were antiretroviral therapy naive.

Mitogen-stimulated IL-2, IFN-γ, IL-10, and TNF-α production

Mitogen-stimulated cytokine production by PBMC was examined in Ugandan and Italian patients and controls. The production of IL-2, IFN-γ, and IL-10 was measured after stimulation with PHA whereas LPS was used to stimulate TNF-α. production. Geometric

Table 1. Phenotypic analyses in Ugandan and Italian HIV-seropositive individuals (arithmetic means are shown)

Marker	Ugandan patients		Italian patients		*P* value*
	Mean	95% CI	Mean	95% CI	
CD3	79.6	75.7–83.4	77.3	73.4–81.3	NS
CD19	4.6	3.5–5.7	7.8	4.9–10.7	NS
CD16	11.1	6.6–15.7	7.7	5.5–10.0	NS
CD3/CD4	19.1	14.0–24.2	17.5	14.1–20.8	NS
CD3/CD8	54.2	49.0–59.4	54.8	51.3–58.4	NS
CD4/DR	2.1	1.4–2.9	1.1	0.8–1.4	**0.011**
CD4/CD45RO	15.5	11.7–19.2	9.2	6.3–12.0	**0.029**
CD4/CD45RA	4.7	2.2–7.2	6.3	3.4–9.2	NS
CD4/CD7$^-$	8.2	6.0–10.4	4.2	2.5–5.9	**0.034**

*Significant *P* values (multivariate analysis taking into account CD4 and CD8 counts, sex, and age) are indicated in bold. NS, not significant.

means of mitogen-stimulated cytokine production and 95% confidence intervals for Ugandan and Italian patients are shown in Fig. 1, together with the statistical significance of the differences between the two groups of patients evaluated using multiple linear regression models and controlling for CD4 and CD8 counts, sex, and age.

The production of IL-2 was not statistically different in Ugandan and Italian individuals, although a higher level of production was seen in African patients (Fig. 1, panel A). PHA-stimulated IL-10 production was reduced in Ugandan patients ($P = 0.002$) (Fig. 1, panel B), whereas LPS-stimulated TNF-α production as well as PHA-stimulated IFN-γ production was significantly

