Immunovirological parameters and cytokines in HIV infection

Karen Ingrid Tasca[1], Sueli Aparecida Calvi[1] and Lenice do Rosário de Souza[1]

[1]. Departamento de Doenças Tropicais, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, SP.

ABSTRACT

Although modern combined antiretroviral therapies (cART) result in lower morbidity and mortality and a visible improvement of clinical and laboratory parameters in HIV-infected, it is known that their long-term use contributes to appearance of the many events unrelated to AIDS such as cardiovascular diseases, cancer and osteoporosis, comorbidities which have been proposed as some of the most important that deprive the majority of infected to present an even better prognosis. This is because even with a decrease in inflammation and immune activation after drug intervention to the patient, these parameters remain higher than those shown by healthy individuals and the imbalance of cytokine profiles also persists. Therefore, evaluations of other biomarkers in clinical practice are needed to complement the exams already carried out routinely and allow more effective monitoring of HIV patients. This review aims to investigate the role of cytokines as potential markers showing studies on their behavior in various stages of HIV infection, with or without cART.

Keywords: Cytokines. HIV/AIDS. Antiretroviral therapy. Clinical stages of infection.

GENERAL CHARACTERISTICS OF HIV INFECTION

The principal damage caused by the human immunodeficiency virus (HIV) is the progressive deterioration of the cellular immune system that leads to severe immunodepression and renders the individual more susceptible to opportunistic diseases or certain types of neoplasms. Several cell types are susceptible to the virus, such T lymphocytes and some antigen presenting cells (APC) due the presence of cluster of differentiation 4 (CD4+) on its surface, which allows the entry of the virus into host cell.

The infection is characterized by an acute or primary phase, in which a higher viremia peak occurs and a chronic phase, established firstly by the control of infection or what is denominated clinical latency or asymptomatic phase, followed by a symptomatic phase in which intense depletion of CD4+ T lymphocytes and a continual increase of plasmatic ribonucleic acid-human immunodeiciency virus (RNA-HIV) viral load (VL) are observed until the moment that opportunistic infections appear, characterizing the acquired immunodeficiency syndrome (AIDS), the aforementioned disease.

In untreated individuals the average time of development of AIDS is 10 years in typical progressores (TyP), although it can develop in 3 to 6 months in some rapid progressors. A minority of individuals infected by HIV, denominated long term non-progressors (LTNP) remain healthy for more than 10 years without clinical evidence of disease progression in the absence of combined antiretroviral therapy (cART). These cases, represented by approximately 5% to 15% of those infected are characterized by stability or increase in CD4+ T count and strong response of cytotoxic T lymphocytes (CTL) against HIV, different to what occurs in TyP.

The evolution in the therapeutic efficacy occurred principally after the introduction of a highly active cART that promotes the VL reduction to undetectable levels in peripheral blood. Even with the emergence of this therapy, the virus persists in organism reservoirs, mainly integrated into the genome of non-activated T lymphocytes and other latent cells, which leads to the unviability of discontinuing the treatment. Furthermore, the emergence and velocity in the appearance of resistant strains to different drugs combination available on the market constitute a limiting factor in controlling the infection.

Nevertheless, the great research challenge is to discover possible targets that effectively disrupt the HIV cycle without causing damage to the normal cell, since several studies have demonstrated great impairment in the organism of patients due to cytotoxicity caused by these drugs. For this reason the investigation of immunology-based therapies that allow the host to maintain control of the infection should be encouraged. In this sense cytokines arise as a one of the principal targets for an immunomodulatory intervention, or, according to Imami et al, the determination of the cytokine profile in patients HIV seropositive, when properly designed could complement the data from VL and CD4+ T count in the analysis of the disease status, thus aiding in the decision among possible therapeutic interventions.

Some studies suggest that early initiation of cART in HIV infection can reduce the viral reservoir and thus diminish its transmission. However, early intervention has not shown substantial long-term clinical benefits. Given the evidence of longer survival, temporal studies on the causes of death by AIDS, in the pre- and post-cART periods have evidenced that special attention must be given to the prevention of chronic diseases, which arise from the prolonged use of CART such as weight gain, metabolic syndrome, lipodystrophy, insulin resistance, diabetes and obesity, which are risk factors for a series of new comorbidities. Conclusions of a study by Cozzi-Lepri et al. call attention to the significant association between patients that developed non-AIDS comorbidities and the presence of soluble inflammatory markers including interleukin (IL)-6, high sensitivity C-reactive protein and D-dimer, indices that are presented elevated even after years of effective treatment. Although the administration...
of cART is able to reduce these biomarkers levels, it is necessary to observe failures in the normalization of these inflammatory parameters despite prolonged treatment, to understand the long-term prognosis.

