

IL-2 AND IFN- γ , BUT NOT IL-4 SECRETION BY PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) ARE RELATED TO CD4+ T CELLS AND CLINICAL STATUS IN BRAZILIAN HIV-1-INFECTED SUBJECTS

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SUMMARY

It has been reported that production of IL-2 and IFN- γ , known as T-helper type 1 cytokines, by peripheral mononuclear cells (PBMC) decreases with progression of HIV infection. In contrast, IL-4 and IL-10 production, Th2 cytokine profile, increases with HIV disease progression. PBMC were evaluated from 55 HIV-infected subjects from Divisão de Imunologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, to "in vitro" cytokines production after 24 hours of stimulation with PHA. Low levels of IL-4 production in both HIV- infected patients and normal subjects, were detected. The patients with CD4+ T cell counts <200 showed a significant decrease of IL-2 and IFN- γ production compared to controls. Patients with higher counts of CD4+ T cells (either between 200-500 or >500 cells/mm³) also showed decreased production of IL-2 that was not statistically significant. There was a correlation between IL-2 and IFN- γ release with CD4+ T cells counts. HIV-1-infected individuals with CD4+ T cells >500 cells/mm³ showed increased levels of IL-2 and IFN- γ , than individuals with CD4+ T cells <500 cells/mm³. In conclusion, we observed a decline of IL-2 and IFN- γ production at advanced HIV disease. IL-4 production was not affected during HIV infection. Taken together, these findings suggest that the cytokine profile might be influenced by the HIV infection rather than the cause of disease progression.

KEYWORDS: HIV infection; Th1; Th2; interleucin (IL)-2; interferon- γ ; IL-4.

INTRODUCTION

Multiple mechanisms have been suggested to explain the progressive immune dysfunction during HIV-1 infection, including the impairment of the cytokine production⁸. It has been reported that phytohemagglutinin (PHA) induced production of IL-2 and IFN- γ , known as T-helper type 1 (TH1) cytokines, by peripheral blood mononuclear cells (PBMC) decreases with progression of disease, whereas IL-4 production, a Th2 cytokine, increases with HIV disease progression. It was also suggested that a Th1 cytokine profile might be related to a delay in disease progression^{8,9}.

Although some immunological parameters in HIV-1 infected individuals from Brazil have already been described⁶, no studies on the cytokine profile of Brazilian HIV-1-infected patients have been reported to date. The purpose of the results reported was to determine the cytokine profile in Brazilian patients in different phases of HIV infection.

MATERIALS AND METHODS

Patients and Controls. Fifty-five HIV-1-infected patients, from the Divisão de Imunologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, were evaluated. The subjects were classified according to the Centers for Disease Control (CDC, Atlanta, 1986) criteria and number of T CD4+ cells (CDC, 1993)⁷. All participants gave informed consent. Ten healthy volunteers from the laboratory staff were evaluated as control group.

Peripheral blood mononuclear cell cultures. PBMC were purified by Ficoll-Hypaque gradient, and adjusted to 2×10^6 of PBMC/mm³ in RPMI supplemented with 10% FCS. PBMC were stimulated with PHA for 24 hours and the supernatant fluids harvested, frozen at -70 °C until assayed for cytokines. Cytokines (IL-2, IL-4 and IFN- γ) were measured using commercial EIA kits (Endogen, Cambridge, MA, USA) following the manufacturer's recommendations. PBMCs from healthy HIV-1-seronegative individuals matched by age were used as controls in all experiments.

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CD4+ cells count. The CD4+ T cells counts in PBMC were done by indirect immunofluorescence using a mouse monoclonal antibody (Dakopatts, Glostrup, Denmark) against the cell surface molecule CD4 as described earlier¹⁷.

Statistical analysis. Statistical analysis was done using Kruskal-Wallis test with Dunn's post-test to compare groups and IL-2 and IL-4 *in vitro* production and one way analysis of variance to calculate IFN- γ secretion. Spearman's rank correlation test was used to analyze the relationship between the CD4+ cell count and cytokine production.

