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Analysis of the systemic immune response in HIV-1-infected patients suffering from opportunistic Candida infection

Abstract

Infection by HIV-1 is a major risk factor predisposing for fungal infection. However, few studies have addressed the immunological status of HIV-1 patients suffering fungal infections. This study examines the status of polymorphonuclear phagocytes (PMN) and T cells in HIV-1-infected patients suffering from mucosal Candida infections. These patients had a more immature

population of blood PMN, as detected by lower CD18 expression, than HIV asymptomatics or healthy controls. They also had a selective defect in T cell activation in response to phytohemagglutinin (PHA), but not to stimulation through the T cell receptor by anti-CD3 crosslinking, when compared to HIV-1 asymptomatic patients. This was shown by a decrease in cellular proliferation and cell surface expression of CD69, CD25 and CD71 activation antigens. There was also a severe impairment of IL-2 production upon activation by PHA. IL-10, and TNF secretion was also reduced, whereas IFN-gamma and IL-5 production was not affected. No correlation with viral load, CD4 or CD8 T cell number or clinical stage was found. In conclusion, our results indicate that Candida-infected HIV patients have a selective defect, independent of viral load, CD4 or clinical status, involving some aspects of T cell activation, IL-2 production being severely impaired.

Full text

INTRODUCTION

Infection by HIV-1 has emerged as the major risk factor predisposing patients to serious fungal infections. Many mycoses that previously were rarely seen are now commonplace, those caused by *Candida* spp being among the most prevalent [1]. Many abnormalities arise as consequence of HIV-1 infection [2]. As infection progresses, there is a defect in cell-mediated immunity which is manifested *in vitro* by a sequential loss of proliferation responses to recall antigen and mitogens [3, 4]. Peripheral blood mononuclear cells (PBMC), also exhibit a dysregulation in cytokine production upon activation. Thus, IL-2 production has been shown to be depressed in HIV-1 patients, and this correlates with progression of the disease [3-8]. Results with IFN-gamma are more controversial since both increases [9] and decreases [10, 11] have been reported. Moreover, a shift from a Th1 to a Th2 pattern of lymphokine secretion have been observed [3, 4, 10, 10], although this has been questioned by some groups [12, 13].

$CD4^+$ T cell depletion, the immunological hallmark of AIDS, either indirectly or directly, probably accounts for most of the immunological dysfunctions that lead to opportunistic mycoses. This is because of the critical role of $CD4^+$ T cells in the defense against fungi. Studies in mice have clearly defined the role of different T cell subsets and cytokines in controlling fungal infection. Thus, type I

immune responses induced by IL-12 and characterized by IFN-gamma and TNF secretion by Th1 cells, have been shown to be protective against *Candida* [14], *Histoplasma* [15] and *Aspergillus* [16]. This response is thought to be required to activate macrophages and neutrophils to phagocytize invading fungi warding off invasion. In contrast, elevated type 2 responses, characterized by high IL-4 and IL-5 levels produced by Th2 cells, are either non-protective or in some instances exacerbate the infection and/or the symptoms [14, 16, 17]. Nevertheless, the specific mechanisms underlying the susceptibility to opportunistic mycoses in HIV-1 infected individuals are not yet clear. Other factors besides $CD4^+$ depletion may account for this.

Although new antiretroviral therapies have decreased the impact of opportunistic *Candida* infections in HIV-1 infected patients, those treatments are not readily available to the great majority of HIV-infected patients in developing countries [18]. Moreover, new antifungal agents are in developmental stages, but resistance and treatment failure are likely to be an ongoing problem [19]. An alternative approach to treat this disease is to identify the underlying cause of the immunodepression in order to correct it. In this study, we have tried to identify putative specific defects in the activation and function of the most relevant cells involved in the fight against fungi, *i.e.* T cells and polymorphonuclear leukocytes (PMN), in HIV-1 infected patients suffering opportunistic candidiasis, as compared to $HIV\text{-}1^+$ asymptomatic and healthy controls. We performed a comprehensive analysis of several parameters of T cell activation in the same set of patients. Our results indicate that despite the lower number of CD4 cells or higher viral load, all patients with fungal infection had a selective defect involving certain aspects of T cell activation. Among these, the most significant fact is that IL-2 production, but not IFN-gamma or IL-5, by mitogen-activated T cells is severely impaired. Moreover, differences in response to the stimuli used to activate T cells were also seen. In addition, lower numbers of mature PMN were observed in the circulating blood of these patients.

MATERIALS AND METHODS

Patients and control subjects

Twenty-one HIV-1 infected patients having opportunistic *Candida* mucosal infections from the Hospital Gregorio Marañón were studied. As controls, 12

healthy individuals, as well as 12 HIV-1-infected, asymptomatic patients were included. Informed consent was obtained from all subjects and the guidelines of the European Union and those of the Hospital Gregorio Marañón, Madrid, Spain, for human experimentation were followed. The characteristics of these patients are listed in Table 1. All were sex and age-matched.