**CONSTANT AND INTENSE INFLAMMATORY STATUS IN BOTH INITIAL AND LATE INFECTION WITHOUT cART**

According to Keating et al. in 2012, it is known that HIV causes a profound disruption in the cytokine network from the earliest points of HIV infection, shortly after first detection of systemic virus. Therefore, in the complex cytokine network the balance between viral suppressors, such as type I interferons (IFN-α, IFN-β and IL-10) and the viral inductors, including IL-6, IL-15, IL-1β and tumoral necrosis factor (TNF-α) contribute to establishing in each individual, an equilibrium point of viral replication that is determinant for the prognosis of AIDS. For example, an in vitro study revealed that several cytokines with inductor effects of viral replication can be mediated, as in the case of TNF-α, by activating the binding of kappa B nuclear transcription factor (NF-kB) to the LTR region (long terminal repeat) of the virus. For this reason the augmented production of TNF-α was verified in HIV patients with and without treatment in comparison to those seronegative, but this difference was greater in treatment naïve patients, probably due the inadequate viral suppression. The TNF-α is a potential inflammatory cytokine, such IL-1β and IL-6 and the first and last exhibit synergistic effects. Relative to this, it is clear that the rise in serum levels of pro-inflammatory and inflammatory cytokines contributes to viral replication and many manifestations of immunodeficiency. Others cytokine production can increase viral replication, due the activation role in HIV infected cells, including IL-2, IL-4 and interferon type II (IFN-γ) which, primarily, are required in the support to expansion of antiviral T cells and antibody responses.

During the acute phase of HIV-1 infection, it was showed that elevated plasma levels of inflammatory cytokines are associated with viremia peaks and can even estimate the time at which the CD4+ T count will drop below 350 cells/mm³. Thus it would be important low levels of these cytokine in this critical phase. Some authors reported that in this initial phase occurs an increase of cells producing IFN-γ, a potent antiviral cytokine, as well as the increase on this cytokine in serum, while the frequency of IL-4 producing cells and the amount of the cytokine secreted are decreased. This cytokine displays both stimulatory and inhibitory effects on HIV replication and leads to Th2 profile. Beyond IFN-γ, Noris et al. demonstrated significant initial increases of TNF-α and IL-10, while Rychert et al. evaluated the correlation of plasma gp120 with change in the cytokine profile and found that plasma gp120 levels were related to the increase of TNF-α, IL-10 and IL-6. These last studies reinforce the importance of low viremia peak for the infection being directed to a better profile. In addition, a review by Katsikis et al. showed the evidence that viral set-point can be manipulated by cytokine interventions during acute infection by the simian immunodeficiency virus (SIV) and HIV and they raised the question: would be possible use of cytokines through immunotherapeutics and potentially vaccines to alter viral set-point during acute HIV infection? It is an interesting question that deserves to be studied, once the cytokines can both change and/or be changed during acute HIV infection? It is an interesting question that deserves to be studied, once the cytokines can both change and/or be changed.
less inflammatory cytokines with regard to HIV-1-infected progressors. TNF-α levels were significantly increased in both the TyP and rapid progression groups compared with the LTNP group in a study release in naive patients from Africa. Differently from typical progressors, those patients that can naturally control the infection are able to maintain highly functional HIV-specific CD8+ T cells and the author highlighted that the quality from these cells is more important than quantity of them to a slower disease progression. Another difference between TyP and LTNP was documented by Li et al. in Chinese CART naive. In vitro, the activation levels of CD4+ T cells on first group was higher than that presented in second group, and it was direct correlated with VL and indirectly correlated with T CD4+ counts. In the same study, the expression of Th1 cytokines and the frequency of Th1 cells were lower in TyP than the other group, showing that the LTNP maintain the control of infection progression due the less activation and Th1 cells conservation. It is therefore important that the immune system cells preserve the specificity and polyfunctionality and be able to balance the cytokines production to provide a favorable environment for viral clearance.

**PERSISTENT INFLAMMATORY STATUS AFTER cART INITIATE**

According to many authors such Hunt et al., French et al., Nixon et al. and Kamat et al., persistent chronic inflammation and immune activation are factors potentially determinant of morbidity and mortality not associated with AIDS, even during effective cART. Thus, it is necessary that these parameters be minimized to augment the patient survival. In this manner the direct or indirect dysregulation in the cytokine network produced due the HIV infection have important consequences from an immunological and clinical perspective.

