Immune deficiency and autoimmunity in patients with CTLA-4 mutations

Nisha Verma[1], Siobhan O Burns [1,2], Lucy SK Walker[2] and David M Sansom[2].

[1]Clinical Immunology Department, Royal Free Hospital, Pond St, London, NW3 2QG, UK

[2]Institute of Immunity and Transplantation, Division of Infection & Immunity, School of Life and Medical Sciences, University College London, Royal Free Hospital, Rowland Hill Street, London, NW3 2PF, UK

Correspondence to:

Prof. DM Sansom
UCL Institute of Immunity and Transplantation
Royal Free Hospital
Rowland Hill Street
London
NW3 2PF
UK

Email: d.sansom@ucl.ac.uk
Tel: 0207 794 0500 x22469

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/cei.12997

Abstract

Immune deficiency disorders are a heterogeneous group of diseases of variable genetic aetiology. Whilst the hallmark of immunodeficiency is susceptibility to infection, it is increasingly clear that autoimmunity is prevalent suggestive of a more general immune dysregulation in some cases. With the increasing use of genetic technologies the underlying causes of immune dysregulation are beginning to emerge. Here we provide a review of the heterozygous mutations found in the immune checkpoint protein CTLA-4, which was originally identified in cases of Common Variable Immunodeficiency Disorders (CVID) with accompanying autoimmunity. Study of these mutations provides insights into the biology of CTLA-4 as well as suggesting approaches for rational treatment of these patients.

Biology of the CTLA-4 pathway

The CTLA-4 pathway can be thought of as an integrated system involving two opposing receptors CD28 (stimulatory) and CTLA-4 (inhibitory). CTLA-4 and CD28 are transmembrane proteins expressed predominantly by T lymphocytes that interact with two shared ligands, CD80 and CD86, found on antigen presenting cells (**Figure 1**). The two ligands differ in both expression pattern and interaction affinities, however there is still limited data on how they might differentially affect immune function. In line with their relatively small size and their role in regulating T cell responses, both CD28 and CTLA-4 can be found interacting with their ligands at the immune synapse, where the higher affinity CTLA-4 interactions can compete with CD28 for ligand binding[1, 2].

Whereas CD28 is found at the plasma membrane on T cells, CTLA-4 expression is more complex. CTLA-4 is induced following activation of conventional T cells and is constitutively expressed by Treg. Approximately 10% of CTLA-4 is expressed at the membrane at any given time, with 90% occupying a variety of intracellular compartments. This is as a result of CTLA-4 being internalized via endocytosis, due to the presence of a “YVKM” motif in its cytoplasmic tail [3]. This motif interacts constitutively with the clathrin adaptor AP-2 resulting in rapid internalization of CTLA-4, in the absence of ligand binding[4]. Internalization exposes CTLA-4 to both re-cycling and degradation within the cell and it is becoming increasingly clear that correct trafficking is critical to its function.

Whilst CTLA-4 internalisation is not well understood, it is nonetheless in keeping with the ability of CTLA-4 to carry out a process, termed transendocytosis(TE)[5]. Here CTLA-4 binds its ligands at the plasma membrane and subsequently internalises them resulting in their removal from the APC. This results in APCs that have impaired expression of ligands and are therefore unable to stimulate T cells through CD28. Such a process potentially explains why CD28 and CTLA-4 share ligands and is consistent with a mechanism whereby CTLA-4 is highly expressed by Treg and can suppress T cell responses in a cell-extrinsic manner.

In summary, CD28 and CTLA-4 are functionally opposing receptors expressed by T cells, which interact with the same ligands but with differing affinities and cell biology. These interactions play a central role in balancing both effective immune responses to pathogens as well as regulating autoimmune responses against self-tissues.

The Immunology of CTLA-4 and CD28.

It is increasingly apparent that thymic selection fails to remove all self-reactive T cells [6, 7], resulting in significant numbers of potentially autoimmune T cells in the circulation of healthy individuals. Not only is the TCR repertoire highly cross reactive, making tolerance by deletion difficult [8] but autoreactive T cells appear to be present at frequencies similar to other antigen specificities [9], arguing deletion is incomplete. Additional levels of control of T responses (and subsequently B cell responses) to self-antigens are therefore required and it is here that the CTLA-4 receptor is of critical importance [10-12]. It is now established that complete loss of

CTLA-4 in mice causes fatal autoimmunity within ~3 weeks of birth [13, 14] demonstrating a key, non-redundant role for CTLA-4 in preventing autoimmune responses. Furthermore, the description of humans carrying CTLA-4 mutations and suffering from profound autoimmunity [15, 16] suggests that the autoimmune protective effects of CTLA-4 are evolutionarily conserved. Similarly, anti-CTLA-4 antibodies used to block CTLA-4 function in the cancer setting can be thought of as treatment-induced autoimmunity against tumour antigens[17]. Taken together these data argue unequivocally that CTLA-4 is an essential regulator of immune responses to our own tissues.