Reagents

Formyl-methionyl-leucyl-phenyalanine (fMLP) and phytohemagglutinin (PHA) were purchased from Sigma (St. Louis, MO, USA); penicillin/streptomycin mixture, L-glutamine, fetal calf serum, were from BioWhittaker (Walkersville, MD, USA). Human recombinant TNF was from Genzyme. Monoclonal antibodies (mAb), against the following cell surface antigens were used: CD3epsilon, CD67, CD18, CD43, CD95, CD25, and CD71 and were obtained from Becton Dickinson. The corresponding FITC conjugated mouse IgG isotype control were all from Becton Dickinson

Cell populations

Human mononuclear cells were obtained from heparinized venous blood by Ficoll Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation. The layer containing mononuclear cells was washed thoroughly by centrifugation in Dulbecco's modified Eagle's medium (DMEM), and finally resuspended in DMEM 2% fetal calf serum (FCS). Human blood monocytes were separated by adherence to plastic dishes for 2 hours at 37° C. T cells were further purified by passing the non-adherent population through a nylon fiber wool column. The purity of this population (detected by flow cytometry) was always greater than 95% $CD3^+$ cells.

PMN were obtained from the pellet of Ficoll centrifugation. Red blood cells were eliminated by hypotonic shock. In all cases, cell viability, determined by trypan blue exclusion, was greater than 99%. Purity was always greater than 98%.

PMN activation

PMN were activated or not with fMLP (1 muM) or TNF (10 ng/ml) for 30 min at 37° C. After this, the cells were stained for CD18, CD67, and CD43 expression.

Proliferation assay

Purified T cells (10^6/ml in DMEM medium containing 10% FCS) were stimulated with immobilized anti-CD3 antibody (1 mug/ml coated wells) (SPV3Tb, kindly provided by Dr. J.E. de Vries, DNAX, Palo Alto, CA, USA) or with PHA (1 mug/ml). For this, purified T cells were seeded in 96-well U-bottom microtiter plates (10^5/100 mul per well), and activated with immobilized anti-CD3 antibodies or PHA for 72 hours at 37° C. Cell proliferation was evaluated by incorporation of (^{3}H) thymidine (New England Nuclear, Boston, MA, USA) into DNA during the last 16 hours of culture. The cells were pulsed with 1 muCi of (^{3}H) thymidine and harvested on glass fiber filters using an automatic cell harvester. Radioactive incorporation was measured in a liquid scintillation spectrometer. The assay was carried out in triplicate.

Cytokine production assays

Purified T cells were seeded in 96-well U-bottom microtiter plates (10^5/100 mul per well), activated with immobilized anti-CD3 antibodies or PHA as described above. After 3 days of culture, supernatants were harvested and their cytokine (IL-2, TNF-alpha, IFN-gamma, IL-5 and IL-10) levels were quantified using commercially available specific ELISA kits used according to manufacturer's instructions (IL-2, R&D Systems, Minneapolis, MN, USA; IL-10, IL-12 and TNF-alpha , Bender MedSystems, Vienna, Austria; IFN-gamma and IL-5, Endogen Inc., Cambridge, MA, USA). Cytokine levels were tested in duplicate.

Analysis of cell surface expression

Cell surface expression in T cell cultures or in PMN was evaluated by direct flow cytometry as previously described. PMN and T cells stimulated as mentioned above were analyzed by flow cytometry using fluoresceine isothiocyanate-conjugated (FITC) antibodies (Becton Dickinson, San Jose, CA, USA) in a FACS Scan flow cytometer (Becton Dickinson, San Jose, CA, USA). Surface expression of the CD69, CD25 CD 95 and CD71 T cell antigens was analyzed at 0, 24 or 48 hours after activation. A minimum of 5,000 cells per point were analyzed. The final percentage of positive cells was obtained by subtracting the values of negative control FITC conjugated obtained with the corresponding isotype-matched, irrelevant antibodies.

Statistical analysis

To analyze the differences among the groups we used the U test of the Mann-Whitney non-parametric statistical test. This type of analysis is as potent as parametric analysis when applied to small groups.

RESULTS

PMN activation

PMN are thought to represent the first line of defense against fungi [20]. Thus, we investigated the numbers, as well as the activation status of these cells in the 3 groups: healthy controls, asymptomatic HIV-1- infected patients (HIV/Asymp) and HIV-1 patients having *Candida* infections (HIV/Cand). As shown in Figure 1A and Figure 1B, the mean number of mature PMN in blood, as detected by the percentage of CD18 positive cells in the granulocytic purified fraction, was not significantly different among control (90%) and HIV/Asymp (86%), but the HIV/Cand group showed a significant reduction in the granulocytic cell fraction expressing CD18 (mean 62%) ($p = 0.02$). Interestingly, the levels of CD18 expression that are a measure of the density of CD18 expression (mean fluorescence intensity (MFI)), and which reflects the maturity of PMN, were also lower in patients suffering fungal infections as compared to controls ($p = 0.01$). This effect seems to be specific, since CD43 or CD67 expression was not affected (data not shown). Further more, be PMN were assayed for their ability to be activated by proinflammatory stimuli. Activation of PMN by fMLP and TNF is characterized by an increase in the number of membrane CD18 molecules (measured as MFI). PMN from control subjects responded by increasing the MFI of CD18, as expected. PMN from both groups of HIV-infected patients did not show a defect in their relative ability to become activated (measured proportionally as stimulation index) although, CD18 MFI never reached the absolute values of healthy controls (not shown). These results suggest that patients suffering fungal infections have a rather immature population of PMN circulating in the blood, although they can respond to inflammatory stimuli.