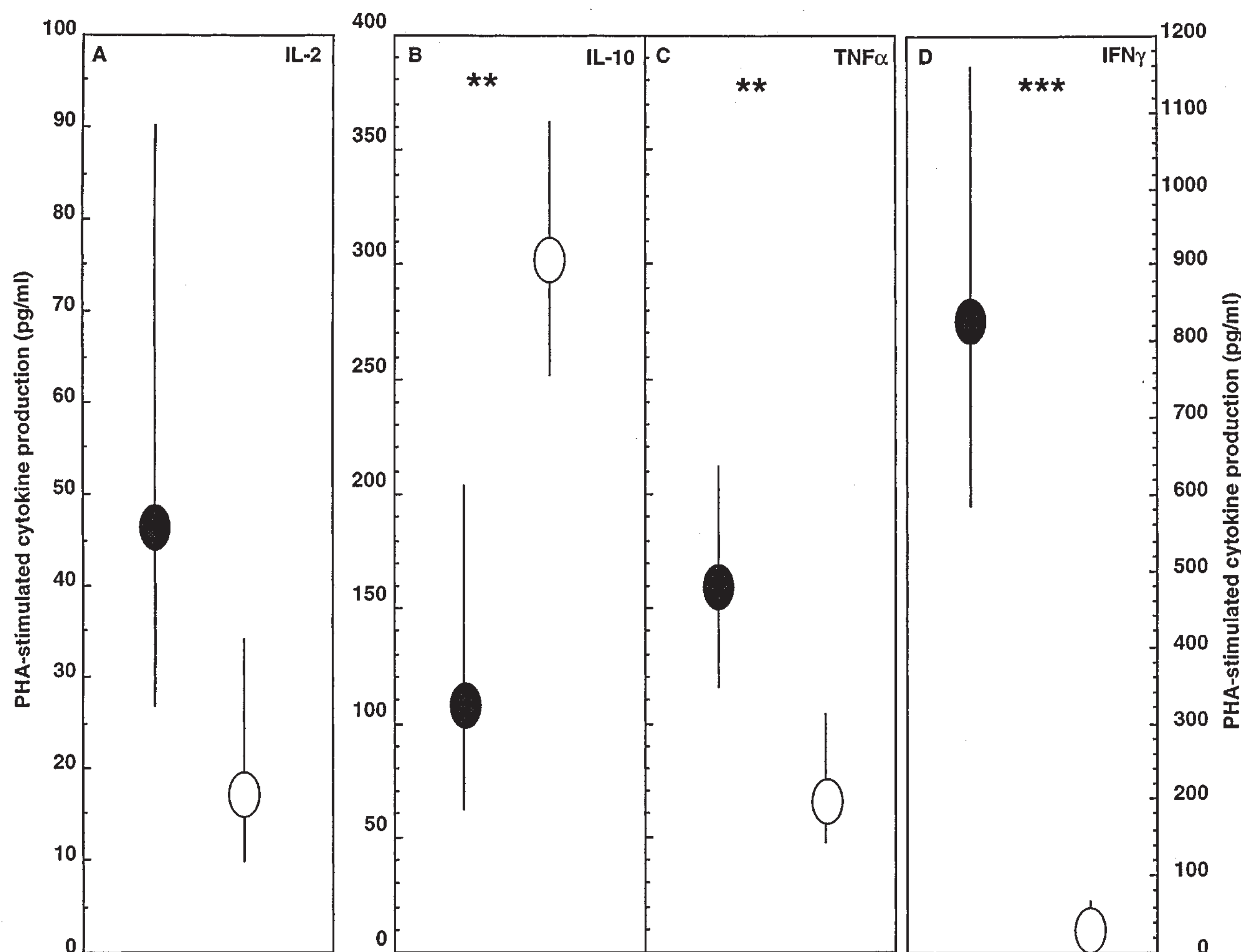

Fig. 1. PHA-stimulated production of IL-2 (panel A); IL-10 (panel B); TNF-α (panel C); and IFN-γ (panel D) in African (●) and European (◯) HIV-positive individuals. Geometric mean and 95% confidence intervals are shown for each cytokine. $^{**}P < 0.01$; $^{***}P < 0.001$.