When analyzing studies with cARV and cytokines, Gay et al. found minimal effects from medications on the levels of cytokines and chemokines after 16 to 24 weeks of treatment. Some authors did not observe an influence of cARV on circulating levels of IL-6 and reactive protein C, while others associated the decrease of IL-1β, IL-6 and TNF-α after initiating cART. Sachdeva et al. in 2010, found similar results in serum levels of IL-1β, IL-6, TNF-α, IFN-γ and IL-12, which decreased after 6 months of cART, but some of these values were still higher than those of control individuals. Still, in a study by Haissman et al., 4 months after cART initiation there was a statistically significant reduction, mainly to the TNF-α, IL-1ra and IL-6 in individuals with elevated CD4+ T, and these values were similar to those found in the group not infected by HIV. It is noted that the results of cART in serum cytokine level are conflicting. Probably this divergence of data in cytokines studies can be attributed to the various factors responsible for not restore a complete immune response despite cART, as late infection stage to initiation of therapy, old age of onset therapeutic, low T CD4+ nadir, residual viremia results from cellular reservoirs, slow time to viremia became undetectable and poor therapeutic adherence.

It was demonstrated by Haissman et al. that the progression of the disease exerts an effect on the levels of TNF-α, IL-6, IL-1ra and IL-8, which were significantly higher in the patient group with CD4+ T counts lower than 200 cells per mm³. In multivariate analysis adjusted for sex and age, high VL levels predicted high levels of TNF-α, IL-1 receptor antagonist (IL-1ra) and IL-8 and when were utilized univariate analysis, the pro-inflammatory cytokines were positively correlated with VL and, TNF-α, IL-8, IL-6 e IL-1ra were negatively correlated with the CD4+ T count, with the exception of IL-10. The latter finding did not concur with a study by Sachdeva et al., which reported a negative correlation between CD4+ T and IL-10, both pre- and 6 months after cART, in addition to a positive correlation with IL-2 measured prior to CART. By observing only the behavior of cytokines in the presence of CART at 6 months, the last authors found no difference between IL-10 levels in the treated group which presented variable responses to ARV. In contrast, Resino et al. observed in children a greater IL-10 production in the treated group when compared to the subjects not treated with cARV, results contrary even to those found by other authors in adults, who observed a decrease of this cytokine upon initiation of cART. It is important to establish the role of IL-10 in the infection, given that some studies indicate the existence of a relationship between the high production of IL-10 and slower disease progression or your importance to control the inflammation and maybe, leading to an effectiveness enhance of antiretroviral treatment over time.

An analysis of the cytokine profile at the peak moment of VL in the primary infection and another peak, after the interruption of treatment, showed significantly higher levels of IFN-γ, TNF-α and IL-10 in the primary infection. The analysis of Cozzi-Lepri et al. in 2011 also demonstrated a high correlation between VL increase, following treatment interruption, versus a decline of CD4+ T and increases in the production of IL-6, TNF-α and IL-10 by macrophages and/or monocytes. This suggests that the effect of HIV replication in these cells can constitute a critical pathway of HIV infection mediated by immune activation and inflammation, as well as a consequent immunological deficiency. Nevertheless, under drug compliance and a good response to therapy, studies have shown that the impact of CART in immune reconstitution is closely related to the reduction of immunological activation due to a decline in VL.

Still along the same lines, under the medication influence on the cytokine profiles, Meira et al. observed that the IL-2 level in peripheral blood mononuclear cells (PBMC) of individuals infected by HIV-1 treated with ARV remained low even after years of therapy, and that the average of IFN-γ was higher than in control individuals, with Th0 being the dominant profile in these patients. Other authors observed an unbalance between Th1 and Th2, with the involvement of Th2-profile cytokines in late stages of HIV infection, in which reverse dysregulation of cytokines occurred in patients with CART and the Th1 profile becomes highlight. On the other hand, this condition is only partially reversed and after years of therapy it is lost again, which leads to faster progress to AIDS. Whence, the type 1 cytokines were associated with protection against infection and constituted the objective in vaccination strategies or therapeutic intervention with immunomodulators.

In relation to IL-18, a pro-inflammatory cytokine that induces IFN-γ production in T cells and enhances NK cytotoxic activity, high levels of this cytokine have been related to the symptomatic condition of individuals in comparison to asymptomatic ones and controls and which could also be to disease progression, regardless of cARV. Treated patients presented lower levels of IL-18 than no treated, increased values have been found in late stages and its plasma levels were directly correlated with VL, constituting a really good marker for disease progression.
TH17: PROMOTION OF HOMEOSTASIS OR INFLAMMATORY STATUS?