Activation of T lymphocytes

As expected HIV-infected patients, especially those suffering from fungal infections, had lower mean $CD4^+$ values (Table 1). In order to see if some specific

defect beside the lower number of circulating T cells exist in patients with opportunistic Candida infections, we studied the response of T cells to different stimuli. Equal number of T cells were activated with PHA or plastic-bound anti-CD3 antibodies, and proliferation as well as cell surface expression of activation markers and cytokine secretion were evaluated. The results for all patients are shown in Figure 1, Figure 2, Figure 3 and the mean values are summarized in Table 2.

Regarding T cell proliferation, HIV/Asymp have a slight impairment in response to PHA, and anti-CD3 (Figure 1C, Figure 1D), which is in agreement with previously reported data [3, 4]. The HIV/Cand group had a more pronounced defect in the proliferative response to PHA. However, no significant difference in proliferation to anti-CD3, between HIV/Asymp and HIV/Cand groups was found.

Comparing the expression of cell surface activation markers in HIV/Asymp and healthy controls, we observed more pronounced defects in anti-CD3 than in PHA-induced T cell responses. Thus, induction of cell surface expression in T cells from HIV-1/Asymp patients in response to mitogens, was not reduced (rather increased) when compared to control individuals for the 3 markers studied: CD69 (Figure 1E, Figure 1F) an immediate early activation marker, CD25 (Figure 1G, Figure 1H), an immediate activation marker, and CD71, a late activation antigen (see Table 2). In contrast, there was a significant defect in the induction of all 3 markers in response to anti-CD3. Thus, CD69 (mean 34 *versus* 53% in the control group), CD25 (mean 25 *versus* 53% in the control group) and to a lesser extent, CD71 (mean 18 *versus* 38% in the control group) were significantly impaired (Figure 1E-Figure 1H and Table 2).

In contrast to what was found in HIV/Asymp patients, the activation of T cells from HIV/Cand patients in response to PHA was reduced for the 3 activation markers tested, although only CD25 was significantly different from controls (Figure 1E-Figure 1H and Table 2). Surprisingly, expression of activation antigens in response to anti-CD3 stimulation, although reduced in HIV/Cand compared to healthy controls, was less drastically reduced than in HIV-1/Asymp. Despite higher CD4 and better clinical status of the latter patients, but these differences did not reach statistical significance.

In basal conditions without stimulation only 1-3% of cells expressed CD95 and no difference was observed between the 3 groups indicating that T cells were

not in an activated state. The basal expression of CD95 in HIV/Asymp was 23%, in HIV/Cand 9%, in controls 12%.

Lymphokine secretion

Lymphokine content (TNF-alpha, IFN-gamma, IL-2, IL-5 and IL-10) in the supernatants of the T cell cultures was assayed by ELISA. As shown in Figure 2 and Figure 3, despite the similar induction of proliferation and activation markers after PHA activation in HIV/Asymp and controls, the production of cytokines by the same cultures was different. Thus, in response to PHA: a) the production of IL-10 and IFN-gamma was significantly reduced, b) TNF production was mildly depressed, without reaching statistically significance, c) The production of IL-5 and IL-2 was not affected. By comparing the effect of anti-CD3 stimulation in controls and HIV/Asymp, there was the same tendency than for the response to PHA, excepted for IL-5, which was increased in HIV patients.

When HIV/Cand were compared to HIV/Asymp patients, the mean values for cytokine production in PHA-stimulated cultures were as follows: a) IL-5 was about the same, b) TNF and IL-10 were slightly more depressed than in asymptomatic patients; c) IFN-gamma was in contrast, slightly upregulated, d) IL-2 production was severely impaired (Figure 3C). In contrast with the effect observed on activation markers, which were increased in HIV/Cand as compared with HIV/Asymp, anti-CD3, stimulated cultures of HIV/Cand patients showed similar or lower production of cytokines than HIV-Asymp. Thus, the production of IL-5 and IFN-gamma was the same, whereas IL-10 production was further reduced in HIV/Cand. IL-2 production with anti-CD3 was also reduced in HIV/Cand, although values were too low and close to the limit of detection ELISA assay to reach statistical significance. No correlation between any of the parameters tested in HIV/Cand, either with viral load, CD4 or CD8 T cell number or clinical stage was found (data not shown).

