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The soluble CTLA-4 receptor: a new marker in autoimmune diseases

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Summary

A soluble form of cytotoxic T lymphocyte-associated antigen-4 (sCTLA-4) was recently found and shown to possess B7 binding activity. sCTLA-4 is generated by alternatively spliced mRNA. The mRNA encoding sCTLA-4 consists of 3 exons: exon 1 encodes a leader peptide, exon 2 the ligand binding domain, and exon 4 the cytoplasmic tail, but it lacks the transmembrane domain encoded by exon 3. The altered transcript is detected in resting CD4 and CD8 T cells and its expression is inhibited after 24–48 h of activation and returns to the prestimulation level after 72–120 h of activation. Low levels of sCTLA-4 have been detected in normal human serum and increased serum levels have been observed in several autoimmune diseases (e.g. Graves' disease, myasthenia gravis, systemic lupus erythematosus, and systemic sclerosis). The biological significance of increased sCTLA-4 serum level has not been clarified. On one hand, sCTLA-4 may bind B7 expressed on antigen-presenting cells and is thus able to interfere with the B7:CD28-mediated costimulation of T cell responses. On the other hand, sCTLA-4 may also be capable of interfering with B7:CTLA-4 interactions, thereby blocking the negative signal imparted via the full-length form of CTLA-4. This double-edged nature of B7 blocking by sCTLA-4 may result in different outcomes of the clinical course of disease.

Key words: sCTLA-4 • alternative splicing • autoimmune diseases

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INTRODUCTION

The activation of T lymphocytes requires T cell receptor (TCR)/CD3 recognition of an antigen/major histocompatibility complex molecule located on antigen-presenting cells (APCs) as well as costimulatory signals. Triggering TCR/CD3 alone in the absence of a costimulatory signals not only fails to induce an immune response, but may also lead to a state of hyporesponsiveness or anergy⁴. CD28 is known to be the major costimulatory molecule expressed constitutively on 95% of CD4⁺ and approximately 50% of T cells. Ligation of CD28 with its ligands CD80 and CD86, expressed on APCs, results in T cell proliferation, the generation of cytotoxic lymphocytes, and lymphokine production⁴². Cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152) is a T cell costimulatory receptor that functions as a negative regulator of T cell activation^{5, 7, 40, 45}. The fundamental inhibitory function of CTLA-4 has been clearly demonstrated with CTLA-4-deficient mice that suffer from a lymphoproliferative disorder^{7, 43, 48}.

CTLA-4: EXPRESSION, STRUCTURE, AND FUNCTION

On resting T cells, CTLA-4 protein levels are low or undetectable. Human CTLA-4 is expressed on activated CD4⁺ and CD8⁺ T cells and is predominantly localized in intracellular compartments. Its membrane expression is maximal after 48–72 h of stimulation^{17, 23, 26, 39, 45}. Recently it was also shown that CTLA-4 expression appears on normal, activated B cells¹⁸, malignant B cells from non-Hodgkin's lymphomas⁴⁹, placental fibroblasts¹⁶, and cultured muscle cells³¹. Weak CTLA-4 expression was observed on the cell surface of fresh monocytes⁴⁶.

CTLA-4 binds the same ligands as CD28, but its affinity to CD80 and CD86 molecules is 10–50-times higher than that of CD28^{5, 17, 25}. CTLA-4 can down-regulate T cell responses by two separate mechanism. One is CTLA-4-mediated negative signaling in response to TCR stimulation²¹. This mechanism requires the cytoplasmic tail of CTLA-4 and can occur in the early stages of an immune response, when the expression of CTLA-4 and B7 is limited⁶. In late stages of immune response, when there is increased expression of B7 and CTLA-4, another mechanism operates which depends on cell-surface competition between CTLA-4 and CD28 for B7 binding. In this case, the level of the surface expression of CTLA-4 is decisive⁶. Binding of B7 to CTLA-4 leads to the termination of the immune response by the limitation of CD28-mediated signaling and may lead to T cell anergy and T cell apoptosis^{35, 38}. The CTLA-4/B7 interaction has a key role in maintaining

peripheral immune tolerance status, and hence in autoimmunity³⁸.

