

## CHAI and LATAIE: new genetic diseases of CTLA-4 checkpoint insufficiency

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**CTLA-4 is a critical inhibitory “checkpoint” molecule of immune activation. Several recent reports have described patients with immune dysregulation and lymphoproliferative disease resulting from 2 different genetic diseases that directly or**

**indirectly cause CTLA-4 deficiency. Numerous articles have also been published describing CTLA-4 blockade in cancer immunotherapy and its side effects, which are ultimately the consequence of treatment-induced CTLA-4 deficiency. Here, we**

**review these 2 diseases and CTLA-4 blockade therapy, emphasizing the crucial role of CTLA-4 in immune checkpoint regulation. (Blood. 2016;128(8):1037-1042)**

### Introduction

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a critical and potent inhibitor of T-cell proliferation that serves as a “checkpoint” of immune responses.<sup>1</sup> The essential role of CTLA-4 in lymphocyte homeostasis and tolerance is illustrated by *Ctla4* knockout mice, which develop rapidly fatal destructive multiorgan lymphocytic infiltration.<sup>2,3</sup> *CTLA4* recessive disease has not yet been reported in humans. However, disease because of partial loss of CTLA-4 was recently described.<sup>4,5</sup> “CTLA-4 haploinsufficiency with autoimmune infiltration” (CHAI) patients have heterozygous loss-of-function mutations in CTLA-4 and develop lymphocytic infiltration of multiple nonlymphoid organs similar to *Ctla4* knockout mice. Additionally, prior reports have described patients clinically resembling CHAI disease, but with recessive inheritance. These patients harbor biallelic mutations in the “lipopolysaccharide-responsive vesicle trafficking, beach- and anchor-containing” (*LRBA*) gene rather than in *CTLA4*.<sup>6-14</sup> However, we discovered that *LRBA* deficiency causes a secondary loss of CTLA-4 by affecting the internal trafficking of this protein, thus elucidating the underlying molecular link between these 2 similar diseases.<sup>12</sup> We call this recessive disease “*LRBA* deficiency with autoantibodies, regulatory T (Treg) cell defects, autoimmune infiltration, and enteropathy” (LATAIE), which emphasizes the most prominent features of this disorder. Here, we review CHAI and LATAIE disease, emphasizing their shared mechanism of autoimmunity, and discuss potential treatment strategies. We also discuss CTLA-4 blockade in cancer immunotherapy, which may induce clinical symptoms of CHAI and LATAIE disease.

### Clinical manifestations

Patients with CHAI and LATAIE present with autoantibody-mediated cytopenias, lymphadenopathy/splenomegaly, hypogammaglobulinemia, organ-specific autoimmunity, and lymphocytic infiltration

of nonlymphoid organs. Although features of CHAI and LATAIE are similar, a notable difference is the typically earlier age of onset with LATAIE, where disease onset is often apparent in preschool-age children, whereas CHAI presents in older children or young adults. The autoantibody-mediated cytopenias (ie, autoimmune hemolytic anemia, autoimmune thrombocytopenia, and neutropenia), lymphadenopathy, and splenomegaly resemble the autoimmune lymphoproliferative syndrome (ALPS) in many patients.<sup>15</sup> Table 1 and Figure 1A summarize the common clinical features of CHAI and LATAIE disease and their frequencies in published reports. The clinical phenotypes for LATAIE patients have been comprehensively reviewed, and their frequencies are comparable to that which we report here.<sup>17,18</sup> Notably, frequencies of different clinical features and organ involvement vary greatly between different clinical cohorts. Patients with hypogammaglobulinemia, often diagnosed as common variable immune deficiency, have increased infections, especially of the respiratory tract. The frequency of patients with interstitial lung disease may potentially be higher for LATAIE disease in which patients are frequently described with chronic lung disease and/or digital clubbing, but interstitial lung disease was not always documented.<sup>6,7,16</sup> Furthermore, disease penetrance can vary. Penetrance for CHAI disease is incomplete, with an estimated 40% of *CTLA4* mutation-positive family members appearing clinically healthy.<sup>5</sup> By contrast, LATAIE disease shows nearly complete penetrance, with only 1 clinically healthy *LRBA* mutation-positive individual reported.<sup>11</sup> Despite near universal penetrance, the severity of LATAIE disease varies substantially, even within individual kindreds.