In contrast, to CTLA-4, CD28 provides signals that enhance T cell activation and is widely recognised as the archetypal "co-stimulatory" receptor, alongside the TCR. Since CD28 ligands are upregulated by inflammatory signals such as those following Toll-like receptor recognition, CD28 signalling represents "danger" in the context of antigen recognition, driving T cell activation, differentiation and effector function [18]. Thus CD28 engagement by its ligands on antigen presenting cells is responsive to inflammation, providing a direct connection between the innate and adaptive immune systems. Interestingly one of the major functions of CD28 costimulation is to facilitate help for B cells via the generation of Tfh cells. Accordingly in the absence of CD28, class switched antibody responses are virtually absent[19], germinal centers are impaired [20] and even heterozygous loss at the CD28 locus impacts on B cell responses[21]. A second major feature of the requirement for CD28 costimulation is in the generation and survival of regulatory T cells. Accordingly, in situations where CD28 costimulation is prevented, for example in mice lacking CD28 or its ligands,or in the presence of ligand blockade [22-24] [25] there is an accompanying loss of Treg. Thus, CD28 signalling drives the generation of effector T cell responses, is critical for class-switched antibody generation and supports homeostasis of Treg whilst CTLA-4 opposes these. Together, these features highlight the delicate balance, which is the hallmark of the CD28 / CTLA-4 pathway.

Whilst the costimulatory function of CD28 is relatively clear, the biological function of CTLA-4 has been more difficult to discern. What is beyond doubt is that CTLA-4 is a negative regulator of T cell responses. Furthermore, CTLA-4 is needed to oppose CD28 function, since inhibiting CD28 in mice can prevent disease caused by CTLA-4 deficiency and increasingly, such concepts appear to be replicated in humans. Exactly how CTLA-4 functions at the molecular level remains controversial, with discussion focusing on cell-intrinsic (e.g. signaling) vs cell-extrinsic (e.g. regulatory) mechanisms [10-12, 26-28]. Whilst it was initially proposed that CTLA-4 generated inhibitory signals that prevented TCR signaling (cell intrinsic effects), experimental evidence now appears to favour the view that the major CTLA-4 functions are T cell-extrinsic and necessary for the effective function of regulatory T cells[29-32]. For example, in mixed bone-marrow chimeric mice CTLA-4-/- T cells behave indistinguishably from CTLA-4+/+ [33-35] indicating that CTLA-4+/+ cells can extrinsically control the behaviour of CTLA-4-/- T cells in a "regulatory" manner. These concepts become significant when it comes to investigating the impact of CTLA-4 deficiency in patients and how to determine defective CTLA-4 function.

The idea that CTLA-4 on T cells acts extrinsically to regulate the behavior of other T cells, is in keeping with the TE model of CTLA-4 function. Here CTLA-4 acts as a competitor for CD28 stimulation by physically removing their shared ligands from APCs [5] (**Figure 2**),consistent with CTLA-4 as an effector mechanism for Treg suppressive activity. However, a number of other models have also been proposed including inhibitory signalling, triggering of tryptophan metabolism and effects on T cell motility (see[11, 26-28] for reviews). Currently, the actual mechanisms critical to CTLA-4 function in vivo remain to be formally established.

Identification of CTLA-4 deficiency and clinical features

Overactivity of the immune system resulting in autoimmune and inflammatory complications is a well-recognized paradox in Primary immunodeficiency (PID) disorders[36]. For example, in addition to antibody deficiency, a spectrum of immune dysregulation is seen in CVID, which

includes, but is by no means limited to B-cell and auto-antibody mediated conditions. Lymphocytic infiltration and granuloma formation, particularly involving gut, lung and liver are hallmarks of such associated immune dysregulation and likely to involve multiple immune lineages. Recently, loss of function mutations in CTLA-4 have emerged, mainly from cohorts of patients diagnosed with antibody deficiency and severe immune dysregulation[16] [15] providing insight into the pathogenesis of immune dysregulation in a subset of patients.

Schubert et.al. investigated a large kindred with 5 family members affected by hypogammaglbulinaemia, recurrent respiratory tract infections, autoimmune cytopenia, autoimmune enteropathy and infiltrative lung disease (who were found to have heterozygous mutations in CTLA-4 [15]. Genetic analysis revealed the same CTLA-4 mutation in 6 other family members considered to be healthy. Based on clinical phenotype, other families with CVID and enteropathy or autoimmunity were investigated revealing 5 additional index patients with novel CTLA-4 mutations, 3 asymptomatic mutation 'carriers' and a further 4 patients based on family history.

Another study[16], reported 9 patients with CTLA-4 mutations across 4 unrelated families with a history of hypogammaglobulinaemia, CD4+ T cell lymphopenia, autoimmune cytopenias and lymphocytic infiltration. Of these, six individuals were clinically symptomatic with three reportedly healthy. Additional sporadic cases have been reported [37-40] and a further 8 patients with CTLA-4 mutations who underwent stem cell transplantation have also been described [41]. Similar clinical features were reported in most cases, as well as additional complications that extend the spectrum of disease including autoimmune hepatitis, primary sclerosing cholangitis, pure red cell aplasia, adrenal insufficiency, type I diabetes, arthritis, and autoimmune choirodopathy.

