CTLA-4 Expression in CD4+ T Cells From Patients With LRBA Deficiency and Common Variable Immunodeficiency With No Known Monogenic Disease

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Common variable immunodeficiency (CVID) is the most common symptomatic form of primary antibody deficiency [1,2]. The underlying causes of CVID are largely unknown; however, genetic mutations are responsible for the disease in approximately 10%-20% of patients [3]. Furthermore, there is accumulating evidence that at least a subgroup of patients with CVID has a complex rather than a monogenic inheritance pattern [3]. Based on published cases, lipopolysaccharide-responsive and beige-like anchor protein (LRBA) deficiency is one of the most common monogenic disorders found during analysis of mutations in CVID patients [3,4]. Several studies have reported that T-cell abnormalities may be involved in the pathogenesis of immune dysregulation in CVID and LRBA deficiency [4,5]. Regulatory T cell (Treg) abnormalities due to low expression of forkhead box P3 (FOXP3) and CTLA-4 defects have also been reported [6]. Although lower levels of CTLA-4 protein have been found in patients with CVID and LRBA deficiency and associated with clinical presentations [1,7], no studies have compared the expression of CTLA-4 transcripts in the $CD4^+$ T cells of patients with LRBA deficiency and CVID patients with no definitive genetic diagnosis.

The study population comprised 8 LRBA patients and 8 age- and sex-matched CVID patients with no known gene mutations. The genetic analysis involved whole exome sequencing, and LRBA gene mutations were confirmed by Sanger sequencing using a method described previously [4]. Eight equally matched healthy individuals with no history of immunodeficiency were also recruited as healthy controls (HCs). Peripheral blood mononuclear cells were isolated, and $CD4^+$ T cells were purified, stimulated, and evaluated (using flow cytometry analysis) as previously described [8]. The expression levels of the *CTLA4* gene were measured using quantitative real-time polymerase chain reaction with specific primers (Table S1) and normalized relative to levels of glyceraldehyde-3-phosphate dehydrogenase.

The mean (SD) age of the patients in the HC, LRBA, and CVID groups was 19.2 (9.1), 18.2 (9.3), and 17.0 (12.0) years, respectively. The frequency of consanguinity was 75% among CVID patients and 100% among LRBA-deficient patients. A history of noninfectious complications was more common in LRBA-deficient patients than in CVID patients. These included autoimmunity (62.5% vs 0%, P=.02), enteropathy (75.0% vs 12.5%, P=.04), splenomegaly (75.0% vs 25.0%, P=.13), hepatomegaly (62.5 vs 0%, P=.02), and granulomas (37.5% vs 0%, P=.20). However, the frequency of lymphadenopathy was 37.5% in both groups. The results showed that both the relative and the absolute frequency of Tregs ($CD4^+CD25^{high}FOXP3^+CD127^{low}$ T cells) in the peripheral blood of CVID and LRBA-deficient patients were significantly lower than those of the HCs. The absolute frequency of activated $CD4^+$ T cells ($CD4^+CD25^{high}$ $CD127^{high}$ T cell) in CVID patients was lower than in the LRBA-deficient and HC groups; however, the differences were not significant. The relative frequency of activated $CD4^+$ T cells (among total $CD4^+$ T cells) was higher in LRBA-deficient patients, but the absolute count of circulating activated $CD4^+$ T cells was lower than in the HCs, although these differences were not statistically significant (Table S2). As illustrated in the Figure, in the absence of anti-CD3 mAb and anti-CD28 mAb stimulation, the median (IQR) gene expression of *CTLA4* in CVID patients was lower (1.2 [0.08-2.2]) than in LRBA-deficient patients (2.7 [0.32-13.4], P=.293) and HCs (2.4 [1.2-4.0], P=.09), although the difference was not significant. Similarly, after stimulation, expression of *CTLA4* genes in CVID patients (5.8 [3.6-6.4]) was lower than in the HC group (18.8 [9.3-32.3], P<.01) and LRBA-deficient patients (11.7 [2.0-36.3], P=.248). There were no significant differences in *CTLA4* gene expression levels between the LRBA-deficient and HC groups (P=.46). We did not observe any correlation between the fold change of *CTLA4* expression and the frequency of activated CD^+ T cells and Tregs in either the CVID or the LRBA-deficient groups. When LRBA-deficient patients were split according to whether or not they had an autoimmune complication, the frequency of activated $CD4^+$ T cells and the expression level of *CTLA4* were shown to be higher in patients with autoimmunity than in those without autoimmunity (P=.297 for both).