Despite varying clinical manifestations, most CHAI and LATAIE patients have lymphocytic overactivation and infiltration of at least 1 nonlymphoid organ, usually the intestine, lungs, or brain. For both diseases, intestinal involvement, leading to enteropathy, is most common. The lungs are the second most frequently infiltrated organ, and infiltrates in the brain are less common. In our experience, lymphocytic infiltrates in the brain seem to cause disease mainly by

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**Table 1. Characteristic features of CHAI and LATAIE disease**

Patient	Mutation	HGG	AIHA	ITP	LAD	Splenomegaly	ILD	Enteropathy	Brain lesions	Reference
<b>CHAI</b>										
P1	p.R51*	+					+	+		4
P2	p.R51*	+		+			+	+	+	4
P3	p.L28Ffs*44	+	+	+	+	+	+		+	4
P4	Splice site, c.458-1G>C		+	+	+	+		+		4
P5	Splice site, c.567+5G>C		+	+	+	+	+	+		4
P6	Splice site, c.567+5G>C	+	+		+	+		+	+	4
P7	p.C35*	+				+		+		5
P8	p.C35*	+					+	+		5
P9	p.C35*	+				+	+	+		5
P10	p.C35*	+		+				+	+	5
P11	p.C35*	+		+	+	+	+	+		5
P12	Splice site, c.110+1G>T					ND	ND	+		5
P13	Splice site, c.110+1G>T	ND			ND	ND	ND	+		5
P14	Splice site, c.110+1G>T						+	+		5
P15	Splice site, c.110+1G>T	+					+			5
P16	p.R70W	+	+	+	+	+	+			5
P17	p.R70W							+	ND	5
P18	p.T124P	+	+		+	+	+			5
P19	p.R75W	+	+	+	+	+	+	+		5
P20	Start codon, c.2T>C	+	+	+				+		5
P21	p.L28Sfs*40	+						+		16
<b>LATAIE</b>										
P1	p.I2657S	+		+			+		+	6
P2	p.I2657S	+		+						6
P3	p.R1683*	+	+	+	+		+	+	+	6
P4	p.E59*	+	+	+		+		+		6
P5	Exon 1-2 deletion	+						+		6
P6	p.E2219Dfs*3		+	+				+		7
P7	p.E2219Dfs*3							+		7
P8	p.E2219Dfs*3	+						+		7
P9	p.E2219Dfs*3	+	+	+				+		7
P10	p.E2219Dfs*3	+						+		7
P11	Exons 1-30 deletion		+	+	+	+	+	+		8
P12	p.T2388Pfs*7			+	+	+		+		10
P13	p.T2388Pfs*7	+	+					+		10
P14	p.Q678*		+					+		9
P15	p.C289Cfs*3	+		+	+	+	+			9
P16	p.C289Cfs*3			+	+	+	+			9
P17	p.I2657S	+	+	+	+	+				11
P18	p.S2713fs		+	+	+	+				11
P19	p.S2713fs		+	+	+	+				11
P20	p.R1445Q	+	+	+	+	+	+	+		12
P21	p.R1445Q		+	+		+	+	+		12
P22	p.K175*; T1587fs	+	+		+	+	+	+		12
P23	p.R1445*	+			+	+	+	+		12
P24	p.S1233F; Q2506*	+			+	+	+			12
P25	p.V2249F; L1834P		+	+	+	+	+	+	+	12
P26	p.R655*	+	+	+	+	+	+	+		12
P27	p.D248G; c.8502-1G>C			+		+		+		12
P28	p.D1329fs							+		12
<b>Frequency (%)</b>										
In CHAI		75	38	43	40	53	63	81	20	
In LATAIE		57	54	64	50	57	43	71	11	