As seen previously, infection by HIV-1 is characterized by chronic immune activation and is associated with the increase of pro-inflammatory cytokines\(^4\). This chronic activation of CD8+ T and CD4+ T leads to clonal exhaustion of pools of memory T cells and probably increases the number of target cells for the viral infection thus increasing viremia\(^1,2,7\). There are still other factors that contribute to a strong and intense immune activation in individuals seropositive for HIV, for example, the imbalance of Th17 type cells that are currently receiving attention in studies on HIV infection. This subtype produces not only IL-17 but also TNF-\(\alpha\), IL-1, IL-21 and IL-22\(^3,7\). In turn, IL-17 is secreted by several cell types especially CD4+ T cells in immune activation\(^7,27\), besides CD8+ T, eosinophils, neutrophils\(^7,79,82\) and more recently by invariant NK cells\(^80,81\). It is possible that a specific extenuation of the Th17 profile would be accompanied by deficiency of IL-21, IL-23 and TGF-\(\beta\), cytokines that promote this profile\(^82,83\).

Their role of IL-17 has been particularly linked to inflammatory conditions and autoimmunity\(^4\); when there is stimulus in cells by IL-17, occurs a second wave of different proinflammatory cytokines/chemokines and of GM-GSF, leading to neutrophil recruitment\(^24\). Another pathogenic effect is that IL-17 inhibits virus-induced cell apoptosis; this protection of virus-infected cells serves as a powerful means for viral evasion of the immune system\(^85\). The inhibition of apoptosis prolongs the life of infected cells on the one hand, and desensitizes killing by cytotoxic T cells on the other, resulting in enhanced viral replication and persistence\(^85\). Studies\(^46,67\) found that, by interfering with CTL function too, IL-17 blocks vigorous and efficient antiviral immunity thereby permitting sustained viral infection. Although the protective roles of Th1 and its main cytokine, IFN-\(\gamma\) have been previously cited but the roles of Th17 and IL-17 in viral infection, despite these harmful roles of IL-17, this cytokine and especially Th17 generally, also exert beneficial effects in HIV infection, as shown below.

The high susceptibility of Th17 cells to HIV, due to the large tropism of the virus in response to CCR5 chemokine receptor, is reflected in the depletion of this profile, in both the PBMC and gut-associated lymphoid tissue (GALT) of seropositive patients\(^86,89\). However, Th17 cells were found preserved in LTNP individuals\(^86\). Similar results were found in studies\(^46,67\) to SIV infection, when the non-human primate with progressive infection were compared to those with non-pathogenic infection and, the last group presented good control of virus replication and showed the capacity to maintain a normal level of Th17 cells in blood and intestinal mucosa\(^89,91\). In a recent study by Salgado et al.\(^92\), it was verified that the LTNP individuals presented more Th17 cells along with less microbial translocation, lower immune activation and slower disease progression when compared with TyP. Thus, several authors\(^89,91,93,94\) reported that in HIV infection, there is an association between scarce Th17 response with alterations in the balance of this profile with the consequent development of AIDS\(^89,91,93,95\).

It is important to remember that the GALT has been extensively studied in HIV/SIV infection because it harbors almost a majority of all lymphocytes in the body\(^96\) and that the intense depletion of Th17 in this system promotes alterations in the structure and function of this mucosa\(^97,98\) which culminates with the rupture of the epithelial barrier\(^99\) due the loss of local integrity and intense apoptosis of epithelial cells.

Thus, an increase in the secretion of pro-inflammatory cytokines occurs which facilitates the mucosal tissue damage\(^100,101\), alterations in bacterial flora and the microbial translocation of gut lumen to peripheral blood\(^102\). In this manner, the increase of immune activation and general inflammation are induced\(^102,103\) and, consequently, the disease progression\(^104\). Therefore, this fact suggests that Th17 cells can improve host defense through their capacity to maintain local homeostasis, prevent microbial translocation and systemic immune activation as well as elevate the potential to protect against opportunistic infections\(^74,89,91,93,105\).

Rather than imagined, in HIV seropositives with and without cART, Hed et al.\(^86\) demonstrated that the portion of IL-17+ T cells was reduced in those under cART independently of the VL value, which did not occur in naive HIV-infected or in HIV-uninfected. But, among patients that had used cART for a prolonged period of at least 5 years, Maçal et al.\(^94\) demonstrated that some of them were able to reconstitute this Th17 population and increase CD4+ T cells in the blood to normal levels. Nevertheless, it is still unclear whether cART produces an increase of Th17 despite the elevation in the CD4+ T cell count.