RESULTS

The mean (\pm SD) of age of the 55 HIV-1-infected patients was 33 ± 7 years old. Forty-two were contaminated through sexual route (26 men who had sex with men, 16 individuals were heterosexuals), 5 were intravenous drug users, and 8 individuals whose transmission route was unknown. Mean (\pm SE) CD4+ T cell counts were 315 ± 55 cells/mm³, 407 ± 37 and 874 ± 115 cells/mm³ for AIDS patients, asymptomatic individuals and healthy controls, respectively. AIDS (n=20) and asymptomatic (n=41) patients showed low levels of IL-2 production following PHA stimulation as compared to HIV-non-infected controls, but this difference was only statistically significant for AIDS patients ($p < 0.05$) (Table 1). However, IFN- γ production was similar between controls and AIDS patients, while HIV-1-asymptomatic individuals presented higher levels of IFN- γ compared to AIDS patients and HIV-non-infected subjects ($p < 0.05$, for both comparisons) (Table 1). We were not able to detect significant differences in IL-4 production, since levels in most patients and controls were near or under the detection limit of the assay used.

When we evaluated the results from patients grouped according to their CD4+ T cell count, we observed that patients with CD4+ T cells counts < 200 (n=16) showed a significant decrease of IL-2 and IFN- γ production compared to controls. Patients with higher counts of CD4+ T cells (either between 200-500 or > 500 cells/mm³) also showed decreased production of IL-2 that was not statistically significant (Table 1).

Additionally, we observed that there is a correlation between IL-2 and IFN- γ release with CD4+ T cells counts ($p = 0.005$ and $p = 0.02$, respectively). Therefore, HIV-1-infected individuals with CD4+ T cells > 500 cells/mm³ showed increased levels of IL-2 and IFN- γ , than individuals with CD4+ T cells < 500 cells/mm³ (Figure 1).

DISCUSSION

Some investigators have suggested that the TH1 cytokine profile, characterized by increased levels of IL-2 and IFN- γ production may be associated with a delay in disease progression^{5,8,9,10}. In contrast, the presence of increased levels of IL-4 and IL-10, associated with a TH2 cytokine profile, might lead to higher susceptibility to opportunistic infections and disease progression. In this study, HIV-1-asymptomatic subjects produced increased levels of IFN- γ compared to AIDS patients or to uninfected individuals. Also,