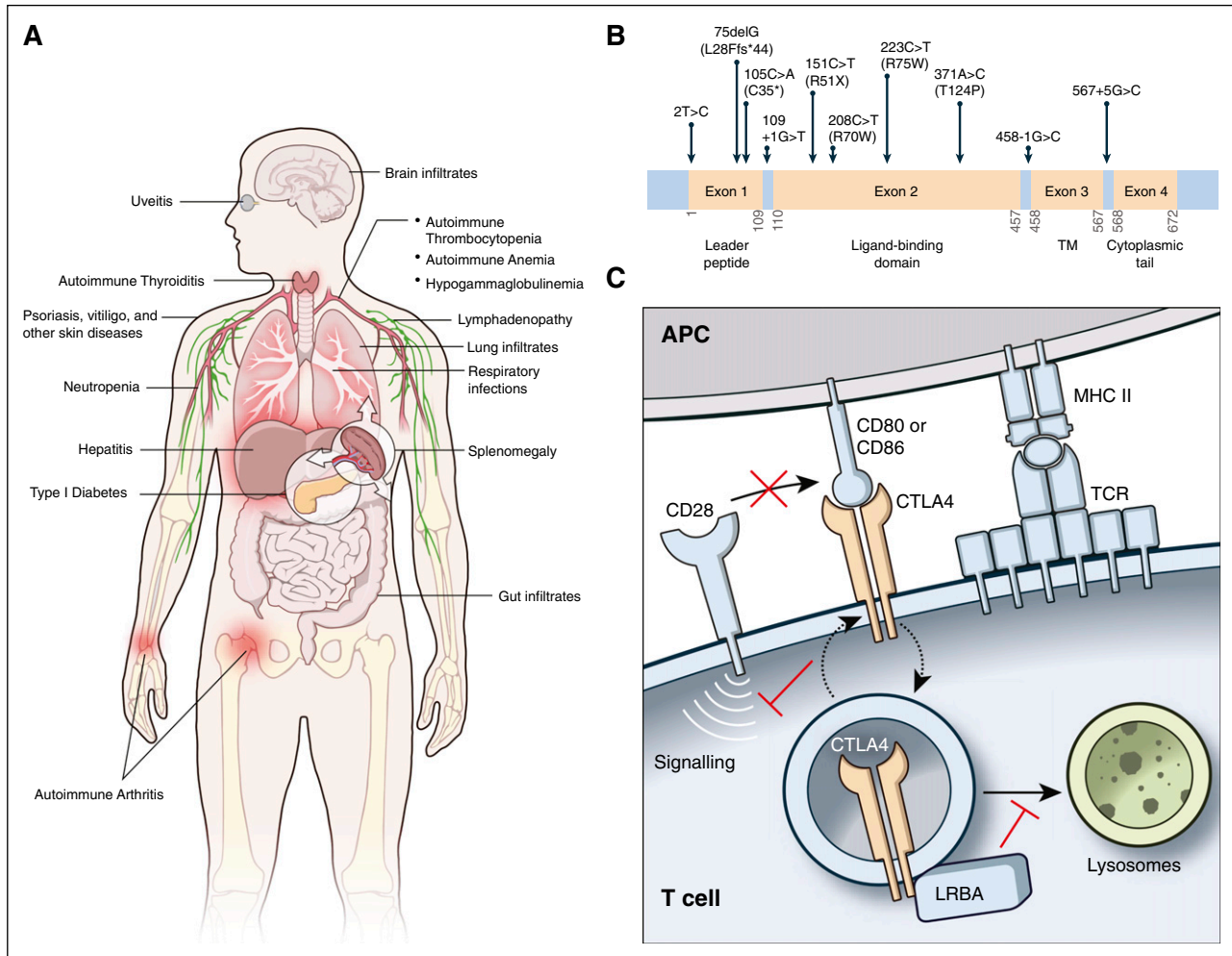
*CTLA4* mutations in CHAI patients are heterozygous. *LRBA* mutations in LATAIE patients are homozygous or compound heterozygous.