The reported clinical features of CTLA-4 deficiency described to date are summarized in Table 1. With the exception of recurrent upper and lower respiratory tract infections, almost all relate to immune dysregulation and autoimmunity. Splenomegaly and lymphadenopathy are common and extensive $CD4^+$ T cell organ infiltration can be seen in multiple organs, including the intestine, lung, bone marrow, central nervous system and kidney. Although opportunistic infections do not appear to be a prominent feature, one patient was diagnosed with CMV gastritis prior to development of gastric carcinoma [39] albeit following steroid treatment. Malignancy risk appears to be elevated with 3 patients developing gastric carcinoma and two Hodgkin's lymphoma. It is not known yet if currently classified asymptomatic/healthy patients progress at a later stage and this will be an important question for follow up studies.

Reduced immunoglobulin levels were noted in most symptomatic patients with heterozygous CTLA-4 mutations [15, 16]. This was associated in the majority of individuals with a low or progressively falling percentage of CD19+ B cells, a low percentage of switched memory B-cells and a predominance of $CD21^{lo}$ B-cells, which are thought to represent exhausted B-cells producing lower levels of immunoglobulin in vitro. In addition, there is evidence of excessive T-cell activation indicated by low levels of naive T-cells and elevated expression of the surface molecule PD-1 (Programmed cell death protein 1). FOXP3+ (forkhead box P3) regulatory T-cells are present at normal or even higher percentages but these express low levels of CTLA-4 and function poorly [42]. Conventional T-cell proliferation and in vitro T-cell differentiation are preserved.

The majority of mutations in CTLA-4 affect the CTLA-4 extracellular domain(**Figure 3**). However, there does not appear to be a clear genotype-phenotype correlation and incomplete clinical penetrance has not been fully explained. Additional factors including genetic and epigenetic modifiers, microbial exposure or environmental factors may therefore influence the clinical outcome of CTLA-4 deficiency. It is interesting to note that bi-allelic loss of function mutations in LRBA (lipopolysaccharide responsive beige-like anchor protein) result in a phenocopy disease with autosomal recessive inheritance and severe early onset [43]. It is now

known that CTLA-4 requires LRBA for regulation of its intracellular trafficking, so that loss of LRBA expression effectively reduces CTLA-4 expression, resulting in functional CTLA-4 deficiency[44].

Lessons from CTLA-4 deficiency in humans

The fact that CTLA-4 deficiency results in highly variable features of autoimmunity appears to result from inappropriate activation of polyclonal T cells. This maybe observed clinically in that generally these patients have expanded populations of memory T cells. Thus whilst presentation of disease is highly variable it is consistent with the concept of increased CD28 costimulation triggering self-reactive T cells against a variety of tissues. Similarly in CTLA-4-deficient mice, T cells are seen to infiltrate multiple tissues and to recognize autoantigens[13, 14, 45]. It may be significant that many of the sites worst affected, eg. Gut, lungs and skin are also sites where there is an interface with the microbiome, where the levels of costimulatory molecules on APCs may be higher.

A key feature of CTLA-4 deficiency in humans is the impact on the regulatory T cell compartment. Consistent with a T cell-extrinsic role for CTLA-4, a Treg functional deficiency is observed in these patients, with an impaired ability to suppress T cell proliferation [15, 16]. This defect relates to impaired CTLA-4 function since suppression in these experiments is abrogated by anti-CTLA-4 antibody[15]. In addition, decreased ability of Treg from CTLA-4 mutation carriers (both clinically affected and unaffected) to carry out transendocytosis or to uptake soluble CTLA-4 ligands was also observed [42]. Since Treg express the highest levels of CTLA-4 it may be that deficiency in one allele is sufficient to compromise the high levels of expression required for effective function.

Given the role of CD28 in Treg homeostasis[25, 46, 47] it might be expected that Treg proportions are affected in CTLA-4 deficiency. Mice deficient in CTLA-4 show marked expansions in Treg numbers due to the increased availability of CD28 signalling[48] and similar expansions are seen in a number of CTLA-4 deficient patients. It should be noted that Treg analysis is frequently confounded by the low expression of CD25, which is seen in many CVID patients, and precludes the use of CD25 as a marker for Treg. However we have observed that Foxp3 staining, particularly following a brief stimulation, reveals that Treg numbers are generally normal or elevated in these patients with some individuals displaying very marked expansions[42].

Whilst the impact of CTLA-4 deficiency on Treg biology is clear, its effects on activated T cells, which also express CTLA-4 are more difficult to determine. It has been suggested that conventional CD4 and CD8 T cells in individuals with CTLA-4 mutations can be hyperproliferative[16] [38, 49]. However, this has not been our experience and such experiments require careful set up and interpretation. We have previously shown CTLA-4 plays little if any intrinsic role in regulating the proliferation of human CD4 T cells[50] and nor is CTLA-4 function readily measured in assays where CD3/CD28 antibodies are used as a stimulus. Given that patients with CTLA-4 deficiency often have a preponderance of activated and memory T cells in their blood, direct comparisons with control samples are confounded unless this is corrected for. Thus further studies are needed andit is not yet clear that in vitro hyperproliferation of conventional T cells is a direct consequence or a useful measure of CTLA-4 deficiency.