CTLA-4 structure

CTLA-4 is the structural homologue of CD28, which belongs to the Ig superfamily^{5, 8, 9, 19}. It is a trans-membrane glycoprotein consisting of 186 amino acid (aa) residues, with 125 aa residues in the extracellular region and 37 in the cytoplasmic domain^{8, 14}. CTLA-4 exists as a homodimer. The CTLA-4 molecule is linked by a disulfide bridge between the cysteine residues at position 120. It contains a conserved motif (MYPPPY), located in the extracellular domain, critical for B7 molecule binding^{8, 14}. CTLA-4 consists of one V-like domain flanked by two hydrophobic regions, one of them having a structure probably responsible for membrane anchoring^{5, 37}.

Counterreceptors for CTLA-4

The best characterized costimulatory molecule for CTLA-4 is the B7 antigen, a member of the immunoglobulin family. Two members of the B7 family, called B7-1 (CD80) and B7-2 (CD86), play a critical role in the regulation of immune responses. They provide activatory or inhibitory signals to T cells through ligation with CD28 or CTLA-4 receptors, respectively²⁴. CD80 and CD86 are expressed on APCs and there is 25% homology between them. CD86 is constitutively expressed on resting dendritic cells, B cells, and macrophages, and its expression increases 1 h after stimulation. In contrast, CD80 is expressed 3–4 days after activation on T, B, and natural killer cells²⁴. CD80 and CD86 are also expressed by salivary gland epithelial cells at both the mRNA and the protein levels³⁰. Both CD80 and CD86 molecules can provide effective T cell costimulation²⁰, but CD86 is the dominant stimulatory ligand for CD28 and CD80 for CTLA-4^{2, 15}.

CTLA-4 gene

The human *CTLA-4* gene was mapped on chromosome 2q33-34¹⁹. It exists as a single copy per haploid genome. It consists of four exons: exon 1 encodes a leader peptide (37 aa), exon 2 the ligand-binding domain (116 aa), exon 3 the transmembrane domain (37 aa), and exon 4 the cytoplasmic tail (34 aa)⁴⁴. Introns 1, 2, and 3 span 2.5, 0.5, and 1.1 kb respectively⁸ (Fig. 1.).

The human *CTLA-4* gene is known to contain several polymorphisms. The following three *CTLA-4* polymorphisms have been the most frequently studied: 1) position –319 (C/T) of the promoter region (Gen-

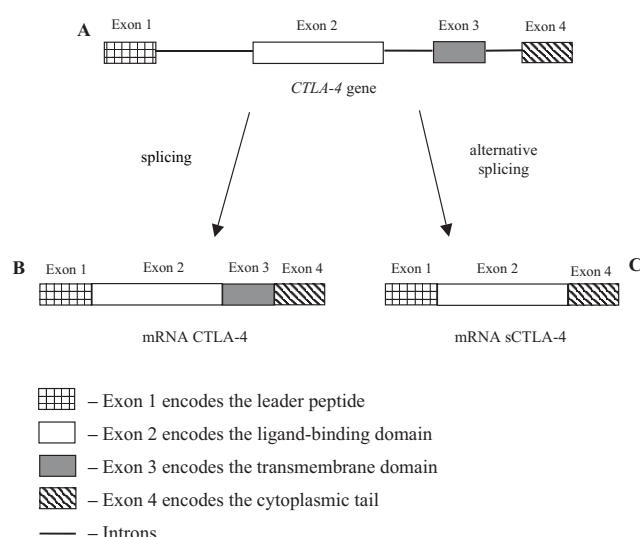


Figure 1. The generation the full-length CTLA-4 and sCTLA-4 mRNA. **A** – *CTLA-4* gene, **B** – full-length CTLA-4 transcript consists of 4 exons: exon 1, 2, 3 and 4, **C** – sCTLA-4 transcript consists of 3 exons: exon 1, 2 and 4 generated by alternatively spliced mRNA. The net effect of alternative splicing is 110-bp deletion corresponding to the entire transmembrane domain of the CTLA-4 molecule and the frame-shift mutation within the cytoplasmic domain of sCTLA-4.

bank no. M74363), 2) position 49 (A/G) of exon 1 leading to substitution of threonine to alanine in codon 17 of the leader peptide (Genbank no. M74363), and 3) a dinucleotide (AT)_n repeat sequence (Genbank no. M37243, locus HUMIGCTL3) located in the 3'-untranslated region (3'-UTR) of exon 4 at position 642. In recent years, several other *CTLA-4* polymorphisms within the *CTLA-4* gene have been discovered⁴⁴.