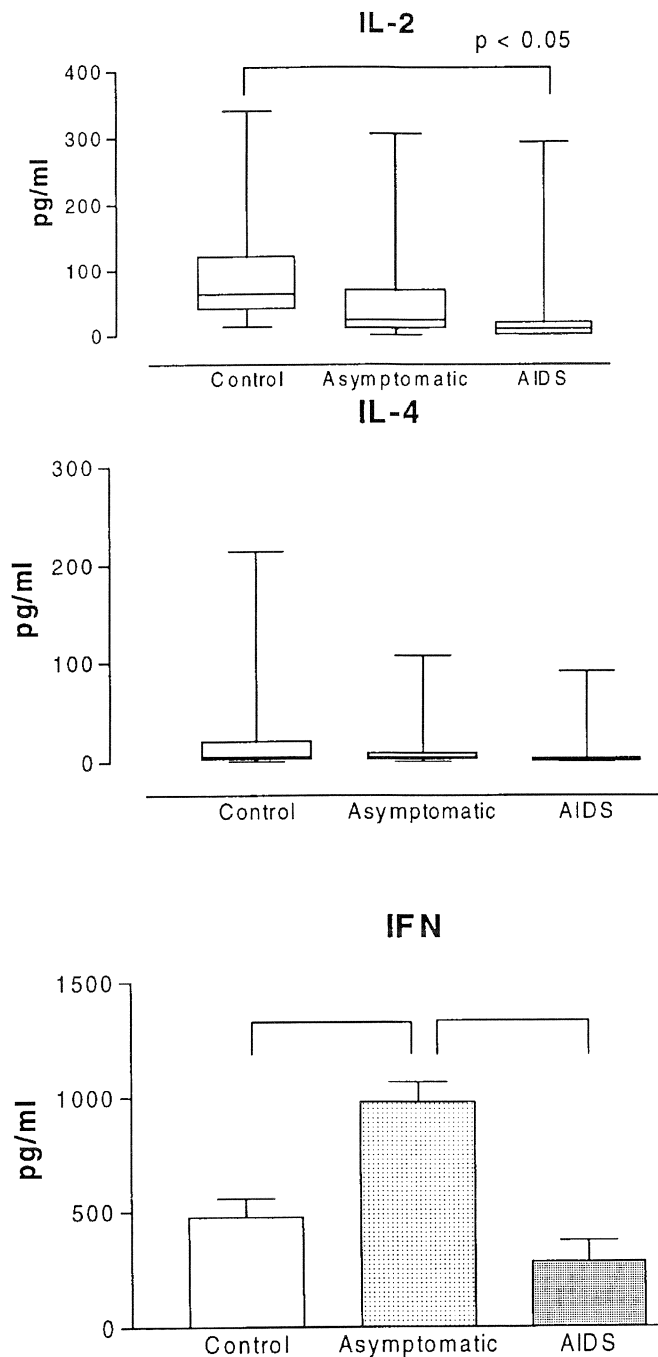


Fig. 1 - Cytokine production by PBMC from HIV-1-infected patients according to clinical status (CDC, 1988)

patients with AIDS produced IFN- γ at the same level than healthy control subjects. It seems IFN- γ production was not decreased during the late stage of HIV infection. It may be due to T CD8+ cells production, as shown previously^{5,12}.

In this study, we detected very low levels of IL-4 production in both HIV- infected patients and normal subjects, a finding similar

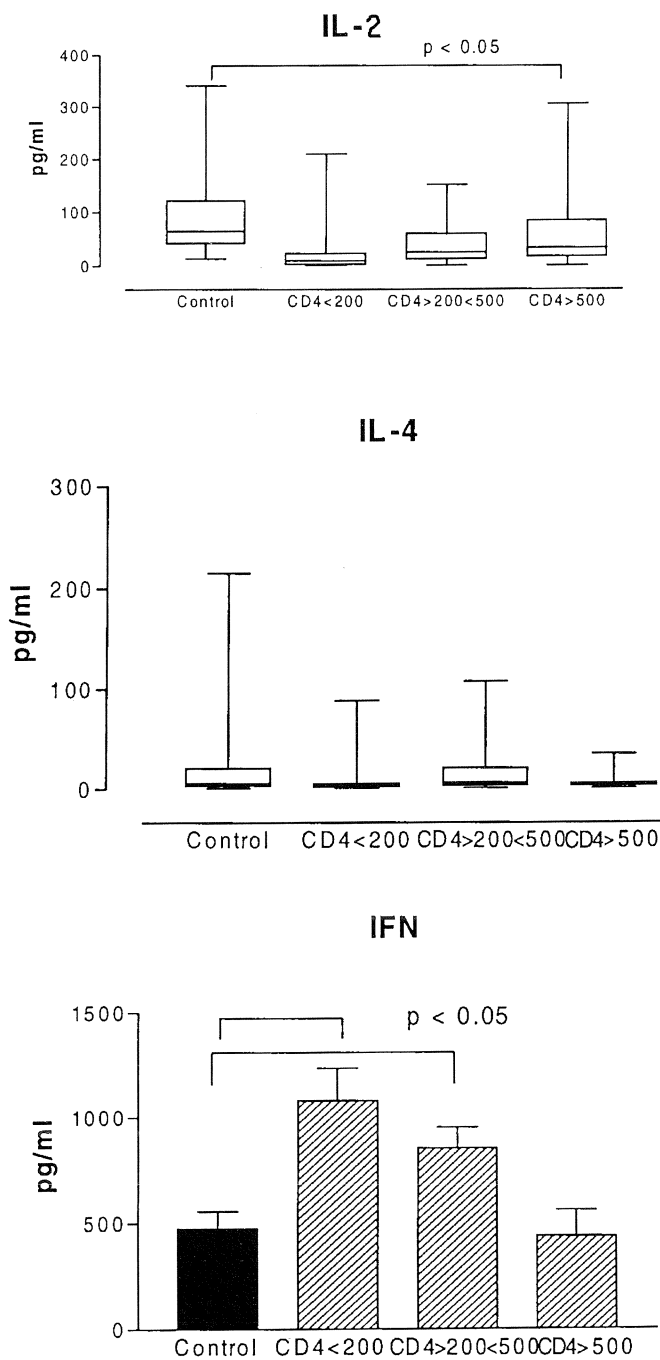


Fig. 2 - Cytokine production by PBMC from HIV-1-infected patients according to CD4+ T cells count

to those previously reported¹². However, other groups have shown increased IL-4 production in infected patients along with disease progression^{11,13,14}. Furthermore, we also tested supernatant of 48-72 hours with PHA incubation and IL-4 release was not detected (data not shown). Therefore, our finding favor the hypothesis that increased production of IL-4 is not a marker of the Th2 patterns during HIV disease progression.