AIHA, autoimmune hemolytic anemia; HGG, hypogammaglobulinemia; ILD, interstitial lung disease; ITP, immune thrombocytopenia; LAD, lymphadenopathy; ND, not determined; P, patient.

edema and compression rather than direct tissue destruction as seen in multiple sclerosis.

Importantly, there are some LATAIE patients that present only with ALPS-like disease and no other autoimmune manifestations or lymphocytic infiltration.<sup>11</sup> These mutations may retain residual LRBA function and/or protein expression leading to a milder phenotype.

In addition to the ALPS-like disorder and lymphocytic infiltration of gut, lungs, and/or brain, some CHAI and LATAIE patients have other autoimmune manifestations or infiltration of other organs (ie, kidney, liver, or bone marrow) less frequently. Autoimmune disorders reported include type 1 diabetes, autoimmune thyroiditis, arthritis, psoriasis or other skin disorders, uveitis, vitiligo, and myasthenia gravis. These



**Figure 1. CHAI and LATAIE disease phenotype and mechanism.** (A) Clinical features of CHAI and LATAIE disease. (B) Schematic of the *CTLA4* exons showing the mutations in CHAI patients. TM, transmembrane domain. A schematic displaying *LRBA* mutations causing LATAIE can be found in Lo et al,<sup>12</sup> Alkhairy et al,<sup>17</sup> and Gámez-Díaz et al.<sup>18</sup> (C) Model depicting the function of CTLA-4 and its regulation by LRBA.

additional autoimmune disorders appear to be more common in LATAIE than CHAI disease.

### Genetic mutation spectrum

CHAI and LATAIE disease are caused by loss-of-function mutations in *CTLA4* and *LRBA*, respectively. CTLA-4 is a single-pass type I transmembrane cell surface receptor of 223 amino acids (~25 kDa). The majority of *CTLA4* reported mutations causing CHAI resulted in loss of protein expression and include insertions/deletions (frameshift), nonsense, missense, splicing mutations, and 1 substitution mutation disrupting the start codon (Figure 1B).<sup>4,5,16</sup> Two mutations led to alternative splicing, which removed the transmembrane domain resulting in soluble CTLA-4 rather than the more functional membrane-bound form (Figure 1B).<sup>4</sup> Two missense mutations appeared to interfere with ligand binding.<sup>5</sup> LRBA is an enormous protein (2863 amino acids, ~319 kDa) with multiple lipid and protein interaction domains. Biallelic *LRBA* mutations in LATAIE disease include frameshift (insertions/deletions), nonsense, missense, splice site mutations, and large multiexon deletions.<sup>6-14,18</sup> All mutations decrease LRBA protein levels, and missense mutations that permit residual LRBA protein expression cause milder clinical disease.<sup>12</sup> Thus, the common mechanism shared by these 2

disorders is that the mutations, whether directly in the *CTLA4* gene or in its regulator LRBA, lead to deficient CTLA-4 surface expression or function.

### Pathogenesis

CTLA-4 is a crucial T-cell inhibitory receptor. It restrains immune responses by negative signaling or competing with its homolog CD28, the principal T-cell costimulatory molecule, critical for inducing maximal T-cell proliferation.<sup>19</sup> CD28 and CTLA-4 compete for the same ligands, CD80 and CD86, on the surface of antigen-presenting cells (Figure 1C). Moreover, CTLA-4 binds CD80 and CD86 with significantly higher affinity and avidity than CD28 and outcompetes CD28 for its ligands. CTLA-4 can also strip these ligands from antigen-presenting cells by transendocytosis, via which the ligands are internalized and degraded in the T cell.<sup>20-22</sup> CD28 is expressed constitutively on all naive and most resting T cells; however, CTLA-4 is expressed only after activation in conventional T cells while it is constitutively expressed on Tregs.<sup>23-25</sup> Thus, Tregs are the chief mediator of CTLA-4 inhibitory function. Most CTLA-4 is stored in recycling endosomes, which cycle to the cell surface following T-cell activation.<sup>26,27</sup> This allows CTLA-4 to spatially and temporally control

