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A soluble form of CTLA-4 is present in serum of paediatric patients with acute lymphoblastic leukaemia

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Abstract

CTLA-4 can regulate and maintain self-tolerance, providing a negative signal limiting immunoresponses. Acute lymphoblastic leukemia is a clonal disorder of lymphoid progenitors representing the most frequent malignancy of childhood. Here, we show the presence of significantly elevated levels of a soluble form of CTLA-4 in 70% of B-ALL patients. A possible role of this soluble molecule in the pathogenesis of this neoplastic disease can be envisaged.

Introduction

Acute lymphoblastic leukemia (ALL) is a clonal disorder of lymphoid progenitors with distinctive morphologic, immunophenotypic and genotypic features and represents the most frequent malignancy of childhood [1]. Immunophenotype allows further subdivision of B lineage ALL into pro-B, common, pre-B and mature B. However, this classification has little value for survival prediction [2]. Thus, beyond a better understanding of molecular mechanisms of disease and resistance to chemotherapy, the identification of markers suitable for chemotherapy and/or immunotherapeutic approaches may be useful.
Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) is a homodimeric glycoprotein belonging to human Ig gene superfamily [3]. The majority of *in vitro* and *in vivo* studies on CTLA-4 support its negative role on T-cell activation contributing to the physiologic termination of the immune response [4]. CTLA-4 inhibitory function occurs upon interaction with its ligands, CD80/CD86, expressed on antigen-presenting cells [4].
A native soluble form of CTLA-4 (sCTLA-4), deriving from lack of transmembrane sequence, has been described [5]. The presence of high concentration of sCTLA-4 was observed in sera of patients with autoimmune diseases, with allergy to hymenoptera venom, but not in allergic rhinitis [6, 8, 9].
sCTLA-4 may have important immunoregulatory functions. The effect of sCTLA-4 binding to CD80/CD86 molecules might depend on the activation state of the cells involved. Thus, sCTLA4 might act indirectly both as inhibitor or as enhancer of the immune response [5-9].
To the aim of further evaluate the immunopathological roles of T-cell costimulatory molecules and in searching for potential surrogate markers in ALL, we investigated the serum concentration of sCTLA-4 in ALL paediatric patients.

Materials and methods

Patients. Fifty-three children with B-ALL and 45 normal controls were enrolled in this study [10].
ELISA. Specific ELISA kits were used for measuring serum sCTLA-4 (Bender), as previously described [7-9].
Western blotting. Western blotting was used to detect serum sCTLA-4 as previously described [7-9].
Statistical analysis. Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., CA, USA).

Results

Plasma sCTLA-4 levels in patients with ALL. The presence of circulating sCTLA-4 was evaluated in the serum of all normal donors and patients enrolled in this study (Figure 1A). Serum sCTLA-4 levels in 45 controls ranged 0.01-36.06 ng/ml (1.071±5.318 ng/ml). Among B-ALL patients, sCTLA-4 levels ranged 0.02-870.8 ng/ml (132.0±208.7 ng/ml). The majority of B-ALL patients (42%) had sCTLA-4 levels higher than normal donors ($P < 0.0001$). In addition, presence of s CTLA-4 was confirmed by Western-blot (Figure 1B).

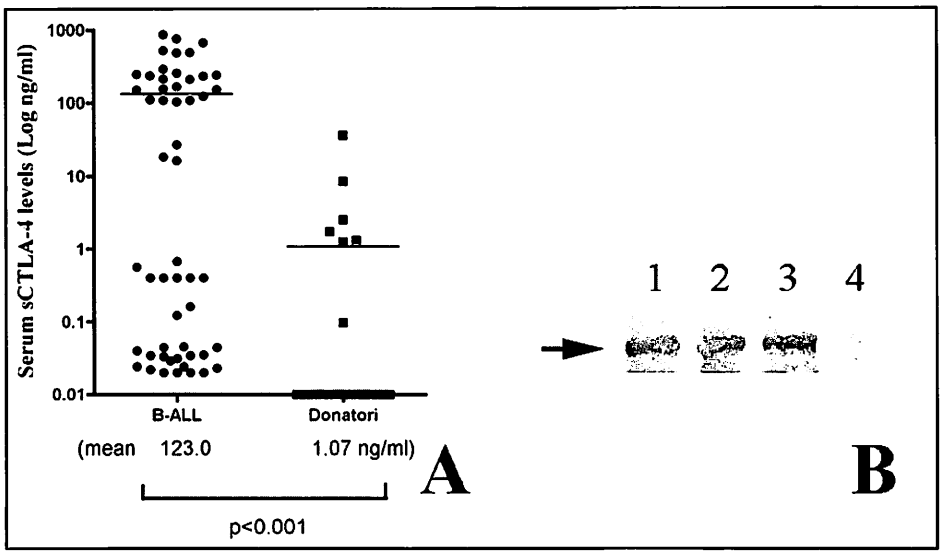

Figure 1. sCTLA-4 is found in serum of B-ALL patients. Panel A, sCTLA-4 was evaluated by ELISA on sera collected from B-ALL patients (n = 53), and healthy donor (n = 45). Deviation between triplicates was <10% for any reported value. Panel B, Immunoprecipitation of sCTLA-4 was performed on sera from a representative group of B-ALL patients. 1, 2 and 3 sera positive during ELISA analysis; 4 negative. Arrow marks 23-kDa species.

Discussion

CTLA-4 was first described as a negative regulator of T-cell activation and proliferation, interacting with B7 molecules on antigen-presenting cells. In addition, alternative splicing of mRNA encoding the CTLA-4 receptor leads to the production of a molecule (sCTLA-4) that lacks a membrane anchor and is therefore secreted into the extracellular space. There is abundance of literature showing the presence of sCTLA-4 in autoimmunity diseases and in non-autoimmune diseases [6-9]. Thus, sCTLA-4 may constitute a strategy for immune-surveillance escape.

In the present study we demonstrate the presence at significantly elevated levels of a circulating sCTLA-4 in 70% of B-ALL patients. Unfortunately, we are not able at this moment to clarify the possible role of this soluble molecule as a marker of progression of malignancy. It should be interesting to follow-up the serum expression of this molecule and, possibly, to correlate its levels to the decreasing of neoplastic cell numbers following therapy.

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