Treatment options for CTLA-4 deficiency

Management of patients with CTLA-4 deficiency requires attention to both the immunodeficiency

and immune dysregulation features of disease. Immunoglobulin replacement therapy, with or without prophylactic antibiotics, represents the main conservative management approach for infection prophylaxis. However, given that CTLA-4 deficiency is in essence a T cell hyperactivation disorder, immunosuppressive compounds are frequently required, despite the potential for worsening immunodeficiency. Given the rare nature of the condition, there are no agreed treatment guidelines for managing the associated immune dysregulation and very little evidence for long-term response to different immunosuppressive agents that could be used to rationalize drug selection.

In CTLA-4 deficiency, limited information from case reports indicates that that corticosteroids have been the most consistently utilised immunosuppressive for autoimmune cytopoenias, enteropathy, infiltrative lung disease and CNS inflammation [15, 16]. Reported responses are variable and the need for high dose and recurrent doses of steroids is apparent. A number of steroid sparing agents have also been employed including sirolimus in multiple patients, rituximab, MMF, Cyclosporin A, anti-thymocyte globulin, anti-TNF drugs and vedoluzimab, with improvement in some but not all cases [15, 16] [37, 38]. Even for individual patients, some features of immune dysregulation may respond to a given drug while other complications remain resistant. The need for recurrent, multiple and serial steroid sparing agents in CTLA-4 deficiency highlights the severity of immunopathology in this condition as well as the inadequacy of current treatment approaches.

Abatacept, a CTLA-4-immunoglobulin fusion protein licensed for the treatment of rheumatoid arthritis[51], holds promise as a more rational and targeted approach to treatment. Since abatacept is a soluble version of CTLA-4 itself it may be considered as a CTLA-4 replacement therapy. Additional experience has been published for use of Abatacept to treat infiltrative lung disease in LRBA deficiency with an improvement in the clinical status, HRCT chest radiological appearances and pulmonary function tests in 3 patients using a dosing regime of 20 mg/kg every 2-4 weeks for up to 8 years[44]. Chronic norovirus was observed in two of the patients but otherwise there were minimal infectious with no deterioration in lung disease whilst on treatment. In that study an additional 3 patients commenced Abatacept for gastrointestinal disease with reported improvement in 2/3 over a short follow up of less than 6 months on treatment, with up to 30mg/kg dosing 2 weekly. To date, there are only two instructive published reports of Abatacept use in CTLA-4 deficiency. In one case, an unspecified dose of Abatacept was used for autoimmune choroidopathy with good response within 3 months [40]. In the second case, treatment was instigated for enteropathy and autoimmune hemolytic anemia (dose 10mg/kg two weekly) with good response and no reported side effects [49]. In addition, sustained remission over several months was achieved with a maintenance dose of 5mg/kg monthly. It is still not clear whether Abatacept is sufficient as a sole long-term immunosuppressive agent for patients with CTLA-4 deficiency and future trials should also consider Belatacept which is a similar molecule with a higher ligand affinity, currently in trial for prevention of renal transplant rejection(https://clinicaltrials.gov).

Haematopoetic stem cell transplantation (HSCT) has been used as a treatment strategy for life threatening, treatment resistant immune dysregulation in CTLA-4 deficiency[41]. 8 patients with severe clinical disease were transplanted with an age range of 10 - 32 years. 6 were alive and well in April 2016 (3.5mo, 4mo, 2yr, 4yr, 4.75yr and 10.2yr post transplant respectively), one with ongoing sirolimus for resolving chronic GvHD. In total GHVD was seen in 4 patients, and led to death in one case. A second patient died two and half years post-transplant from diabetic ketoacidosis. These results are encouraging and demonstrate that CTLA-4 deficiency can be potentially cured by HSCT, although GVHD risk may be higher because of the inflammatory burden at transplantation making autologous gene therapy approaches an attractive proposition for the future.

Conclusions

CTLA-4 deficiency is a novel PID that joins the growing subset of inherited immunodeficiencies associated with prominent autoimmunity and immune dysregulation. It is evident that Treg dysfunction is a key mechanism of the immune activation associated with CTLA-4 loss of function mutations although the cellular causes of immunodeficiency remain unclear. The availability of pathway-specific treatments that provide a soluble form of CTLA-4 hold promise for targeted therapy and highlight the importance of gene discovery to improve treatment for PID presenting outside, as well as during, childhood. Finally, lessons learnt from CTLA-4 in PID have application for other common autoimmune diseases and potentially cancer immunotherapy.

The authors have no competing financial interests.

Table1: Main clinical features associated with confirmed CTLA-4 mutations.

Lung: - Granulomatous Lymphocytic Interstital Lung Disease (infiltrative lung disease) - Fibrosis - Bronchiectasis
Cytopenias: - Autoimmune Haemolytic anaemia - Autoimmune thrombocytopenia - Autoimmune Neutropenia
Gut: - Diarrhoea/Enteropathy - Gastric cancer
Autoimmunity: - Type 1 diabetes - Autoimmune thyroiditis - Arthritis - Psoriasis - Uveitis - Vitiligo - Myasthenia gravis.
Others: - Lymphadenopathy - Splenomegaly - Lymphocytic infiltration of non lymphoid organs (Lung, bone marrow, gut, brain) - Malignancy