T-cell responses. The multiorgan lymphocytic infiltrative disease in *Ctla4* knockout mice and CTLA-4 haploinsufficient (CHAI) patients illustrates the importance of CTLA-4 in restraining T-cell responses. In mice with complete germ-line deficiency of CTLA-4, unrestrained T-cell expansion causes death at 4 weeks old,<sup>2,3</sup> whereas the loss of CTLA-4 selectively on Treg cells leads to fatal lymphoproliferation by ~7 weeks of age.<sup>28</sup> In CHAI patients, compromising a single allele can predispose them to severe infiltrative T lymphoproliferative disease, with the onset of disease varying among patients.<sup>4,5</sup> Thus, CTLA-4 performs vital quantitative regulation of T lymphocyte expansion.

LATAIE patients have lymphocytic infiltrative disease resembling that of CHAI patients, and we now understand why.<sup>12</sup> LRBA is a member of a gene family involved in vesicle trafficking,<sup>29,30</sup> and we found that LRBA regulates CTLA-4 turnover in endosomes (Figure 1C).<sup>12</sup> LRBA helps maintain an intracellular vesicular pool of CTLA-4 for immediate mobilization to the cell surface as needed.<sup>12</sup> Loss of LRBA leads to the rapid shuttling of CTLA-4 vesicles to lysosomes for degradation. We found that inhibiting lysosomal degradation with the drug chloroquine or other inhibitors of the lysosome rescued CTLA-4 loss in vitro.<sup>12</sup> Interestingly, CTLA-4 degradation because of defective LRBA in LATAIE patients can lead to lower total overall levels of CTLA-4 than seen in CHAI, which may explain why disease is more penetrant in LATAIE than in CHAI patients.

In both CHAI and LATAIE disease, hypogammaglobulinemia and low circulating B-cell numbers are observed. However, because optimal humoral immunity depends on CD28 signaling and T-cell help, CTLA-4 loss might have been expected to augment antibody levels and B-cell numbers as seen in knockout mouse models.<sup>2,31-33</sup> Instead, patient B-cell numbers and antibody levels progressively decline over time. These findings appear to be explained, at least in part, by a corresponding increase in CD21<sup>lo</sup> B cells, which have previously been described as “exhausted,” characterized by a state of functional unresponsiveness following persistent activation.<sup>4,34,35</sup> Thus, the heightened T-cell help and chronic B-cell stimulation because of CTLA-4 loss ultimately compromises B-cell function. Although B-cell abnormalities in LATAIE patients resemble that found in CHAI patients, indicating CTLA-4 loss as the culprit, LRBA is expressed in B cells and other non-CTLA-4-expressing cell types and, therefore, may also have yet undiscovered CTLA-4-independent cellular effects.

## Therapeutic considerations

Since both CHAI and LATAIE disease result from a functional loss of CTLA-4, drugs targeting the CD28/CTLA-4 pathway or Tregs, which constitutively express CTLA-4, could be effective treatments for either disease. Abatacept, a CTLA-4-immunoglobulin fusion drug, which may act as a pharmacologic “replacement” of CTLA-4, has achieved substantial and lasting improvement of interstitial lung disease in LATAIE patients.<sup>12</sup> Abatacept has also been reported to mitigate autoimmune symptoms in a CHAI patient.<sup>36</sup> CTLA-4 is critical for the proper immunosuppressive function of Tregs, which preserve immune homeostasis and tolerance.<sup>28</sup> Sirolimus, a widely used mechanistic target of rapamycin inhibitor, suppresses conventional T-cell responses but allows Tregs to preferentially expand and maintain their suppressive function because they resist its inhibitory effects.<sup>37-40</sup> Accordingly, sirolimus has been reported to alleviate autoimmune symptoms in some CHAI patients.<sup>4</sup> In our unpublished experience, sirolimus has also been observed to benefit some LATAIE patients, though not all. A variety of other immunosuppressive drugs have been used for treating CHAI and LATAIE with varying results, and those for LATAIE patients have been briefly reviewed by Gámez-Díaz et al.<sup>18</sup>