SOLUBLE FORM OF CTLA-4

Recently, a soluble form of CTLA-4 (sCTLA-4) was described which was found to be a functional molecule with CD80 and CD86 binding ability^{28, 32, 33}. sCTLA-4 is generated by alternatively spliced mRNA (Fig. 1). The nature of this process has been investigated by Oaks et al.³³ in a rat model. Restriction enzyme analysis and sequencing of the cloned genomic DNA revealed two introns. Splicing between the splice-donor/splice-acceptor (SD/SA) sites of both intron A and intron B results in the transcript encoding the transmembrane form of the molecule, while a splicing event between the SD site of intron A and the SA site of intron B gives the soluble form of CTLA-4. The net effect of this splicing event is a 110-bp deletion corresponding to the entire transmembrane domain of the CTLA-4 molecule. The A→B splicing event introduces a frame-shift mutation within the

cytoplasmic domain of sCTLA-4. Thus, although the nucleotide sequences of the normal and soluble molecules are identical, the change of reading frame produces an amino acid tail which is unique to the sCTLA-4 molecule. CTLA-4 showed 95% homology between human and rat³³. The mRNA encoding sCTLA-4 consists of 3 exons: exon 1 encodes a leader peptide, exon 2 the ligand-binding domain, and exon 4 the cytoplasmic tail, but it lacks exon 3 encoding the transmembrane domain³³ (Fig. 1). The spliced transcript produces a 23-kDa soluble form of CTLA-4. This form of CTLA-4 has a unique cytoplasmic tail, characteristic for sCTLA-4, which is 22 aa long and 12 aa shorter than that of the full-length form of CTLA-4 antigen. sCTLA-4 lacks the cysteine residue at position 120 and is expressed as a monomer²⁸. sCTLA-4 contains the MYPPPY motif and it may bind B7 there and participate in the B7/CTLA-4/CD28 signaling pathway of T cell regulation²⁸.

sCTLA-4 transcripts have been detected in lymph nodes, spleen, CD4 and CD8 subsets of T cells, B lymphocytes in both humans and rats³³, and in monocytes⁴⁶. sCTLA-4 has not been detected in a wide variety of non-lymphoid tissues, including adrenal, brain, eye, heart, kidney, liver, lung, ovary, pancreas, salivary gland, seminal vesicles, skeletal muscle, testes, and thyroid³³. A distribution analysis of the soluble and full-length CTLA-4 transcripts among the CD4 and CD8 subsets of T cells showed that CD4 cells express both transcripts at the same level, whereas CD8 cells appear to express nearly 2.5 times more full-length product than sCTLA-4³³. Low serum levels of sCTLA-4 were detected in normal human serum by Liu et al.²⁷, Magistrelli et al.²⁸, Oaks and Hallett³² and Wang et al.⁴⁷. Ueda et al.⁴⁴ studied the association of sCTLA-4 mRNA level and the exon 1 +49A/G and CT60A/G *CTLA-4* gene polymorphisms in healthy subjects and found that the sCTLA-4 mRNA level in unstimulated CD4 T cells was higher for the allele A at position +49 and CT60 A variant.

It is difficult at present to explain why autoimmune disease-susceptibility genotypes (+49G/G and CT60G/G)^{1, 3, 13, 22, 36, 44} are associated with lower levels of sCTLA-4 transcripts. The exact mechanisms of this phenomenon are still unknown. It can be suggested that abnormal translation of both CTLA-4 transcripts (sCTLA-4 and full-length CTLA-4) and/or intracellular trafficking and release of sCTLA-4 may occur in these patients.

REGULATION OF SCTL4-4 EXPRESSION

Cellular activation appears to regulate the relative level of each CTLA-4 transcript. mRNA sCTLA-4

expression is inhibited after 24–48 h of activation with concanavalin A plus phytohemagglutinin³³. After 72–120 h of activation the sCTLA-4 transcript appears to increase, but it does not exceed about 2/3 of total mRNA CTLA-4³³. Similar results were obtained upon activation of human peripheral blood mononuclear cells with anti-CD3 plus anti-CD28^{28, 33}. Short-time T cell stimulation with phorbol 12-myristate plus ionomycin for 6 h or anti-CD3 plus anti-CD28 monoclonal antibody led to inhibition of mRNA sCTLA-4 expression²⁸. It was also observed that interferon (IFN)- β 1a can enhance the expression of the soluble isoform of CTLA-4 in human mononuclear (MNL) cells from healthy subjects¹¹. Analysis of unstimulated MNL cells incubated in complete medium with and without IFN- β 1a for 72 h did not show any differences in cDNA full-length CTLA-4 band intensity, whereas the amount of sCTLA-4 transcript was higher after IFN- β 1a treatment. This result showed a selective induction of sCTLA-4 by IFN- β 1a in human MNL cells which might exert immunomodulatory effects¹¹.

