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Research Paper

Serum interleukin-6 levels are increased in HIV-infected patients that develop autoimmune disease during long-term follow-up

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ABSTRACT

Background: Elevated IL-6 levels have been associated with both autoimmune diseases and treated HIV-seropositive (HIV^+) subjects. However, few data on classic and *trans-signaling* IL-6 in autoimmune thyroid diseases and HIV^+ subjects developing autoimmune disorders are currently available.

Materials and methods: A total of 102 patients were included in the study. They were subdivided into two groups. Group A consisted in 51 HIV^+ patients, who were followed-up for a period of five years in search of possible occurrence of autoimmune diseases. Ten of them, treated with antiretroviral therapy (ART), developed an autoimmune disorder, namely Hashimoto's thyroiditis, and psoriasis. Group B consisted in 51 patients affected by Hashimoto's thyroiditis (HT). Serum levels of the free form of IL-6 were analyzed by ELISA in all patients and for HIV^+ patients at the beginning of the follow-up, before initiation of ART.

Results: Mean serum levels of IL-6 were similar in Group A and in Group B. In Group B, IL-6 levels showed a 5.8% increase compared with assay minimum detectable dose corresponding to 1% of full serum IL-6 level. However, serum levels of free IL-6 were increased in those HIV^+ patients who developed autoimmune disorders (5.8 ± 2.8 pg/ml) and in these patients, the highest levels of free IL-6 correlated with age and CD4 cellular counts.

Conclusions: The present study indicates a correlation between serum free IL-6 levels and the occurrence of autoimmune disease in HIV^+ population, treated with ART during a long-term follow-up. The increased levels of serum free IL-6 were observed before ART treatment was initiated, indicating that IL-6 measurement in such patients may represent an early predictor of development of autoimmune disease.

1. Introduction

Interleukin 6 (IL-6) is a pleiotropic cytokine in the form of a 21–30 kDa glycosylated protein (Hirano, 1998; Hunter and Jones, 2015); its receptor (IL-6R) includes an 80 kD IL-6-binding subunit called IL-6R and a glycoprotein 130 (gp130) subunit which is responsible for downstream signal transduction (Rose-John, 2012; Scheller et al., 2011) (Fig. 1). Upon binding to IL-6R, IL-6 exerts either pro-inflammatory or anti-inflammatory and regenerative effects by means of activation of two distinct pathways namely, the *trans-signaling* and the *classic* IL-6, respectively. Pro-inflammatory effects are *trans-signaling* IL-6 consequences, whereas regenerative and anti-inflammatory functions are inferred to classic IL-6 *signaling* (Rose-John, 2012; Dienz et al., 2009). Classic signaling via membrane IL-6R and gp130 is limited to a few cell types, since membrane IL-6R is only expressed on hepatocytes and immune cells, as well as thyrocytes (Rose-John, 2012; Scheller et al., 2011; Dienz et al., 2009; Ruggeri et al., 2002; Ruggeri et al., 2006; Jones et al., 2010) (Fig. 1). In contrast, *trans-signaling*, in which a complex is formed between IL-6 and a soluble form of IL-6R, sIL-6R and then joins with membrane gp130, may be elicited in all cells due to ubiquitous expression of membrane gp130 (Rose-John, 2012; Dienz et al., 2009; Borges et al., 2014; Borges et al., 2015) (Fig. 1). Serum free IL-6 level is the floating parameter monitoring anti-inflammatory reactions promoted through classic signaling IL-6, while serum IL-6/sIL-6R complex levels identify the *trans-signaling* IL-6 implied in pro-inflammatory activities (Rose-John, 2012).

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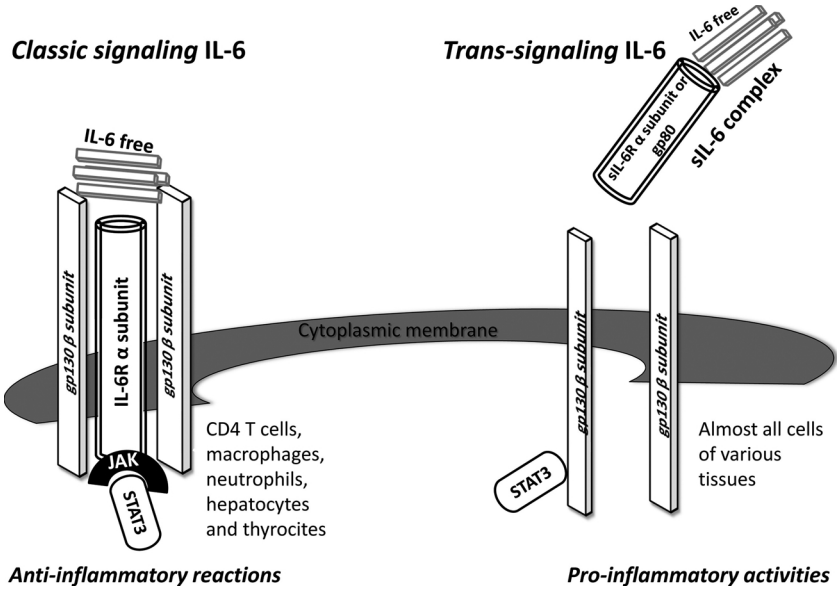

Fig. 1. Classic and *trans*-signaling IL-6.
Note: Activation of *classic signaling* IL-6 requires IL-6R and gp130 receptors on cellular surface as well as IL-6 medium free to trigger jak/STAT3 pathway in the cytoplasm of cells. By binding gp130 receptors, IL-6/sIL-6R complex is responsible for *trans-signaling* IL-6.
Abbreviations: IL-6, Interleukin 6; gp130, Glycoprotein 130; IL-6Rα subunit, Interleukin 6 Receptor α subunit; sIL-6R, soluble form of IL-6Rα; gp80, Glycoprotein 80 also known as sIL-6R; JAK, Janus kinase; STAT3, Signal Transducer and Activator of Transcription 3.