DISCUSSION

Many studies have addressed the immune defects in HIV-infected patients. However, to our knowledge, no studies have been performed in a subset of HIV-infected patients suffering from opportunistic fungal infections. We have shown in this study that there are some immune defects which are specific to the

subset of HIV patients that also harbors fungal infections. These defects can be summarized as follows:

a) Lower number of mature PMN circulating in the blood, although they can respond to proinflammatory stimuli.

b) A defect in T cell activation. This defect is not general but selective, present in response to mitogens but not to TcR stimulation. Moreover, this defect is not at the level of proliferation, but at the level of early activation markers such as CD25, CD71 and CD69.

c) No significant effect on basal CD95 (Fas) expression, suggestive of no increased activation-induced cell death (AICD).

d) A defect in cytokine production; IL-5 production was not affected, whereas IFN-gamma, IL-10 and TNF were slightly reduced. Importantly, IL-2 production was severely impaired in response to mitogens.

Thus, our results indicate that T cells from *Candida*-infected HIV-1 patients have a defect in PHA and T cell receptor stimulation in contrast to HIV asymptomatic patients that only demonstrate a defect in anti-CD3 but not in PHA stimulation. This is in agreement with published reports that indicate that as AIDS infection progresses, there is a sequential loss of response, first to mitogens and later to recall antigens [3, 4]. PHA is a more complete stimulus able to crosslink and activate the costimulatory molecules that may compensate TcR-signalling defects.

On the other hand, it is thought that some opportunistic infections such as *Candida* in HIV-infected patient take hold when the number of $CD4^+$ T cells drops below a certain number. Our results, although not contradictory to this, indicate that T cells from these patients have a qualitative defect involving T cell activation as well the maturity of their circulating PMN. Despite the lower number of CD4 and CD8 cells in these patients, the relative expression of cell surface activation markers/per cell (%) was not as severely impaired. These patients respond similarly, if not better, to anti-CD3 stimulation than HIV/Asymptomatic patients on a per cell basis. Moreover, no correlation was found with viral load, antiviral treatment or the absolute or relative number of CD4 and CD8 cells (data not shown).

It is unlikely that variations in the number or circulating $CD4^+$ and $CD8^+$ cells among the different patients can explain the low CD69, CD71 and CD25 expression in T cells observed in *Candida*-infected patients. Firstly, different responses were observed in anti-CD3 or in PHA-stimulated T cells. So, depending on the stimulus and not on the original number of cells, there was a normal response or a defective one. Secondly, the number of T cells was adjusted after isolation and before the assays. Thirdly the correlation coefficient of CD69, CD25 and CD71 expression with absolute CD4 or CD8 counts in blood was low (data not shown), indicating that CD4 or CD8 counts cannot solely explain the defect, thus suggesting than there is a more selective defect in those patients.

In this regard, our results with *Candida*-HIV patients are indicative of a selective failure to produce IL-2 by T cells. The fact that other Th1 cytokines such as IFN-gamma and expression of cell activation surface marker were not as severely impaired, indicate that this phenomenon probably reflects a specific mechanism. These results are in agreement with those found in mice infected with *C. albicans* in which proliferation and especially IL-2 production was severely diminished [21]. Most groups have found that IL-2 production was severely impaired in response to mitogen in all stages of infection, although this decrease was more severe in patients with advanced disease. Interestingly, recent studies have indicated a good correlation between a decrease in PHA-induced IL-2 production and faster progression to AIDS [22]. Our results are in agreement with these results, although we found a more greatly diminished IL-2 response in *Candida*-infected HIV patients than in asymptomatic patients with similar CD4 counts, supporting the idea that the IL-2 defect caused by HIV-infection is further augmented by *Candida* infection. The fact that IL-2 production is decreased despite the only slightly abnormal T cell proliferation also suggests that other cytokines such as IL-4, a Th2 cytokine, may take the control of T cell proliferation in those patients.

In the few studies on the immune status of patients suffering from *Candida* infections, no clear correlation between any particular systemic immune defect has been reported. Thus, in women with recurrent vulvovaginal candidiasis, decreases in PBMC proliferation in response in response to *Candida* antigen and mitogens have been reported [23]. By contrast, Fidel *et al.* have shown that IL-2 and IFN-gamma production are not depressed in response to mitogens [24]. On

the other hand, in chronic mucocutaneous candidiasis no clear immune defect has been reported [20, 25].

T cells play a central role in regulating the immune response to fungi. In mouse models, the Th1 response induced by IL-2 and characterized by IFN-gamma and TNF production, is protective [14, 17, 26]. By contrast, type-2 responses characterized by IL-4 and IL-5 production are either not protective or can exacerbate the disease [27, 28]. Moreover, a switch in the Th1/Th2 ratio has been hypothesized as a cause of susceptibility to fungal infection [29]. In addition, several studies in patients with chronic and widespread fungal infection also suggested that these patients suffered from an immune imbalance characteristic of a type 2 response [20]. Our results in *Candida*-infected patients tend to agree with that hypothesis and suggest that T cells from *Candida*-infected patients have a reversed Th2/Th1 ratio (lower IFN-gamma and higher IL-5), which may explain why they cannot cope with the infection. HIV-infection may induce *per se* this Th1/Th2 switch that may favor fungal spreading. Moreover, a lower number of mature PMN, another well-known effector cell against fungi [20], are circulating in the blood. How this can affect fungal clearance was not addressed in our studies, but it may also contribute to systemic infection.