CTLA-4 is an inhibitory checkpoint protein that is expressed on activated T cells and Tregs. mRNA and

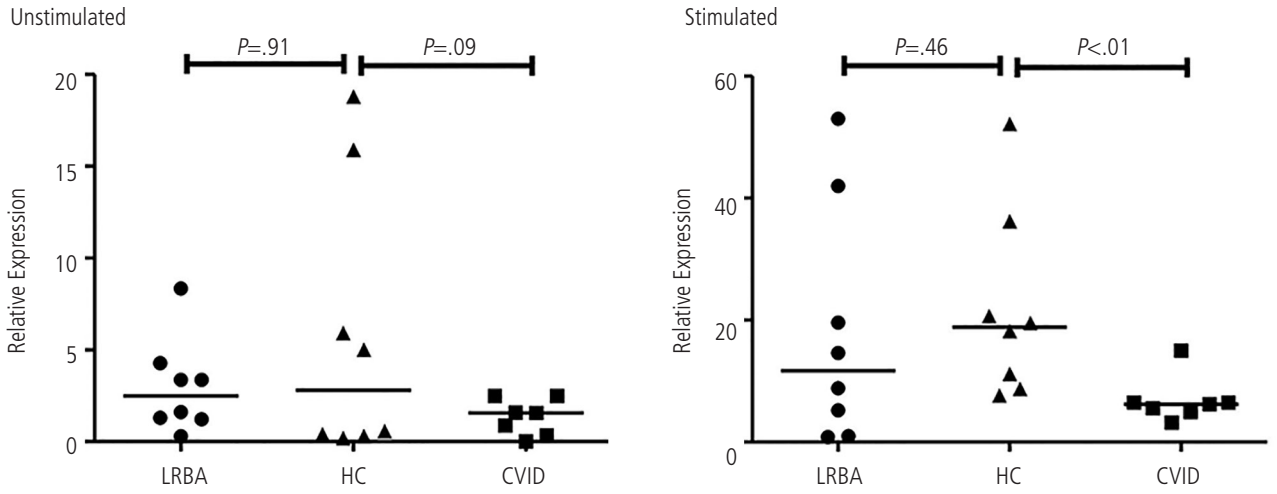

Figure. Expression of *CTLA4* by CD4+ T cells. Comparison of *CTLA4* gene expression with and without stimulation (using anti-CD3 mAb and anti-CD28 mAb) of CD4+ T cells from patients with CVID, LRBA deficiency, and HCs by quantitative real-time polymerase chain reaction. The median is represented by a horizontal line. LRBA indicates lipopolysaccharide-responsive and beige-like anchor protein; HC, healthy controls; CVID, common variable immunodeficiency.

surface expression of CTLA-4 have been reported to be lower in CVID patients than in HCs; this difference may be responsible for the cellular immune dysregulation observed in CVID patients, especially in those with autoimmune manifestations [9]. In the current study, we found a lower level of *CTLA4* expression in CVID patients than in HCs and LRBA-deficient patients, although there was no association with autoimmunity. The difference in *CTLA4* expression in the current study and previous studies may be due to the fact that monogenic disorders such as LRBA deficiency, which have more autoimmune presentations, were excluded during our analysis of mutations in CVID patients [8]. Therefore, the remainder of the CVID patients in whom mutations were not found correspond to patients with milder clinical symptoms.

Patients with LRBA deficiency show loss of CTLA-4 protein [10], because LRBA plays a role in CTLA-4 surface recycling [7]. To the best of our knowledge, this is the first report to evaluate *CTLA4* mRNA in patients with LRBA deficiency. Our results showed that there were no significant differences in *CTLA4* gene expression between LRBA-deficient patients and HCs. Moreover, there was no correlation between the fold change of *CTLA4* expression and the frequency of activated $CD4^+$ T cells or Tregs. We believe that in the circulating $CD4^+$ T-cell compartment, the low frequency of Tregs on the one hand and the increased frequency of activated $CD4^+$ T cells (due to recurrent infection and persistent inflammation) on the other may lead to the absence of changes in total levels of CTLA-4 mRNA in the $CD4^+$ T cells of LRBA patients. CTLA-4 mRNA expression should be evaluated in sorted $CD4^+$ T-cell subsets of patients with LRBA deficiency.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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