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Exaggerated T Follicular Helper Cell Responses in LRBA Deficiency Due to Failure of CTLA4-Mediated Regulation

Fayhan J. Alroqi, MD[1,*], **Louis-Marie Charbonnier, PhD**[1,*], **Safa Baris, MD**[2], **Ayca Kiykim, MD**[2], **Janet Chou, MD**[1], **Craig D. Platt, MD, PhD**[1], **Abdulrahman Algassim, MD**[1], **Sevgi Keles, MD**[1,3], **Bandar K. Al Saud, MD**[4], **Fowzan S. Alkuraya, MD**[5], **Michael Jordan, MD**[6,7], **Raif S. Geha, MD**[1], and **Talal A. Chatila, MD, MSc.**[1]

[1]Division of Immunology, The Boston Children's Hospital. Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA

[2]Marmara University, Division of Pediatric Allergy/Immunology, Istanbul, Turkey

[3]Necmettin Erbakan University, Meram Medical Faculty, Division of Pediatric Allergy and Immunology, Konya, Turkey

[4]Department of Pediatrics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

[5]Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

[6]Division of Bone Marrow Transplantation and Immune Deficiency, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH, USA

[7]Division of Immunobiology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH, USA

Abstract

Purpose—LRBA (lipopolysaccharide-responsive beige like anchor protein) and CTLA4 (cytotoxic T lymphocyte antigen 4) deficiencies give rise to overlapping phenotypes of immune dysregulation and autoimmunity, with dramatically increased frequencies of circulating T Follicular helper (cT_{FH}) cells. We sought to determine the mechanisms of cT_{FH} cell dysregulation in LRBA deficiency and the utility of monitoring cT_{FH} cells as a correlate of clinical response to CTLA4-Ig therapy.

Methods—cT_{FH} cells and other lymphocyte subpopulations were characterized. Functional analyses included *in vitro* T_{FH} cell differentiation and cT_{FH}/naïve B cell co-cultures. Serum

Corresponding author; Talal A. Chatila, Division of Immunology. The Boston Children's Hospital. One Blackfan Circle, Boston, Massachusetts 02115, USA. Tel. (617) 919 3529; Fax: (617) 730 0528. talal.chatila@childrens.harvard.edu.

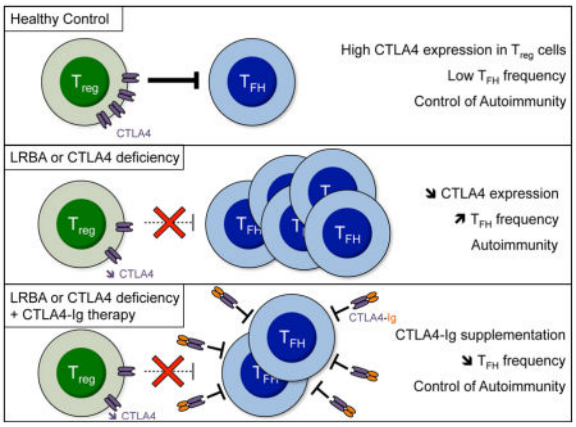
*These authors contributed equally to this work.

soluble IL-2 receptor alpha chain (sIL-2Rα), and *in vitro* immunoglobulin production by cultured B cells were quantified by ELISA.

Results—cT_{FH} cell frequencies in patients with LRBA or CTLA4 deficiency sharply declined with CTLA4-Ig therapy, in parallel with other markers of immune dysregulation including sIL-2R α, $CD45RO^+CD4^+$ effector T cells and auto-antibodies, and predictive of favorable clinical responses. cT_{FH} cells in patients with LRBA deficiency were biased towards a T_H1-like cell phenotype, which was partially reversed by CTLA4-Ig therapy. LRBA-sufficient but not -deficient regulatory T (T_{reg}) cells suppressed *in vitro* T_{FH} cell differentiation in a CTLA4-dependent manner. LRBA deficient T_{FH} cells supported *in vitro* antibody production by naïve LRBA-sufficient B cells.

Conclusions—cT_{FH} cell dysregulation in LRBA deficiency reflects impaired control of T_{FH} differentiation due to profoundly decreased CTLA4 expression on T_{reg} cells, and probably contributes to autoimmunity in this disease. Serial monitoring of cT_{FH} cell frequencies is highly useful in gauging the clinical response of LRBA deficient patients to CTLA4-Ig therapy.