On the other hand, our results with asymptomatic HIV patients are, in general, in agreement with previously published data. In HIV-1-infected patients, a sequential and progressive loss of Th function, in which the Th response through TcR is lost before the response to mitogens as PHA, as disease progress [3, 4], similar to what we found. Moreover, it has been hypothesized that a shift from a Th1 to a Th2 response take place during progression in AIDS [3, 4]. Thus, it has been shown that production IL-2 and IFN-gamma, type 1 cytokines, decreases in stimulated PBMC as the disease progresses, whereas IL-4 and especially IL-10 increase [3, 7, 11]. By contrast, other groups have failed to show any evidence of enhanced production of IL-4 or particularly IL-10 [13, 30-32]. Our results regarding IL-10 are in agreement with these latter studies, since we found that IL-10 was not increased, and was rather decreased, upon stimulation with anti-CD3 or PHA in HIV-1-infected patients. Furthermore, patients with *Candida* infections have even lower IL-10 secretion in response to PHA and/or CD3. However, we have found an increase in IL-5, a Th2 cytokine, and a decrease in IFN-gamma production which was particulary evident in response to anti-CD3, or suggestive of a Th1/Th2 switch.

Alternatively, it has been proposed that the T cell defect that take place in HIV-1 infection may be related to abnormal induction of apoptosis [33] caused by Th2 cytokines [34, 35]. In a later study, CD95 expression was elevated both in CD4 and CD8 cells in basal conditions. We have not seen such asymptomatic patients. Only two fold increases in basal CD95 expression over healthy control were found. More interestingly, CD95 was not elevated in HIV-1 patients with opportunistic fungal infections.

Recently, CD69 expression after T cell activation with anti-CD3, has been shown to be impaired in HIV-infected individuals. Moreover, the decrease was more pronounced in late stage disease patients, and has been taken as an indicator of immune function [36]. Our results confirm that HIV^+ patients have lower CD69 expression upon anti-CD3 activation. However, those infected with *Candida*, having lower CD4 mean numbers than asymptomatic patients, respond better than asymptomatics. The differences among the various studies mentioned above can be ascribed to differences in the cell types (purified T cell, PBL, $CD4^+$, etc.), or stimuli used (mitogens, anti CD3 or CD2), as well as to the kinetics of the response.

Recent data from the literature have shown that antiretroviral therapy only partially restores immune function [37-39]. Therefore, cytokine-based therapy may be a promising adjuvant approach to a long lasting reconstitution of the immune system [5]. Efforts should be made in order to identify those cytokine alterations in the various manifestation of AIDS. IL-2 is one of the cytokines that have been used in clinical trials [5, 40, 41]. Our results could be taken as an indication that IL-2 adjuvant therapy may help to restore immune function in HIV-1-infected patients suffering fungal opportunistic infections.