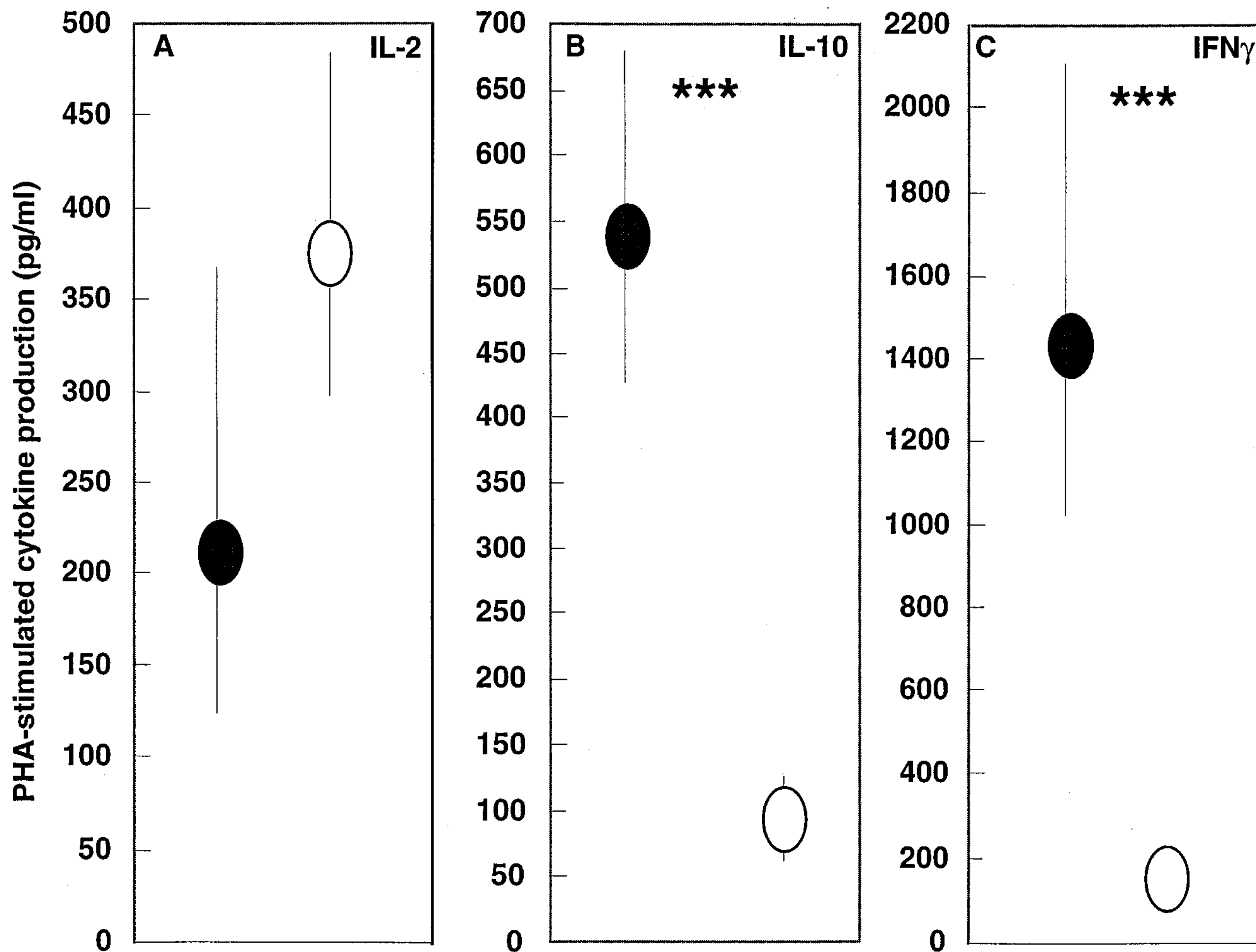

Fig. 2. PHA-stimulated production of of IL-2 (panel A); IL-10 (panel B); and IFN-γ (panel C) in African (●) and European (○) HIV-seronegative healthy controls. Geometric mean and 95% confidence intervals are shown for each cytokine. ***$P < 0.001$.

increased in Ugandan compared to Italian patients (TNF-α: $P = 0.002$; IFN-γ: $P < 0.001$). These data are shown in Fig. 1 (panels C and D). The profile of cytokines produced upon mitogenic stimulation is thus different in Ugandan and Italian patients in that a predominant production of type 1 and proinflammatory cytokine IFN-γ characterizes African patients, whereas IL-10, a type 2 and immune-dampening cytokine, is mainly produced in Italian patients.

Results obtained in African and Italian healthy controls are shown in Fig. 2. Both mitogen-stimulated IL-10 and IFN-γ production were significantly augmented (IL-10: $P < 0.001$; IFN-γ: $P < 0.001$), whereas the production of IL-2 was decreased, albeit not significantly in Ugandan compared with in Italian healthy individuals (LPS-stimulated TNF-α-production could not be measured in Italian controls; the geometric mean of TNF-α production by PBMC of Ugandan controls was 40.1 pg/ml).

Env peptide-stimulated IL-2, IFN-γ, IL-10 and TNF-α production

The analysis of mitogen-stimulated cytokine production allows a precise evaluation of the ability of the immune system to respond maximally to stimulation; to study the HIV-specific immune response in HIV-infected individuals, we stimulated PBMC with a pool of five antigenic peptides from the env region of HIV-1. The results are shown in Fig. 3; geometric means and 95% confidence intervals for each cytokine for both groups of patients are also presented, together with the statistical significance of the differences evaluated using multiple linear regression models. The production of IL-2 was reduced in Ugandan patients ($P < 0.001$) (Fig. 3, panel A). Env-stimulated IL-10 production was greatly increased in Ugandan patients ($P < 0.001$) (Fig. 3, panel B) as was the env-stimulated production of IFN-γ ($P < 0.001$) (Fig. 3, panel D) and TNF-α ($P = 0.006$) (Fig. 3, panel C). In contrast to