One possible explanation for the findings of pro-inflammatory IL-17 increase and protector Th17 decrease is that, higher doses of IL-17 are not always related to greater presence of the Th17 lineage and it is conceivable that the constant immune activation or exhaustion suppresses the effecter functions of Th17\(^98\). Furthermore, Th17 cytokines other than IL-17 may be able to modulate protective antiviral T cell responses\(^86,87\). In fact, in HIV infection, it is seems that the presence of Th17 is important to homeostasis but these cells need to present a normal and regulate IL-17 secretion.

LAST CONSIDERATIONS

Finally, the fact is that ARV induces not only quantitative but also qualitative alterations in the cytokine profile of persons that are living with HIV warrants attention\(^96\). In this manner it can be inferred that, for individuals that have access to cARV, the needs are not only the discovery of new medicaments and the implementation of programs that are intend to encourage and stimulate the medication adherence to patients\(^106\) but also the search for new interventions aimed at persistently decreasing immune activation and chronic inflammation\(^107,108\). After all, studies suggest that the attenuation of inflammation induced principally by products from microbial translocation can promote the improvement of clinical results and, it take together with adequate viral suppression, the AIDS progression and mortality can be reduced\(^109\). These findings can supply a new rational basis for studying different cARV or other anti-inflammatory interventions that can modify the risk of cardiovascular diseases and other complications that would contribute to improved prognoses for patients\(^95\).

Here, it was possible to note the importance in the balance of cytokines profile in HIV infection and the potential biomarker role of them to predict the rate of disease progression. Thus it could be suggested that cytokines, when are studied in isolate forms, does not shed light on the pathogenic or protective role of each one, neither could elucidate the clinical or immunological status of the individual. The biological effect of cytokines depends on their own interaction and, to be considered clear parameters in medical practice would be required study of a cytokines network expressed by the individual.
However, it is need to evaluate the cost versus benefit of this proposal. For this, should considering the low serum levels of these cytokines and low sensitivity of the tests and the high costs involved in these dosages when used in routine clinical monitoring, as both in relation to the investment of the kits acquisition as technical training of a professional for correct analysis.

Many researchers support that the study of cytokines is exceeded, however this review showed that the abundance of studies on the subject does not justify the low of accurate responses, since the divergence of data does not allow a consensus on the real pro-viral and pro-host cytokines and their long-term relationship, in naïve or treated patients. More studies on the inflammatory and regulatory profiles are necessary to clarify the interaction between cytokine levels and the real clinical condition of the HIV infected, mainly whom with the prolonged use of cART.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABSTRACT IN PORTUGUESE

Parâmetros imunovirológicos e citocinas na infecção pelo HIV

Enquanto modernas terapias antirretrovirais (TARV) têm resultado em menores índices de morbidade e mortalidade e na melhora visível dos parâmetros clínicos e laboratoriais em infectados pelo HIV, sabe-se que seu uso em longo prazo contribui com aparecimento dos vários eventos não associados à aids como doenças cardiovasculares, cânceres e osteoporose, comorbidades as quais têm sido propostas como algumas das mais importantes que privam a maioria dos infectados pelo vírus a apresentarem prognóstico ainda melhor. Isso ocorre porque, mesmo com diminuição da inflamação e ativação imune após intervenção medicamentosa ao paciente, tais parâmetros continuam maiores que os apresentados por indivíduos saudáveis e o desequilíbrio dos perfis de citocinas também persiste. Por isso, avaliações de outros biomarcadores na prática clínica são necessárias para complementar os exames já realizados rotineiramente e permitir o monitoramento mais eficaz dos portadores do HIV. Esta revisão tem o intuito de investigar o papel das citocinas como potenciais marcadores, o monitoramento mais eficaz dos portadores do HIV. Esta revisão tem o avanços de outros biomarcadores na prática clínica são necessárias para complementar os exames já realizados rotineiramente e permitir o monitoramento mais eficaz dos portadores do HIV. Esta revisão tem o objetivo de investigar o papel das citocinas como potenciais marcadores, o monitoramento mais eficaz dos portadores do HIV.


REFERENCES


51. French MA, King MS, Tscharma JM, Silva BA, Landay AL. Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells. J Infect Dis 2009; 200:1212-1215.


78. Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A. IL-17, produced by NKT cells constitutively express IL-23 receptor and RORgammat and rapidly produce IL-23 and IL-17 in response to IL-23. J Exp Med 2004; 199:257-266.