CONCLUSION

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Figures

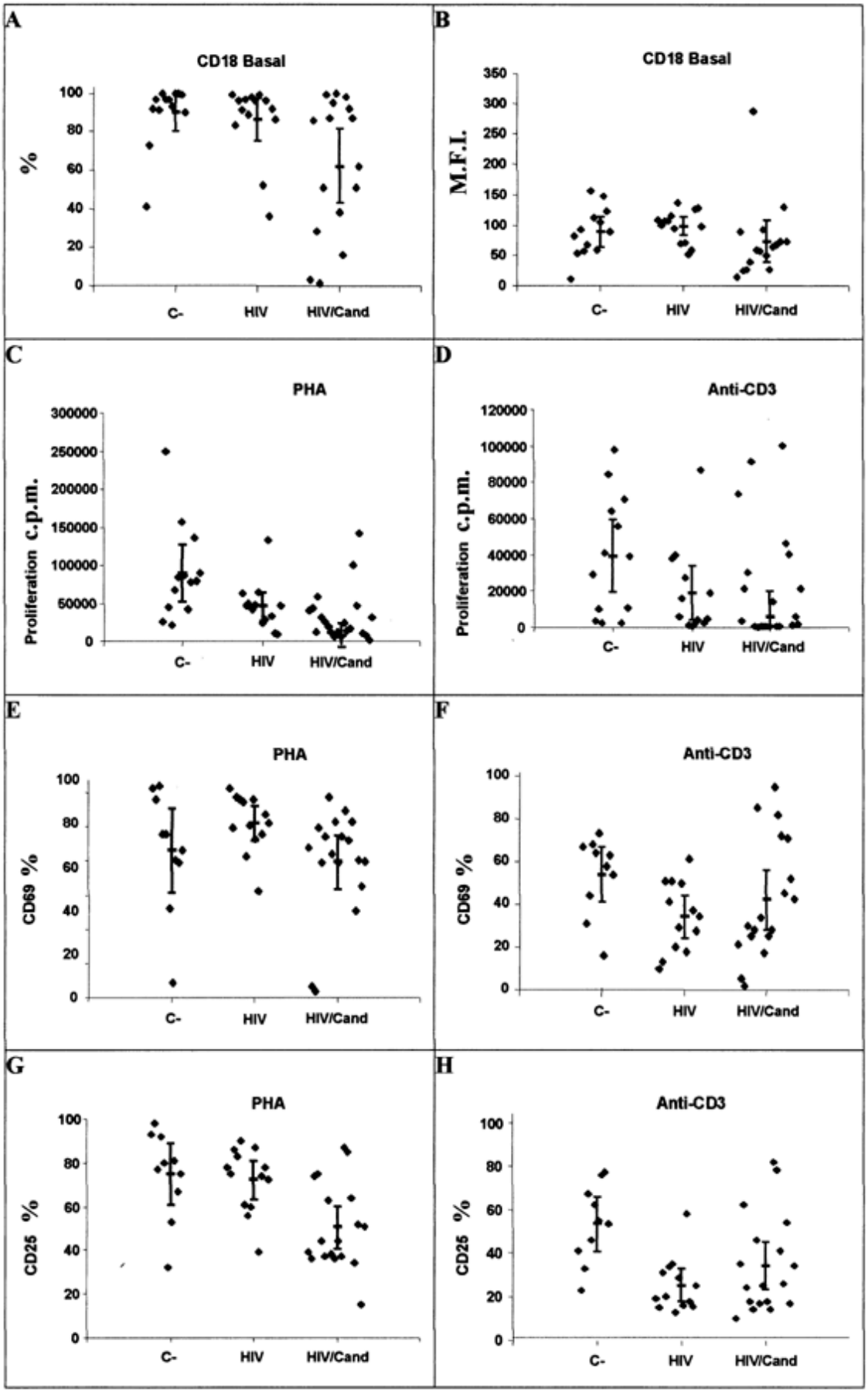


Figure 1A and 1B

Cell surface analysis of PMN.

The blood PMN was analyzed by flow cytometry in the 3 groups of patients by staining with anti-CD18 antibodies and the % of positive cell and the mean fluorescence intensity (MFI) of positive cells was plotted.

Figure 1C and 1D

Proliferative ability of activated T cells.

T cells were activated with PHA or anti-CD3, and proliferation measured 72 hours later.

Figure 1E and 1F

CD69 cell surface expression by activated T cells.

T cells were activated with PHA or anti-CD3, and the percentage of cells expressing CD69 measured by flow cytometry, 48 hours later.

Figure 1G and 1H

CD25 cell surface expression by activated T cells.

T cells were activated with PHA or anti-CD3, and the percentage of cells expressing CD25 assessed by flow cytometry, 48 hours later.

C, healthy control patients, HIV, HIV-1-infected asymptomatic; HIV/Cand, HIV-1-infected suffering from *Candida* infections.