Moreover, Oaks et al.³³ found that recombinant sCTLA-4 inhibits the mixed leukocyte reaction in a dose-dependent manner and is, at high concentrations, capable of complete inhibition of cellular proliferation in this systems. The mechanism of this phenomenon is not fully understood. It has been shown recently^{10, 12} that recombinant soluble CTLA-4 protein (CTLA4-Ig) may work by provoking different types of APCs, including dendritic cells (DCs), to catabolize tryptophan by indoleamine 2,3-dioxygenase. This process is important in inhibiting T cell proliferation²⁹.

SCTLA-4 AND AUTOIMMUNE DISEASES

Abnormal expression of CTLA-4 may lead to the development of autoimmunity in experimental systems and the autoimmune phenotype of the CTLA-4-deficient mouse. In light of this information, the role for sCTLA-4 in activated cells due to its association with autoimmunity has been studied. Recently, increased serum levels of sCTLA-4 have been reported in patients in several autoimmune diseases. The short-splice variant of CTLA-4 was observed significantly more often in patients with autoimmune diseases than in healthy individuals^{27, 32, 47}. The investigated patient groups were patients with Graves' disease³², Hashimoto's thyroiditis³², myasthenia gravis (MG)⁴⁷, systemic lupus erythematosus (SLE)^{27, 41}, and systemic sclerosis (SSc)⁴¹. Study of these patients showed significantly higher circulating levels of sCTLA-4 in their serum compared with healthy controls. The initial studies on sCTLA-4

serum levels in patients with autoimmune disease were performed by Oaks and Hallet³² in 17 patients with Graves' disease and 3 patients with Hashimoto's thyroiditis. Eleven of the 20 patients had circulating levels of sCTLA-4 ranging 28–78 ng/ml, whereas only 1 of the 30 apparently healthy volunteer controls had an sCTLA-4 level greater than 4 ng/ml³². Liu et al.²⁷ showed in a group of one hundred patients with SLE significantly higher serum levels of sCTLA-4 than in healthy controls (21.6 ± 12.3 ng/ml versus 5.9 ± 5.4 ng/ml, $p < 0.001$). By contrast, others⁴¹ found normal serum sCTLA-4 levels in 23 patients with SLE. They could not find a statistically significant correlation between serum sCTLA-4 and lupus disease activity²⁷. Sato et al.⁴¹ observed elevated serum sCTLA-4 levels in 32 patients with diffuse cutaneous SSc compared with healthy subjects ($p < 0.001$), while mean serum sCTLA-4 levels in 27 patients with limited cutaneous SSc did not differ from those found in healthy subjects⁴¹. sCTLA-4 correlated with disease severity and activity of systemic sclerosis.

Patients with MG also showed increased serum levels of sCTLA-4⁴⁷. The median sCTLA-4 level in a group of 96 patients with MG was 6.8 ng/ml (range 0–1200 ng/ml), while in the control group it was 3.0 ng/ml (range 0–600 ng/ml; $p < 0.001$)⁴⁷. The serum levels of sCTLA-4 correlated positively with the serum concentration of antibodies against the acetylcholine receptor ($r = 0.396$, $p < 0.01$)⁴⁷. Comparisons among MG patients classified by thymic histopathology according to Oosterhuis³⁴ showed that patients with thymoma had higher levels of sCTLA-4 than other patients, and all patients had statistically higher levels of sCTLA-4 than healthy volunteers⁴⁷. No difference in serum sCTLA-4 level was found between patients with and without immunosuppressive treatment^{27, 47}. There were also no correlations observed between CTLA-4 gene polymorphisms in the promoter region at position -319²⁷ and in the 3'-UTR of exon 4 at position 642⁴⁷ and the levels of sCTLA-4 in patients with autoimmune diseases.

In a summary, sCTLA-4 levels appear to be constitutive in autoimmune diseases and may have important immunoregulatory functions. On one hand, sCTLA-4 may bind B7 expressed on APC and thus interfere with B7:CD28-mediated costimulation of T cell responses. On the other hand, sCTLA-4 may also be capable of interfering with B7:CTLA-4 interactions, thereby blocking the negative signal imparted via the membrane-bound form of CTLA-4. This double-edged nature of B7 blocking by sCTLA-4, which may result in different outcomes of the clinical course of disease, requires further studies.

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