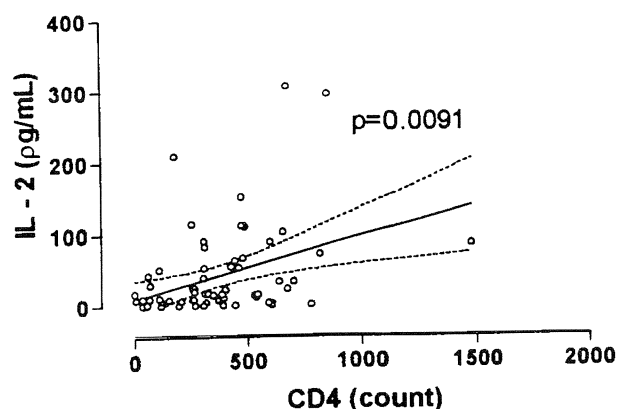


Fig. 3 - Correlation between IL-2 production and CD4+ T cells counts

One possible explanation for the impairment of the production of some cytokines, such as IL-2 and IFN- γ , but not IL-10, would be functional cell subset abnormalities seen CD4+ and CD8+ T cells, caused by the virus. However, other cell types, such as monocytes, which are only markedly affected in the late stages of HIV infection might be responsible for the higher IL-10 levels observed^{2,15}. Thus, the reduction in IL-2 and IFN- γ secretion may be rather the consequence of the progressive immunologic dysfunction caused by the infection rather than that a causative factor in disease progression^{2,4}.

Recently, significant advances in therapeutic approaches to HIV/AIDS have been made, such as the use of cytokines in order to reconstitute the immune system or the use of the combined antiretroviral therapy^{1,16}. Therefore, in vitro measurement of cytokines may be of considerable importance clinical follow-up of HIV-infected patients.

RESUMO

Secreção de IL-2 e IFN- γ , mas não de IL-4 por células mononucleares do sangue periférico (CMN) são relacionadas à contagem de linfócitos T e ao estadiamento clínico em indivíduos brasileiros infectados pelo HIV-1

É relatado que a produção de IL-2 e IFN- γ , conhecidas como citocinas T-auxiliador tipo 1, pelas células mononucleares do sangue periférico fica deprimida no decorrer da infecção HIV. Por outro lado, a produção de IL-4 e IL-10, chamada padrão T-auxiliador tipo 2, aumenta com o avanço da doença (AIDS). Nesse estudo, foram avaliados 55 indivíduos infectados pelo HIV-1 em acompanhamento no Ambulatório de Imunodeficiências Secundárias do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. Células mononucleares foram estimuladas "in vitro" com fitohemaglutina (PHA) por 24 horas e o sobrenadante foi utilizado para a dosagem de citocinas através de kits de ELISA disponíveis comercialmente. Foi observado que a produção de IFN- γ pelos indivíduos assintomáticos HIV+ está aumentada quando comparada aos controles não infectados pelo HIV, enquanto os pacientes com AIDS

tiveram produção similar aos controles. A produção de IL-2 foi diminuída nos pacientes HIV+, porém a diferença não foi estatisticamente significativa quando comparada aos indivíduos controles. Essa produção diminuída das duas citocinas foi relacionada com a queda dos linfócitos T CD4+, onde pacientes com >500 cells/mm³ mostraram níveis aumentados quando comparados aos indivíduos com contagem abaixo <500 cells/mm³. A produção de IL-4 não foi alterada no decorrer da infecção HIV. Esses achados sugerem que o perfil de citocinas pode ser um efeito que ocorre durante a infecção HIV, e não à causa da progressão.

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