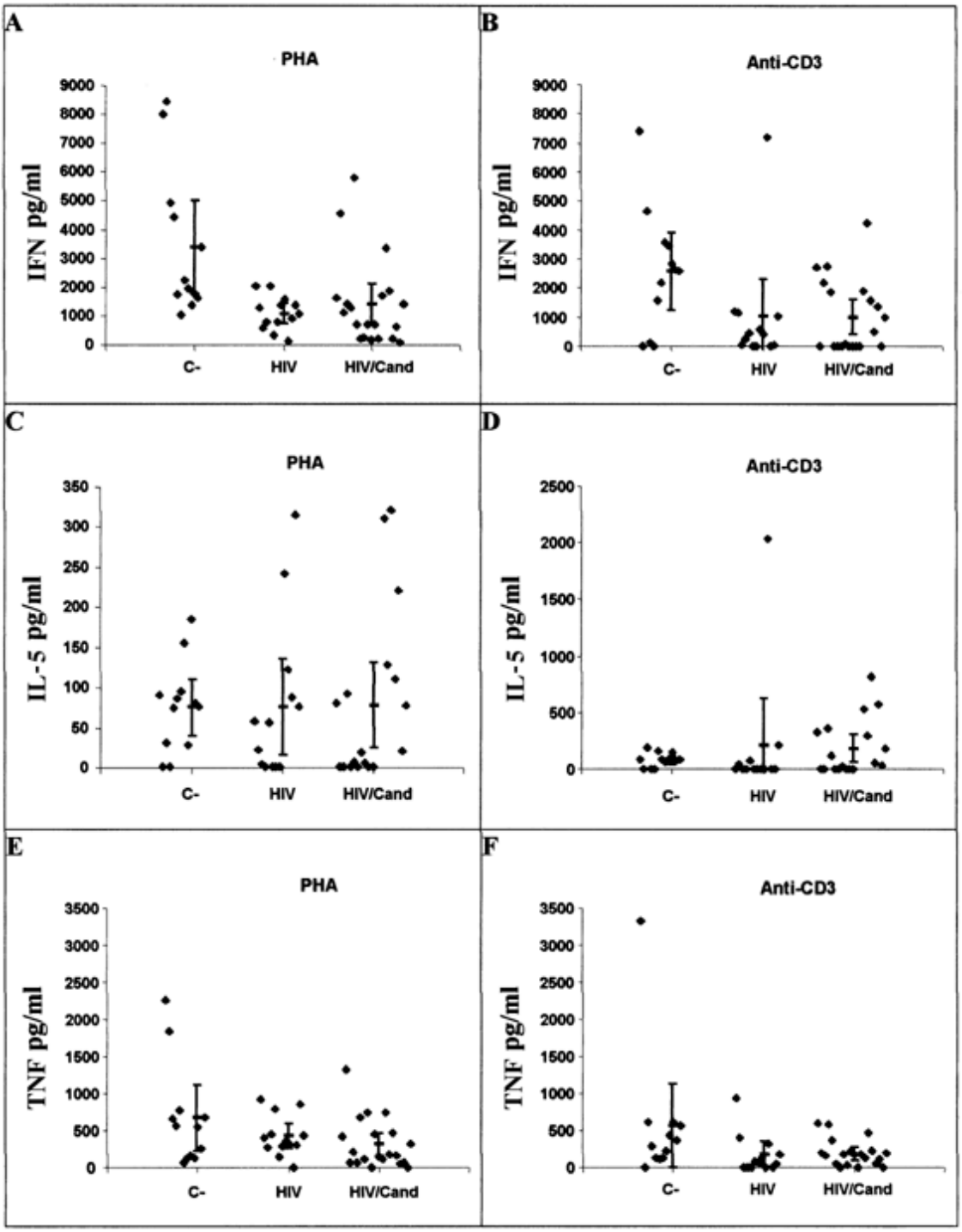


Figure 2A and 2B

IFN-gamma production by activated T cells.

T cells were activated with PHA or anti-CD3, and the amount of IFN-gamma released to the supernatants evaluated by specific ELISA, 72 hours later.

Figure 2C and 2D

IL-5 production by activated T cells.

T cells were activated with PHA or anti-CD3 (lower panel), and the amount of IL-5 released to the supernatants evaluated by specific ELISA, 72 hours later.

Figure 2E and 2F

TNF-alpha **production by activated T cells.**

T cells were activated with PHA or anti-CD3, and the amount of TNF-alpha released to the supernatants evaluated by specific ELISA, 72 hours later.

C, healthy control patient; HIV, HIV-1-infected asymptomatic; HIV/Cand, HIV-1-infected suffering from *Candida* infections.

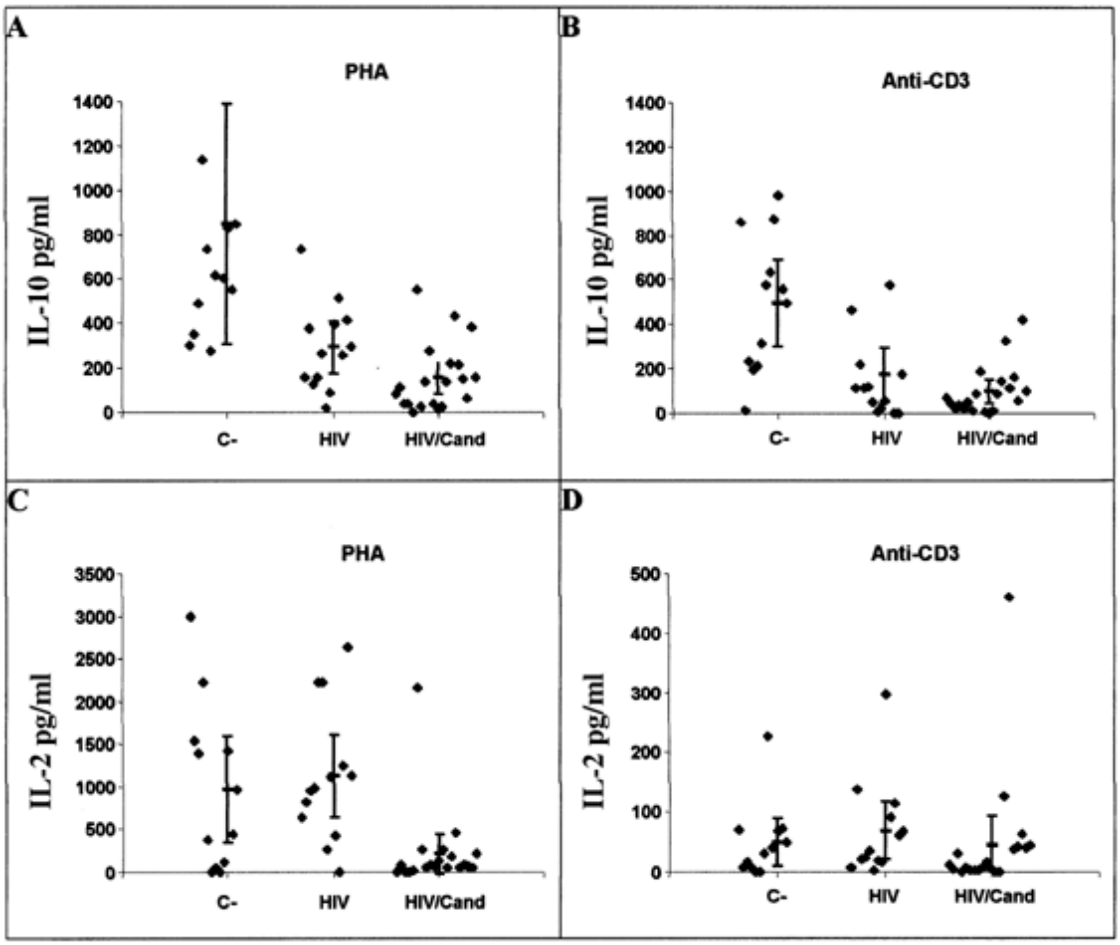


Figure 3A and 3B

IL-10 production by activated T cells.