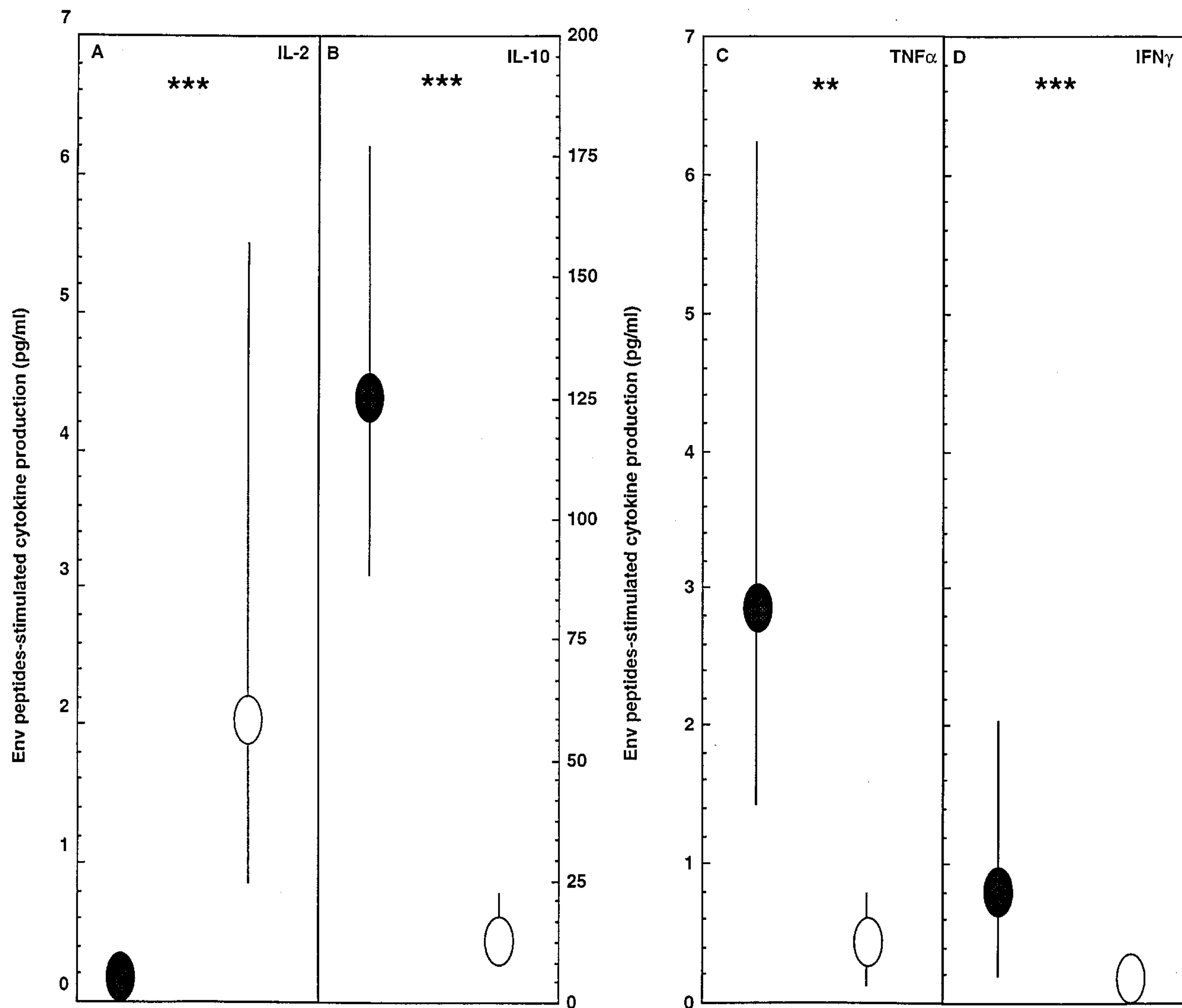

Fig. 3. Env peptide-stimulated production of IL-2 (panel A); IL-10 (panel B); TNF-α (panel C); and IFN-γ (panel D) in African (●) and European (○) HIV-positive individuals. Geometric mean and 95% confidence intervals are shown for each cytokine. $^{**}P < 0.01$; $^{***}P < 0.001$.

mitogen-stimulated cytokine production, the profile of cytokines produced upon stimulation of PBMC with HIV antigens is thus characterized by a predominance of IL-10 production in both groups of patients. Of interest is the fact that, when the mitogen- and HIV antigen-stimulated production of cytokines are compared, a 10- to 100-fold difference is observed except for IL-10 production which, in Ugandan patients, is similar with mitogenic or antigenic stimulation.