References

1. Egen JG, Allison JP. Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. Immunity 2002; **16**:23-35.
2. Yokosuka T, Kobayashi W, Takamatsu M, Sakata-Sogawa K, Zeng H, Hashimoto-Tane A, Yagita H, Tokunaga M, Saito T. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. Immunity 2010; **33**:326-39.
3. Shiratori T, Miyatake S, Ohno H, Nakaseko C, Isono K, Bonifacino JS, Saito T. Tyrosine phosphorylation controls internalization of CTLA-4 by regulating its interaction with clathrin-associated adaptor complex AP-2. Immunity 1997; **6**:583-9.
4. Qureshi OS, Kaur S, Hou TZ, Jeffery LE, Poulter NS, Briggs Z, Kenefeck R, Willox AK, Royle SJ, Rappoport JZ, Sansom DM. Constitutive clathrin-mediated endocytosis of CTLA-4 persists during T cell activation. J Biol Chem 2012; **287**:9429-40.
5. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, Hou TZ, Futter CE, Anderson G, Walker LS, Sansom DM. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science 2011; **332**:600-3.
6. Malhotra D, Linehan JL, Dileepan T, Lee YJ, Purtha WE, Lu JV, Nelson RW, Fife BT, Orr HT, Anderson MS, Hogquist KA, Jenkins MK. Tolerance is established in polyclonal CD4(+) T cells by distinct mechanisms, according to self-peptide expression patterns. Nat Immunol 2016; **17**:187-95.
7. Hogquist KA, Jameson SC. The self-obsession of T cells: how TCR signaling thresholds affect fate 'decisions' and effector function. Nat Immunol 2014; **15**:815-23.
8. Sewell AK. Why must T cells be cross-reactive? Nature reviews Immunology 2012; **12**:669-77.
9. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. Immunity 2013; **38**:373-83.
10. Walker LS, Sansom DM. The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. Nature Reviews Immunology 2011; **11**:852-63.
11. Walker LS, Sansom DM. Confusing signals: Recent progress in CTLA-4 biology. Trends in immunology 2015; **36**:63-70.
12. Schildberg FA, Klein SR, Freeman GJ, Sharpe AH. Coinhibitory Pathways in the B7-CD28 Ligand-Receptor Family. Immunity 2016; **44**:955-72.
13. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science 1995; **270**:985-8.
14. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 1995; **3**:541-7.
15. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, Bulashevska A, Petersen BS, Schaffer AA, Gruning BA, Unger S, Frede N, Baumann U, Witte T, Schmidt RE, Dueckers G, Niehues T, Seneviratne S, Kanariou M, Speckmann C, Ehl S, Rensing-Ehl A, Warnatz K, Rakhmanov M, Thimme R, Hasselblatt P, Emmerich F, Cathomen T, Backofen R, Fisch P, Seidl M, May A, Schmitt-Graeff A, Ikemizu S, Salzer

U, Franke A, Sakaguchi S, Walker LS, Sansom DM, Grimbacher B. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 2014; **20**:1410-6.
16. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, Schickel JN, Tran DQ, Stoddard J, Zhang Y, Frucht DM, Dumitriu B, Scheinberg P, Folio LR, Frein CA, Price S, Koh C, Heller T, Seroogy CM, Huttenlocher A, Rao VK, Su HC, Kleiner D, Notarangelo LD, Rampertaap Y, Olivier KN, McElwee J, Hughes J, Pittaluga S, Oliveira JB, Meffre E, Fleisher TA, Holland SM, Lenardo MJ, Tangye SG, Uzel G. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 2014; **345**:1623-7.
17. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015; **348**:56-61.
18. Soskic B, Qureshi OS, Hou T, Sansom DM. A Transendocytosis Perspective on the CD28/CTLA-4 Pathway. Adv Immunol 2014; **124**:95-136.
19. Shahinian A, Pfeffer K, Lee KP, Kundig TM, Kishihara K, Wakeham A, Kawai K, Ohashi PS, Thompson CB, Mak TW. Differential T cell costimulatory requirements in CD28 deficient mice. Science 1993; **261**:609-12.
20. Walker LS, Gulbranson-Judge A, Flynn S, Brocker T, Raykundalia C, Goodall M, Forster R, Lipp M, Lane P. Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. J Exp Med 1999; **190**:1115-22.
21. Wang CJ, Heuts F, Ovcinnikovs V, Wardzinski L, Bowers C, Schmidt EM, Kogimtzis A, Kenefeck R, Sansom DM, Walker LS. CTLA-4 controls follicular helper T-cell differentiation by regulating the strength of CD28 engagement. Proceedings of the National Academy of Sciences of the United States of America 2015; **112**:524-9.
22. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity 2000; **12**:431-40.
23. Bar-On L, Birnberg T, Kim KW, Jung S. Dendritic cell-restricted CD80/86 deficiency results in peripheral regulatory T-cell reduction but is not associated with lymphocyte hyperactivation. Eur J Immunol 2011; **41**:291-8.
24. Gogishvili T, Luhder F, Goebbels S, Beer-Hammer S, Pfeffer K, Hunig T. Cell-intrinsic and -extrinsic control of Treg-cell homeostasis and function revealed by induced CD28 deletion. Eur J Immunol 2013; **43**:188-93.
25. Tang Q, Henriksen KJ, Boden EK, Tooley AJ, Ye J, Subudhi SK, Zheng XX, Strom TB, Bluestone JA. Cutting edge: CD28 controls peripheral homeostasis of CD4+CD25+ regulatory T cells. J Immunol 2003; **171**:3348-52.
26. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol Rev 2009; **229**:12-26.
27. Wing K, Yamaguchi T, Sakaguchi S. Cell-autonomous and -non-autonomous roles of CTLA-4 in immune regulation. Trends in immunology 2011; **32**:428-33.
28. Bour-Jordan H, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/ B7 family. Immunol Rev 2011; **241**:180-205.
29. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008; **322**:271-5.

30. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, Appleby M, Der SD, Kang J, Chambers CA. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. J Exp Med 2009; **206**:421-34.
31. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med 2000; **192**:295-302.
32. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000; **192**:303-10.
33. Bachmann MF, Kohler G, Ecabert B, Mak TW, Kopf M. Cutting edge: lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous. J Immunol 1999; **163**:1128-31.
34. Bachmann MF, Gallimore A, Jones E, Ecabert B, Acha-Orbea H, Kopf M. Normal pathogen-specific immune responses mounted by CTLA-4-deficient T cells: a paradigm reconsidered. Eur J Immunol 2001; **31**:450-8.
35. Homann D, Dummer W, Wolfe T, Rodrigo E, Theofilopoulos AN, Oldstone MB, von Herrath MG. Lack of intrinsic CTLA-4 expression has minimal effect on regulation of antiviral T-cell immunity. Journal of virology 2006; **80**:270-80.
36. Gathmann B, Mahlaoui N, Ceredih, Gerard L, Oksenhendler E, Warnatz K, Schulze I, Kindle G, Kuijpers TW, Dutch WID, van Beem RT, Guzman D, Workman S, Soler-Palacin P, De Gracia J, Witte T, Schmidt RE, Litzman J, Hlavackova E, Thon V, Borte M, Borte S, Kumararatne D, Feighery C, Longhurst H, Helbert M, Szaflarska A, Sediva A, Belohradsky BH, Jones A, Baumann U, Meyts I, Kutukculer N, Wagstrom P, Galal NM, Roesler J, Farmaki E, Zinovieva N, Ciznar P, Papadopoulou-Alataki E, Bienemann K, Velbri S, Panahloo Z, Grimbacher B, European Society for Immunodeficiencies Registry Working P. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol 2014; **134**:116-26.
37. Navarini AA, Hruz P, Berger CT, Hou TZ, Schwab C, Gabrysch A, Higgins R, Frede N, Padberg Sgier BC, Kampe O, Burgener AV, Marquardsen F, Baldin F, Bigler M, Kistner A, Jauch A, Bignucolo O, Meyer B, Meienberg F, Mehling M, Jeker LT, Heijnen I, Daikeler TD, Gebbers JO, Grimbacher B, Sansom DM, Jeker R, Hess C, Recher M. Vedolizumab as a successful treatment of CTLA-4-associated autoimmune enterocolitis. J Allergy Clin Immunol 2017; **139**:1043-6 e5.
38. Zeissig S, Petersen BS, Tomczak M, Melum E, Huc-Claustre E, Dougan SK, Laerdahl JK, Stade B, Forster M, Schreiber S, Weir D, Leichtner AM, Franke A, Blumberg RS. Early-onset Crohn's disease and autoimmunity associated with a variant in CTLA-4. Gut 2015; **64**:1889-97.
39. Hayakawa S, Okada S, Tsumura M, Sakata S, Ueno Y, Imai K, Morio T, Ohara O, Chayama K, Kobayashi M. A Patient with CTLA-4 Haploinsufficiency Presenting Gastric Cancer. Journal of clinical immunology 2016; **36**:28-32.
40. Shields CL, Say EA, Mashayekhi A, Garg SJ, Dunn JP, Shields JA. Assessment of CTLA-4 Deficiency-Related Autoimmune Choroidopathy Response to Abatacept. JAMA Ophthalmol 2016; **134**:844-6.
41. Slatter MA, Engelhardt KR, Burroughs LM, Arkwright PD, Nademi Z, Skoda-Smith S, Hagin D, Kennedy A, Barge D, Flood T, Abinun M, Wynn RF, Gennery AR, Cant AJ, Sansom D, Hambleton S, Torgerson TR. Hematopoietic stem cell transplantation for CTLA4 deficiency. J Allergy Clin Immunol 2016; **138**:615-9 e1.