T cells were activated with PHA or anti-CD3, and the amount of IL-10 released to the supernatants evaluated by specific ELISA, 72 hours later.

Figure 3C and 3D

IL-2 production by activated T cells.

T cells were activated with PHA or anti-CD3, and the amount of IL-10 released to the supernatants evaluated by specific ELISA, 72 hours later.

C, healthy control patient; HIV, HIV-1-infected asymptomatic; HIV/Cand, HIV-1-infected suffering from *Candida* infections.

Table 1
Clinical and biological characteristics of patients

Group	CDC Clasif.	Treatment	CD4[a] Cells/mm^3 (range)	CD8[a] Cells/mm^3 (range)	Viral load[b] range
Control [12]	–	NO	1,241 ± 279 (890-1421)	720 ± 291 (451-899)	–
HIV [12]	A	5 (NO) 7 (YES)	468 ± 221 (301-803)	1,074 ± 325 (830-1,214)	10,500 ± 5,340 (0-21,400)
HIV/Cand [21]	A1 (3) B1 (3) C2 (4) C3 (11)	3 (NO) 18 (YES)	184 ± 87 (11-531)	345 ± 149 (114-461)	38,720 ± 40,320 (3,200-218,000)

[a] Mean values ± standard deviation and range.
[b] Mean viral copy number ± standard deviation.

Table 2
T cell activation status of the HIV patients

	PHA response			Anti-CD3 response		
	Control	HIV Asymp	HIV /Cand	Control	HIV Asymp	HIV /Cand
Proliferation[a]	69,134	45,836 $p_1 = 0.038$	31,407 $p_2 = 0.001$ $p_3 = 0.062$	39,473	19,018 $p_1 = 0.094$	21,661 $p_2 = 0.043$ $p_3 = 0.392$
CD69[b]	67	80 $p_1 = 0.382$	62 $p_2 = 0.458$ $p_3 = 0.021$	53	34 $p_1 = 0.016$	42 $p_2 = 0.339$ $p_3 = 0.586$
CD25[b]	75	72 $p_1 = 0.554$	50 $p_2 = 0.016$ $p_3 = 0.006$	58	25 $p_1 = 0.001$	34 $p_2 = 0.003$ $p_3 = 0.444$
CD71b[b]	43	53 $p_1 = 0.310$	33 $p_2 = 0.241$ $p_3 = 0.011$	38	18 $p_1 = 0.082$	31 $p_2 = 0.120$ $p_3 = 0.879$
IFN-γ[c]	3,413	1,096 $p_1 = 0.002$	1,400 $p_2 = 0.003$ $p_3 = 0.734$	2,576	1,025 $p_1 = 0.088$	1,005 $p_2 = 0.022$ $p_3 = 0.832$
IL-5 [c]	75	76 $p_1 = 0.525$	78 $p_2 = 0.487$ $p_3 = 0.879$	81	217 $p_1 = 0.152$	186 $p_2 = 0.890$ $p_3 = 0.286$
TNF[c]	671	427 $p_1 = 0.796$	315 $p_2 = 0.112$ $p_3 = 0.152$	568	182 $p_1 = 0.065$	193 $p_2 = 0.103$ $p_3 = 0.395$
IL-10[c]	848	295 $p_1 = 0.004$	153 $p_3 < 0.001$ $p_3 = 0.043$	495	175 $p_1 = 0.013$	98 $p_3 < 0.001$ $p_3 = 0.228$
IL-2[c]	960	1,123 $p_1 = 0.695$	211 $p_2 = 0.064$ $p_3 < 0.001$	49	68 $p_1 = 0.449$	46 $p_2 = 0.250$ $p_3 = 0.059$

p_1 = Statistical differences *versus* control group.
p_2 = Statistical differences between control group.
p_3 = Statistical differences between HIV/ Asymptomatics.
[a] Mean proliferation values expressed as counts per minute of (^{3}H) thymidine incorporation.
[b] Mean cell surface expression expressed as percentage of positive cells.
[c] Mean cytokine production expressed as pg/ml.

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Candida, HIV, cytokine, phagocytes, polymorphonuclear, T cell.

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antioxidant, airway epithelium, cytokine, HIF-1alpha, IL-1beta, mitochondria, oxygen sensing, reactive oxygen species.

Thioredoxin specifically cross-desensitizes monocytes to MCP-1

S. Pagliei, P. Ghezzi, C. Bizzarri, V. Sabbatini, G. Frascaroli, S. Sozzani, G. Caselli, R. Bertini

thioredoxin, monocytes, MCP-1.

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