As expected, env-stimulated cytokine production was undetectable in both Ugandan and Italian healthy controls (data not shown).

Phenotypic analyses

Surface expression of a panel of phenotypic markers was evaluated in the same patients and controls (Tables 1 and 2). No significant differences were observed when the percentage of T ($CD3^+$) and B ($CD19^+$) lymphocytes were compared, whereas natural killer cells ($CD16^+$) were augmented in African individuals. The percentage of $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T lymphocytes was similar, as was the CD4/CD8 ratio. Cell surface activation markers were also evaluated on both $CD4^+$ and $CD8^+$ T lymphocytes. The results demonstrated that the percentage of $CD4^+$/HLA class II (DR^+)-expressing lymphocytes was augmented in Ugandan patients. An alteration of the ratio between naive and memory lymphocytes was also observed in Ugandan patients as $CD4^+/CD45RO^+$ lymphocytes were elevated and $CD4^+$/CD45RA cells were reduced in these patients. Finally, the percentage of the preferentially type 2 cytokine-secreting $CD4^+/CD7^-$

Table 2. Phenotypic analyses in Ugandan and Italian HIV-seronegative healthy individuals (arithmetic means are shown)

	Ugandan individuals		Italian individuals		
Marker	Mean	95% CI	Mean	95% CI	*P* value*
CD3	72.4	69.6–75.2	80.2	78.3–82.2	**< 0.001**
CD19	10.7	8.9–12.5	9.1	7.6–10.6	NS
CD16	11.0	9.0–13.0	9.1	7.6–10.6	NS
CD3/CD4	43.0	37.7–48.3	53.7	51.1–56.3	**0.004**
CD3/CD8	23.1	21.1–25.2	29.1	26.5–31.7	**0.001**
CD4/DR	2.3	1.9–2.6	1.3	1.1–1.4	**< 0.001**
CD4/CD45RO	31.1	29.3–33.0	18.0	14.3–21.6	**< 0.001**
CD4/CD45RA	13.0	11.5–14.5	15.2	12.6–17.7	NS
CD4/CD7–	13.1	11.8–14.5	4.6	3.2–5.9	**< 0.001**

*Significant *P* values (multivariate analysis taking into account CD4 and CD8 counts, sex, and age) are indicated in bold. NS, not significant.

lymphocytes was augmented in Ugandan patients, whereas $CD4^+/CD7^+$ cells were comparable in the Africans and Italians.

The percentage of $CD3^+$ T lymphocytes was significantly reduced in Ugandan compared with Italian healthy controls; this difference was secondary to a decrease in both $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T lymphocytes. In analogy with the results obtained in HIV-infected patients, activation markers–expressing $CD4^+$ T lymphocytes ($CD4^+/DR^+$) were augmented in Ugandan healthy controls; the percentage of $CD4^+/CD45RO^+$ and of $CD4^+/CD7^-$ T lymphocytes was also significantly augmented in these individuals. These results are shown in Table 2.

HIV plasma viraemia in Ugandan and Italian HIV-infected individuals

HIV plasma viraemia was analysed on the frozen plasma of 30 Ugandan, and 29 Italian patients. All precautions were taken to avoid possible technical mishaps in the freezing and shipping of plasma (plasma was separated from whole blood immediately after drawing, frozen immediately after the centrifugation procedures, transported overnight in thermal containers, and was thawed only before the analyses of plasma viraemia). CD4 and CD8 counts were included in the model as potential cofounders for evaluating the statistical significance of the differences observed. HIV plasma viraemia was quantified using a competitive PCR assay which utilizes a pair of primers (SK38/39) from a conserved region of gag of HIV-1 (HIV-2 was never observed in Uganda) [23]. In all cases in which fewer than 1000 copies/ml were detected, a qualitative RT–PCR based on a different set of primers (which amplifies a 233 bp conserved region within gag of HIV-1) was run. Because the A and D subtypes of HIV-1 are predominant in northern Uganda [24–26] and because the SK38/39 primers might be inadequate for the optimal detection of such strains [27–29], frozen plasma specimens from the same patients (20 Ugandan and 20

Table 3. Comparison between HIV plasma viraemia quantified in HIV-infected Ugandan and Italian patients using a competitive PCR assay (qPCR) (SK38/39 primers from a conserved region of gag of HIV-1) or a branched DNA (bDNA) assay

