

RESEARCH NOTE

## Cytokine Profile and Natural Killer Activity among Brazilian HIV-1-Infected Subjects

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The immune system is progressively affected during HIV infection. Several mechanisms have been described regarding the HIV/AIDS immunopathogenesis, some related to the humoral response and others to the cellular immune response. The changes in the pattern of cytokine production by peripheral blood mononuclear cells (PBMC) have been particularly studied in order to better understand the immunoregulation during HIV disease progression.

It has been reported by some authors that PBMC production of interleukin (IL)-2 and interferon gamma (IFN- $\gamma$ ), known as T-helper type 1 cytokines, decreases with progression of HIV infection (M Clerici et al. 1993 *Immunol Today* 14: 107-111, E Maggi et al. 1994 *Science* 265: 244-248). In contrast, IL-4, IL-5 and IL-10 production, which characterizes the Th2 cytokine profile, increases with HIV disease progression (Clerici et al. 1993 *loc. cit.*, M Clerici et al. 1996 *AIDS Res Hum Retrov* 12: 1053-1061). However, this matter is still controversial since C Graziosi et al. (1994 *Science* 265: 248-252) could not describe this shift in the cytokine secretion. These authors reported low levels of IL-2 and IL-4 production, but high

levels of IL-10 and INF- $\gamma$  in patients in different stages of the disease.

We investigated the cytokine secretion patterns of PBMC from a series of Brazilian HIV infected patients in response to the mitogen phytohemagglutinin (PHA). We collected supernatants of BMC cultures from a total of 61 HIV-patients followed at the Immunology Division, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (Hong et al. unpublished results). Cytokine levels were measured by commercial EIAs.

Similarly to data from Graziosi et al. (*loc. cit.*), we were not able to verify a clear Th1 to Th2 pattern shift in our patients. However, we could observe a decrease in the production of Th1 cytokines, namely IL-2 and INF- $\gamma$  that paralleled the fall in the immune status of the patients as assessed by T CD4+ cell counts. Typical Th2 cytokine production, like IL-4, did not increase with disease progression. This cytokine was almost always detected at very low levels, in patients and controls, similarly to data from Graziosi et al. (*loc. cit.*). Our results, therefore, point out the suggestion that many of the observed changes in the immune parameters are rather the consequence than the cause of the disease progression and immune destruction by the infection.

This suggestion is partially reinforced by our results of IL-10 production. This cytokine was increasingly produced in HIV infected patients. However, we interpret this result not as a shift to a Th2 pattern, but as an increased production of this cytokine by monocytes/macrophages, probably secondary to the enhanced immune activation status of HIV infected patients. Thus, monocytes and macrophages would be responsible for the major part of the synthesis of this cytokine, since they are severely affected only in late phases of the disease (TR Mosmann & KW Moore 1991 *Immunol Today* 12: 49-53, E Hagiwara et al. 1996 *AIDS Res Hum Retrovir* 12: 127-133). Indeed, we also observed an increased spontaneous IL-10 production by PBMC from patients compared to controls. In addition, we routinely detect high serum levels of immunoglobulin A in our patients (LFM Brígido 1990, *Imunodisfunção Associada à Infecção pelo Vírus da Imunodeficiência Humana*, Faculdade de Medicina da Universidade de São Paulo, São Paulo, 75 pp., F Cavallin 1996 *Clin Immunol Immunopathol* 81: 224-228), an immunoglobulin class whose synthesis is also driven by IL-10.

In this regard, we also evaluated another important cell subset in HIV infection, the NK cell subset, that presumably is preserved during disease progression (DE Nunes et al. unpublished results). These

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cells present broad specificity, rapid activation and dual role in cell-mediated cytotoxicity and lymphokine production. In contrast to lymphocytes involved in specific (acquired) immunity, i.e., T and B cells, NK cells possess an apparently innate ability to respond to tumors and intracellular pathogens, such as viruses (G Trinchieri 1989 *Adv Immunol* 47: 187). There are controversial reports on the role of the NK cells in the HIV/AIDS pathogenesis. Some investigators reported that NK cells did not show any protective effect during HIV disease progression (Q Cai et al. 1990 *J AIDS* 3: 669-676) and others have shown that NK cells may represent a protective tool to avoid HIV infection or disease progression (PF Hu et al. 1995 *J AIDS* 10: 333-340, B Lucia et al. 1995 *Cytometry* 22: 10-15).

We evaluated NK cell activity from 41 HIV infected-patients against the murine mieloma derived cell line K562 labelled with Chromium 51, as described by Cai et al. (*loc. cit.*). Our results showed that only patients with advanced disease presented a decrease in the NK cell activity.

Literature in this subject is also conflicting. Data for both early and delayed compromise of NK cell function has been presented (Hu et al. *loc. cit.*, H Ullum et al. 1995 *J Exp Med* 182: 789-799, Lucia et al. *loc. cit.*). An interesting additional observation, although still preliminar, was made from the assays with cells from patients in advanced dis-

ease but on regular antiretroviral therapy. Compared to those not taking this medication, there was a clear improvement of the NK function. This observation argues for a role of these cells in disease progression. A similar observation was made by DT Harris et al. (1987 *J Immunol* 138: 889-894) showing that a decrease in viral load was associated with improvement in NK cell activity. This hypothesis will now be tested, by studying patients on more effective antiretroviral therapies with concomitant dramatic decreases in the viral load.

The most plausible explanation for the depression in NK cell activity in late disease may relate to some cytokines, such as INF- $\alpha$ , that play a role in the regulation of this activity. In fact, INF- $\alpha$  is important in the activation of NK cells, and its production also is only affected in the late phase of the disease (DM Howell et al. 1993 *J AIDS* 6: 15-23).

In conclusion, our results indicate that the monitoring of *in vitro* PBMC cytokine production may serve as a parameter of disease progression, since it reflects the evolution of the disease itself, rather than explain its immunopathogenesis. Additionally, we also believe that the role of other immune cell functions, like NK cell activity, deserve further studies in order to better understand the progression of the HIV infection.

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