In particular, IL-6 plasma levels, measured in HIV$^+$ subjects treated with antiretroviral therapy (ART), showed a relevant increase, from 50 to 100%, compared to the general population (Borges et al., 2014). The relationship between IL-6 and HIV infection was recently analyzed in many clinical studies. A large number of individuals receiving ART treatment were enrolled in a cross-sectional study, conducted by the international network for strategic initiatives in global HIV trials from 2001 to 2013 (Borges et al., 2015). In addition, three International HIV trials, namely SMART (NCT00027352), ESPRIT (NCT00004978) and SILCAAT (NCT00013611), reported that the highest serum IL-6 levels of treated HIV+ subjects were found in association with demographic factors such as older age, as well as HIV RNA levels, smoking and ART comorbid conditions such as cardiovascular disease, diabetes mellitus, HBV and HCV infections (Borges et al., 2015).

Even if there is no direct evidence that HIV replication induces IL-6 serum expression and, therefore, the drivers of IL-6 production linked to HIV contagion remain unknown (Shive et al., 2012). All of these observations indicate that IL-6 may represent a potential biomarker of clinical outcomes in HIV-infected persons, and that it can be correlated with disease progression and poor prognosis (Breen et al., 1990; Connolly et al., 2005).

We have previously characterized the cellular distribution of both IL-6 and IL-6R in autoimmune thyroid disease (Ruggeri et al., 2006). Simultaneous expressions of both IL-6 and IL-6R were analyzed in thyrocytes from Hashimoto's thyroiditis (HT) samples by immunohistochemistry (Ruggeri et al., 2002), and higher expression of IL-6 was observed compared to its cognate receptor. In addition, a positive correlation between IL-6 immunostaining and lymphocytes infiltration of thyroid tissue was found (Ruggeri et al., 2002; Ruggeri et al., 2006). Furthermore, we measured the serum levels of total free and bound gp80/IL-6 in patients with HT, reporting an increase of the entire amount compared to healthy controls and goitrous subjects (Ruggeri et al., 2009). Thus, both *trans-signaling* and *classic* IL-6 appear to be involved in the development and progression of HT.

In the present study, we measured free IL-6 serum levels in HIV$^+$ patients before receiving ART therapy, to determine the role, if any, of IL-6 signaling in the development of autoimmune diseases in HIV patients.

2. Materials and methods

2.1. Patients and serum collection

The present study was approved by the Ethics Committee of Messina University. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki. A total of 102 subjects were recruited for this study. They were subdivided into two groups of 51 unrelated patients. HIV infected patients, 46 men and 5 women (mean age ± SD: 45.9 ± 12) showing no autoimmune diseases at first clinical and laboratory evaluation were enrolled in the first group (Group A). Each HIV$^+$ patient was seen at the Unit of Infectious Diseases of the University of Messina, where they received careful medical evaluation, including a detailed anamnesis and physical examination. CD4 count and viral load criteria supported the clinical diagnosis of positivity for HIV. All subjects of the HIV$^+$ group have been followed over five years, with ART, and 10 patients, 9 men and 1 woman (mean age ± SD: 45.5 ± 13) developed autoimmune disorders, such as psoriasis (n = 6) and HT disease (n = 4).

The second group (Group B) was composed of 51 HT patients (9 men and 42 women, mean age ± SD: 42.8 ± 14.1 years). All patients were euthyroid at the time of sampling and none was under L-thyroxine therapy. Each subject received a careful medical evaluation, including a detailed anamnesis, and physical examination. Blood was taken from each subject to perform routine laboratory tests, as well as measurement of thyroid hormone function.

Venous blood (3–5 ml) was drawn from each study participant at the time of the first evaluation, with a 5 ml sterile syringe and immediately transferred to a sterile clot tube. After clotting, samples were centrifuged to obtain serum and this was then aliquoted and frozen at −20 °C until cytokine detection.

Serum concentration of free IL-6 was determined using an antibody recognizing both natural and recombinant human IL-6, by competitive enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (950.030 Human IL-6 ELISA Kit- version 11- from Diaclone SAS, Besancon, France). This assay is designed to measure the total amount of free IL-6 in all sera. The sensitivity or minimum detectable dose (mdd) was 2 pg/ml. Thus, this assay showed a higher specificity than previous ones (Ruggeri et al., 2009). In our previous study, IL-6 serum concentrations were detected by using a recombinant human IL-6 cytokine (Eurogenetics U.K. Ltd, Middlesex, UK) designed to measure total amount of free and bound IL-6 cytokine in serum (Ruggeri et al., 2009). Even if its specificity was lower, the sensitivity of