	HIV plasma viral load			HIV plasma viral load	
	qPCR	bDNA		qPCR	bDNA
Ugandan patients (ID)			Italian patients (ID)		
1	253*	< 500	1	1392	4700
2	1578	1500	2	380 275	596 000
3	388	1479	3	519 783	218 000
4	133	2547	4	95 175	120 555
5	385	< 500	5	16 327	87 950
6	145 938	84 500	6	13 305	19 500
7	2976	5700	7	94 100	89 500
8	188	3350	8	4471	8890
9	919 118	85 110	9	12 732	15 785
10	1792	5005	10	65 356	86 610
11	32 275	47 400	11	311	5850
12	273	< 500	12	4850	5515
13	279	< 500	13	15 850	21 455
14	180	< 500	14	16 736	15 500
15	508	7710	15	178 925	198 540
16	319	< 500	16	35 432	42 125
17	2110	5110	17	125 667	147 550
18	10 000	5801	18	47 619	31 500
19	8818	22 400	19	107 717	82 000
20	113	< 500	20	117 600	92 400

*HIV copies/ml.

Italian individuals) were retested in a bDNA assay, which optimally detects clades A to H of HIV [30,31]. A comparison of the results obtained when quantification of HIV plasma viral load was performed either by qPCR or by bDNA is shown in Table 3. The results suggest that the plasma HIV viral load is lower in Ugandan patients ($P < 0.001$; statistic value obtained from the RT–PCR using SK38/39).

Discussion

HIV infection is among the leading causes of morbidity and mortality in Africa [32]. Many differences are detected when HIV infection in Africa and in Europe are compared. To summarize, African HIV infection is mainly caused by infection by non-B clades of HIV [24–27] (clade B is highly prevalent in European infection) and is mainly heterosexually transmitted [33]. Faster progression to AIDS as well as shortened survival time after AIDS diagnosis are also suggested to characterize African HIV infection [2–10]. An abnormal immune response was suggested to be associated in African patients with faster progression to AIDS [18–20]. Immune activation resulting from chronic infections, poor hygienic conditions, and dietary limitations would thus lead to a chronic activation of peripheral lymphocytes with a consequent increased susceptibility of these lymphocytes to HIV infection [18–20]. Support for this hypothesis stems from observations done on HIV-infected and -uninfected Ethiopian Jews who had moved to Israel. The immune response in these individuals was characterized by a generalized state of immune hyperactivation, with a tendency towards normalization with time after immigration [18,20]. Because of logistical problems, remarkably few data are available on the immune status of HIV-infected African individuals living in Africa. We recently performed immunological analyses on sera from African and Italian HIV-infected patients, and observed elevated serum levels of IL-2 and IL-10 in African HIV-infected individuals [19]. To investigate immune activation in African individuals further we analysed functional and phenotypic parameters in African HIV-infected and -uninfected individuals living in the Gulu District in northern Uganda. Official and non-official data show that HIV prevalence in northern Uganda ranges between 14 and 25%. A recent survey among pregnant women attending the Antenatal Clinic of Lacor St Mary's Hospital in Gulu showed a prevalence rate of HIV antibodies of 13%, whereas the prevalence of HIV infection in the general medical ward was 60% (data collected between 1989 and 1996) [34]. To avoid variables secondary to freezing and thawing of cells we analysed fresh PBMC by hand carrying blood specimens overnight from Uganda to Italy; HIV plasma viral load was analysed in the same patients.

Our results confirm that immune activation accompanies HIV infection in Africa and is present even in Ugandan HIV-seronegative healthy controls. Therefore (1) significantly increased amounts of TNF-α and IFN-γ are produced by PBMC of HIV-infected Ugandan patients; (2) IFN-γ and IL-10 production is augmented in HIV-seronegative African compared to Italian controls; and (3) the percentage of activation markers expressing T lymphocytes (CD4/HLA DR$^+$), of CD4$^+$/CD45RO$^+$ T cells, and the memory/naive ratio are augmented both in African HIV-infected and -uninfected compared to Italian individuals.