42. Hou TZ, Verma N, Wanders J, Kennedy A, Soskic B, Janman D, Halliday N, Rowshanravan B, Worth A, Qasim W, Baxendale H, Stauss H, Seneviratne S, Neth O, Olbrich P, Hambleton S, Arkwright PD, Burns SO, Walker LS, Sansom DM. Identifying functional defects in patients with immune dysregulation due to LRBA and CTLA-4 mutations. Blood 2017; **129**:1458-68.
43. Lopez-Herrera G, Tampella G, Pan-Hammarstrom Q, Herholz P, Trujillo-Vargas CM, Phadwal K, Simon AK, Moutschen M, Etzioni A, Mory A, Srugo I, Melamed D, Hultenby K, Liu C, Baronio M, Vitali M, Philippet P, Dideberg V, Aghamohammadi A, Rezaei N, Enright V, Du L, Salzer U, Eibel H, Pfeifer D, Veelken H, Stauss H, Lougaris V, Plebani A, Gertz EM, Schaffer AA, Hammarstrom L, Grimbacher B. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. American journal of human genetics 2012; **90**:986-1001.
44. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, Zhang Y, Liu Z, Fritz JM, Marsh R, Husami A, Kissell D, Nortman S, Chaturvedi V, Haines H, Young LR, Mo J, Filipovich AH, Bleesing JJ, Mustillo P, Stephens M, Rueda CM, Chougnet CA, Hoebe K, McElwee J, Hughes JD, Karakoc-Aydiner E, Matthews HF, Price S, Su HC, Rao VK, Lenardo MJ, Jordan MB. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science 2015; **349**:436-40.
45. Ise W, Kohyama M, Nutsch KM, Lee HM, Suri A, Unanue ER, Murphy TL, Murphy KM. CTLA-4 suppresses the pathogenicity of self antigen-specific T cells by cell-intrinsic and cell-extrinsic mechanisms. Nat Immunol 2010; **11**:129-35.
46. Zhang R, Huynh A, Whitcher G, Chang J, Maltzman JS, Turka LA. An obligate cell-intrinsic function for CD28 in Tregs. The Journal of clinical investigation 2013; **123**:580-93.
47. Gogishvilli T, Luhder F, Goebbels S, Beer-Hammer S, Pfeffer K, Hunig T. Cell-intrinsic and -extrinsic control of Treg-cell homeostasis and function revealed by induced CD28 deletion. Eur J Immunol 2013; **43**:188-93.
48. Schmidt EM, Wang CJ, Ryan GA, Clough LE, Qureshi OS, Goodall M, Abbas AK, Sharpe AH, Sansom DM, Walker LS. Ctla-4 controls regulatory T cell peripheral homeostasis and is required for suppression of pancreatic islet autoimmunity. J Immunol 2009; **182**:274-82.
49. Lee S, Moon JS, Lee CR, Kim HE, Baek SM, Hwang S, Kang GH, Seo JK, Shin CH, Kang HJ, Ko JS, Park SG, Choi M. Abatacept alleviates severe autoimmune symptoms in a patient carrying a de novo variant in CTLA-4. J Allergy Clin Immunol 2016; **137**:327-30.
50. Hou TZ, Qureshi O, Wang CJ, Baker J, Young SP, Walker LS, Sansom DM. A Transendocytosis model of CTLA-4 function predicts its suppressive behaviour on regulatory T cells. . J Immunol 2015; **194**:2148-59.
51. Chitale S, Moots R. Abatacept: the first T lymphocyte co-stimulation modulator, for the treatment of rheumatoid arthritis. Expert opinion on biological therapy 2008; **8**:115-22.

Figure 1

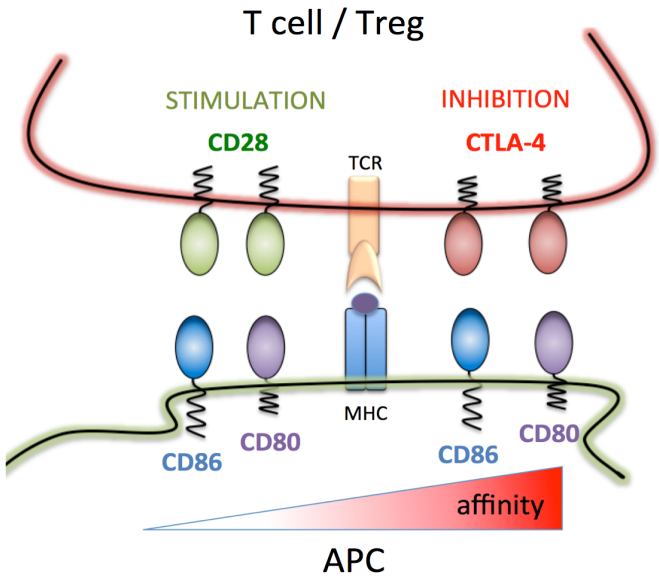

Figure 1. Interactions between CD28, CTLA-4 and their natural ligands. Cartoons shows the "immune synapse between a CTLA-4-expressing T cell or Treg and an APC expressing the two ligands CD80 and CD86. The affinity of the interactions is depicted from right to left with the lowest interaction CD86-CD28 on the left and the highest CD80-CTLA-4 on the right.