Chloroquine, a lysosomal inhibitor drug, was found to augment CTLA-4 levels in vitro in Treg or activated T cells, especially for LRBA-deficient cells.<sup>12</sup> Therefore, chloroquine or hydroxychloroquine, a more widely used derivative, warrant further study of their utility as a therapy for CTLA-4-deficiency diseases. Although chloroquine/hydroxychloroquine are most widely used as antimalarials, they have also shown efficacy in treating rheumatoid arthritis and lupus.<sup>41-43</sup> It would be interesting to investigate whether the latter may be attributable to CTLA-4.

Another therapeutic strategy for CHAI and LATAIE patients has been hematopoietic stem cell transplantation (HSCT). At least 8 CHAI and 8 LATAIE patients have undergone HSCTs.<sup>10-13,18,44,45</sup> Eleven of the transplants were successful, although 1 died 2.5 years post-HSCT because of diabetic ketoacidosis. Three other transplants resulted in death shortly after transplant, and 2 outcomes have yet to be reported. Four of the CHAI patients experienced graft-versus-host disease with 1 being fatal. Thus, aggressive graft-versus-host disease prophylaxis may be recommended for future patients. Overall, HSCT may be an effective treatment option, but because of its mortality risk and the variability of patient phenotypes, it should perhaps be reserved for more severe cases.

## CTLA-4 blockade in cancer immunotherapy

Immune checkpoint therapy has shown considerable promise in enhancing antitumor responses that improve clinical outcome for cancer patients.<sup>1</sup> However, the severe clinical phenotypes of CHAI and LATAIE patients underscore the importance of these negative regulatory molecules in preventing autoimmunity, lymphoproliferation, and unnecessary tissue damage in humans. Ipilimumab, a monoclonal antibody directed against CTLA-4, was recently approved by the US Food and Drug Administration in 2011 for the treatment of metastatic melanoma. This approval was the result of multiple late-phase clinical trials demonstrating that ipilimumab significantly increased the mean and long-term survival of patients with advanced melanoma.<sup>46,47</sup> Notably, approximately two-thirds of patients treated with ipilimumab experienced immune-related adverse events associated with CTLA-4 blockade. Similar to individuals with genetic CTLA-4 deficiency, these immunotoxicities often affected the gastrointestinal tract with approximately one-third of ipilimumab-treated patients having diarrhea or colitis. CTLA-4 blockade in melanoma patients also commonly caused skin-related disorders associated with increased lymphocytic infiltration and melanocyte apoptosis, which differs from those afflicted with CHAI and LATAIE disease.<sup>46,48</sup> These side effects were reported to be successfully managed with systemic corticosteroids and/or anti-tumor necrosis factor  $\alpha$  antibody (for diarrhea or colitis).<sup>46</sup> Depending on the length of CTLA-4 blockade treatment, other organ-specific pathologies or autoantibody-mediated disorders observed in CHAI and LATAIE disease may emerge. In the investigations thus far, the survival benefits for advanced-stage cancer patients outweigh the risks because the immune-related adverse effects have generally not been life-threatening.

## Conclusion

Although the immune dysregulation disorders CHAI and LATAIE are caused by mutations in 2 different genes, they share a common underlying etiology, the functional loss of the critical immune regulatory molecule CTLA-4. Thus, they share similar clinical phenotypes, and the same treatment strategies may be effective for either disease. CTLA-4 blockade in cancer immunotherapy can result in adverse events that

resemble the autoimmune phenomena of CHAI and LATAIE disease. Overall, these 2 genetic diseases and the autoimmune adverse events of CTLA-4 blockade therapy illustrate the crucial role of CTLA-4 in suppressing autoimmunity and lymphoproliferation in humans.

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