Immune activation augments in-vitro susceptibility of peripheral lymphocytes to HIV infection [35,36]; this is also suggested by the observation that activation of the immune system can be associated with an increase in virus replication in HIV-infected patients receiving vaccines [37–42]. In addition, TNF-α is known to be a soluble factor favouring cachexia, and can have a potent activity on HIV-infected cells enhancing viral replication *in vitro* by activation of the transcription factor NF-$\kappa\beta$ upon binding of the long terminal repeat sequences of HIV [43,44]. Both immune activation and increased production of TNF-α are present in this African population in which a faster progression of HIV infection is present, suggesting a possible causal relationship between these observations. The results also suggest that the PBMC of HIV-seronegative healthy African individuals could be more susceptible to HIV infection because of IFN-γ-associated immune activation; the massive production of IL-10 present in the same individuals would impair antiviral-specific cell-mediated immunity allowing for an inefficient immune control over disease progression.

Mitogen-stimulated cytokine production is an estimate of the ability of the immune system to respond to supra-optimal antigenic stimulation. We also stimulated PBMC from African and Italian individuals with a pool of antigenic peptides from the env region of HIV, thus evaluating the HIV-specific immune response. Results showed a significantly augmented env peptide-stimulated production of IFN-γ, TNF-α, and IL-10 in African patients, whereas env-stimulated IL-2 production was higher in Italian HIV-infected individuals (as expected env-stimulated cytokine production could not be detected in either African or European controls). An increased generation of cytokines potentially capable of facilitating HIV infection and replication is therefore evident even when cytokine production by HIV-specific lymphocytes is analysed. Cytokines produced upon mitogen stimulation are polyclonally generated and include both antigen-specific and antigen-non-specific clones. It is thus surprising that the amounts of mitogen- and antigen-stimulated IL-10 overlap. Because: (1) an augmented percentage of CD4/CD7$^-$ lymphocytes is observed in African

patients; and (2) CD4/CD7⁻ T lymphocytes produce low amounts of IL-2 and high levels of IL-4 and IL-10 [45], it is possible that the elevated levels of env-stimulated IL-10 production seen in these patients is supported by HIV-specific CD4/CD7⁻ T lymphocytes. In addition, because HIV replicates better in T helper (Th) type 2 clones [46]; because IL-10 is produced by such clones [47,48]; and because IL-10 dominates the profile of cytokines produced upon env peptide-stimulation (see Fig. 2) it can be hypothesized that HIV-specific T cells in African patients could mostly belong to the subpopulation that allows HIV replication. On the other hand, the lower levels of viraemia observed in African patients could be secondary to an inhibition of HIV replication induced by the high quantities of IL-10 produced in these patients. IL-10 was in fact suggested to reduce HIV viral load by impeding virus replication in monocytes [49,50], and by suppressing the nuclear activity of NF-κβ/Rel in T lymphocytes [51].

Taken together these data suggest that immunopathogenetic mechanisms could play a predominant role in African HIV infection. This is further suggested by the observation that plasma HIV viral load might be reduced in African compared to Italian HIV-infected individuals. A word of caution is needed in the interpretation of these data, as HIV viral load was quantified in most sera with methods that might not be optimally sensitive when HIV infection is supported by non-B clades. On the other hand, that lower levels of viraemia were present in our African patients was confirmed by bDNA, a method which accurately quantifies multiple HIV-1 subtypes including the A and D clades that are prevalent in north Uganda [30,31]. In the light of the impairment of the immune response observed in African patients, it is nevertheless not surprising that a lower amount of HIV could support infection because in these patients disease progression would be facilitated by: (1) polyclonal activation and augmented susceptibility of lymphocytes to HIV infection; (2) augmented TNF-α and cachexia; and (3) a high prevalence of HIV-specific Th2 lymphocytes suggested to allow HIV replication. In this scenario HIV infection of, and replication inside, target cells would be less efficiently blocked by immune defences, resulting in disease progression even in the presence of lower levels of viraemia.

Conclusion

These data underline the fact that an abnormal immune activation is present in African individuals and suggest that reduced plasma HIV viral load might be present in HIV disease in Africa. A speculative interpretation of these results is that in African HIV infection lower loads of virus could support a mainly immunologically driven pathology; this immune activation, along with numerous and recurrent co-infections and poor nutritional and hygienic conditions, might therefore explain the faster progression of HIV infection to AIDS suggested to characterize African patients.

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