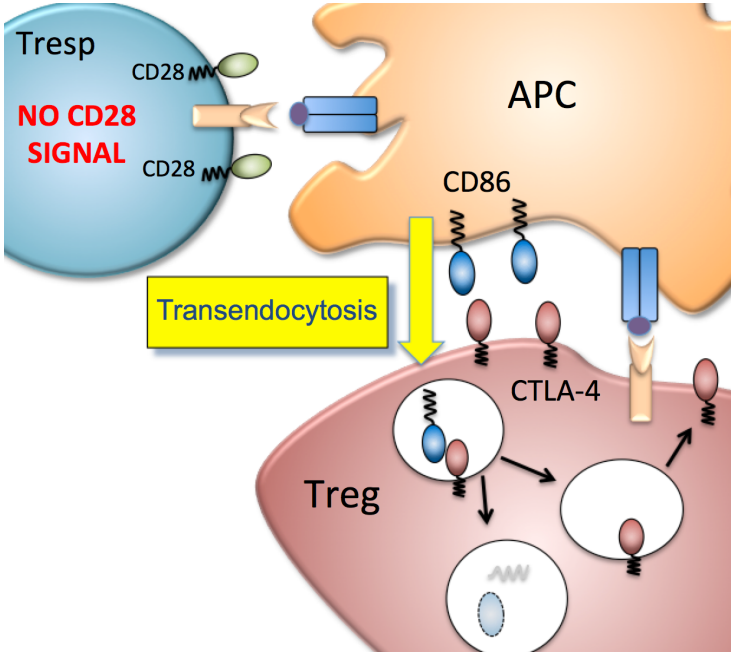

Figure 2. Mechanism of action for CTLA-4. A Regulatory T cell (Treg) expressing CTLA-4 can capture and internalise stimulatory ligands (CD86 shown) by transendocytosis. Ligands are internalised into the CTLA-4 expressing cell and degraded whilst CTLA-4 is recycled. This results in reduction of available CD86 and therefore loss of CD28 costimulatory signals in the responder T cell (Tresp). This mechanism is compatible with much of the biology of CTLA-4 but is not exclusive of other mechanisms.

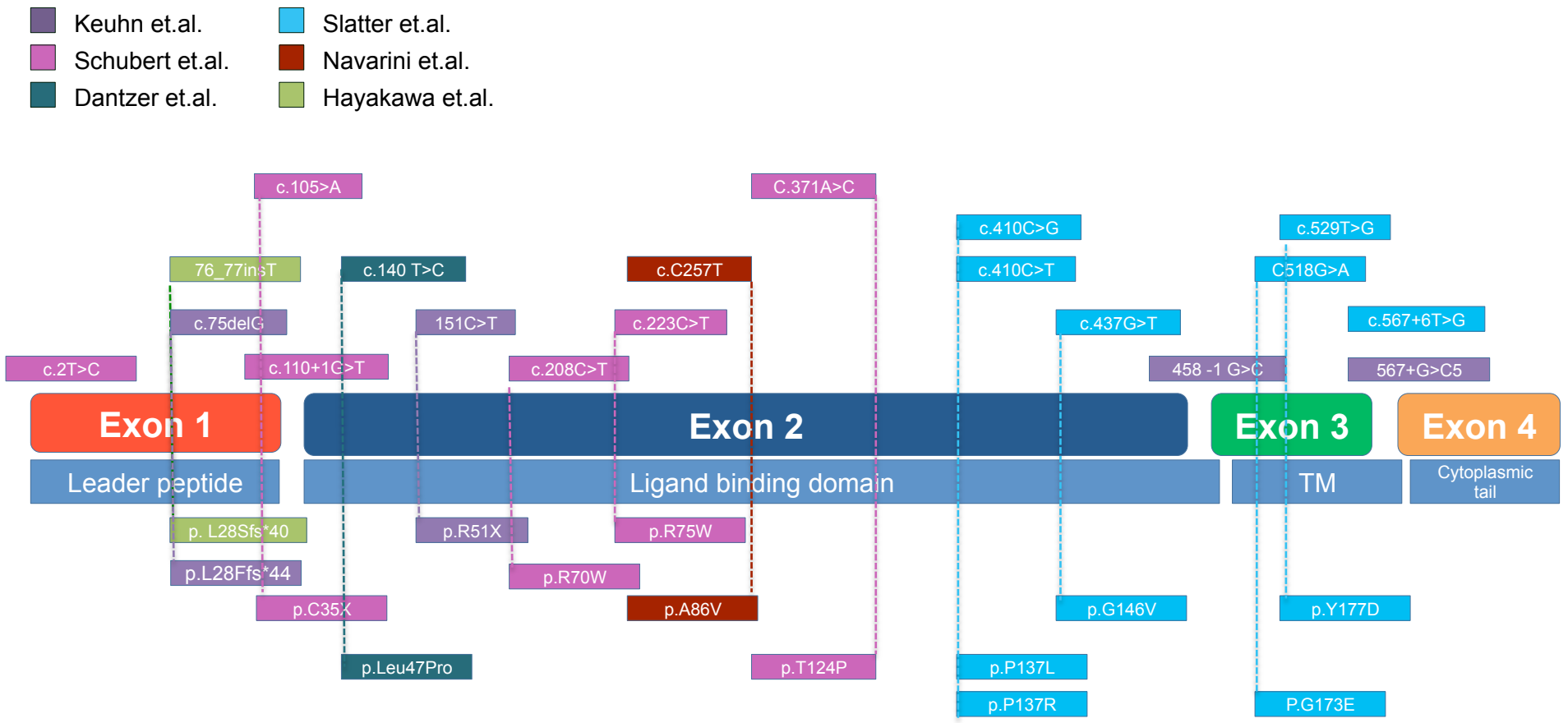

Figure 3. Schematic view of reported CTLA-4 mutations. DNA mutations are shown in the upper half of the diagram and aligned with amino acid changes below. The alignment between exons and protein functional domains is shown.Mutations are color coded according to the report in which they are described.