Graphical abstract



Keywords

Autoantibodies; LRBA; CTLA4; T Regulatory Cells; T Follicular Helper Cells; T Follicular Regulatory Cells

Introduction

Follicular helper T (T_{FH}) cells are a distinct subset of T cells that have a crucial role in humoral adaptive immunity [1]. This specific T helper (T_H) lineage has a unique phenotype, expressing high levels of CXCR5, PD-1, ICOS and CD40L[2, 3]. T_{FH} cells constitutively express high levels of B cell lymphoma 6 (Bcl-6), whereas other T helper cell populations including T_H1, T_H2 and T_H17 cells express high levels of the antagonizing transcription factor Blimp-1 [4]. Various signaling pathways have been implicated in T_{FH} cell differentiation including signals from dendritic cells, B cells, cytokines (IL-6 and IL-21 in mice; IL12, 1L23 and TGF-β in human) and surface molecules (ICOS, CD28, CD40L, PD-1, BTLA, and SAP) [1]. T_{FH} cells are essential for germinal center formation and B cell

differentiation into long-lived memory B cells and plasma cells within secondary lymphoid tissues. Up-regulation of CXCR5 and down-regulation of CCR7 guide T_{FH} cell migration into the B cell follicle [1]. The interaction between T_{FH} cells and their cognate B cells provides fundamental signals for high-affinity antibodies production through affinity maturation and class switch recombination. The importance of the T_{FH} cell lineage in promoting antibody responses was established by the observation of defective antibodies class switching in mice lacking T_{FH} cells [5], whereas unbridled T_{FH} cell responses trigger humoral autoimmunity due to generation of autoantibodies by autoreactive B cells [5–10]. Circulating T_{FH} (cT_{FH}) cells were recently identified in human subjects as reflective of T_{FH} cells [3, 11]. Increased cT_{FH} cells have been reported in several human autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjogren's syndrome and autoimmune thyroid disease [12–16]. Reciprocally, decreased cT_{FH} have been found in several monogenic immunodeficiency disorders associated with humoral deficiency [17].

T follicular regulatory (T_{FR}) cells, that originate from $FOXP3^+$ T cells and express high levels of CXCR5, play a pivotal role in controlling immune dysregulation by regulating T_{FH} and activated B cell responses through the inhibitory effect of cytotoxic T lymphocyte associated antigen 4 (CTLA4) [18–22]. CTLA4 deletion in mice results in exaggerated B cell response and increased T_{FH} cells frequencies [21–24]. A number of inherited disorders of immune dysregulation that potentially impact T_{FR} function result in high cT_{FH} cell frequencies, which positively correlate with autoantibodies production. The best characterized of these are monogenic defects involving the LRBA-CTLA4 pathway [25–30]. LPS-responsive beige-like anchor (LRBA) deficiency is a primary immunodeficiency characterized by recurrent infections with hypogammaglobulinemia, giving rise to a common variable immunodeficiency-like phenotype, and immunodysregulation with autoimmunity, including inflammatory bowel disease, autoimmune endocrinopathies and cytopenias [25, 26, 29–31]. LRBA regulates the intracellular trafficking of CTLA4 [30]. Its deficiency results in dysregulation of the T_{FH} cell response and is associated with intense immune dysregulation and auto-antibody production [29]. The precise mechanism for T_{FH} cell dysregulation in LRBA deficiency, whether it involves enhanced T_{FH} cell differentiation, impaired T_{FR} regulation or both, remains unclear. CTLA4-Ig therapy has emerged as an effective treatment for the immune dysregulation associated with both gene defects [30]. The capacity to rapidly and sensitively monitor the immune status of these patients and their response to CTLA4-Ig therapy would be of great clinical utility in optimizing their care.

In this report, we examined the mechanisms of T_{FH} dysregulation in human subjects with LRBA deficiency and the utility of using cT_{FH} cell frequencies as a clinical indicator of response to treatment with CTLA4-Ig. Serial cT_{FH} cell analysis was carried out on LRBA deficient patients before and after starting CTLA4-Ig therapy. Our results show that T_{FH} dysregulation in LRBA deficiency was not due to an intrinsic abnormality in T_{FH} cells. Rather, it reflects failure of LRBA-deficient T_{reg} cells to control T_{FH} cell differentiation. cT_{FH} cells were found to be a sensitive marker of the clinical response to CTLA4-Ig therapy. Our finding supports the use of cT_{FH} cell frequencies as a marker for monitoring the clinical status of LRBA deficient patients and their response to therapy.

Materials and Methods

Patients

Patients P1 and P2 are two previously described Saudi Arabian siblings with LRBA deficiency due to a homozygous deletion in the BEACH domain of *LRBA* that abolished protein expression (patients P5 and P6; Family C, in our original report) [29]. Patient P3 is an 11 year old girl with chronic immune dysregulation who was diagnosed with LRBA deficiency due to exon 57 deletion as confirmed by genomic analysis, cDNA sequencing and absent LRBA protein on flow cytometry and immunoblotting (Fig. E1 in this article's Online Repository). Her clinical presentation is detailed in the Online Repository Text. Patient P4 is a 6 year old Saudi Arabian boy who developed severe Crohn's-like illness and type 1 diabetes and was found on whole exome sequencing to have a 4 base pair (bp) deletion in *LRBA* (c.4757_4760del, p.L1586fs). LRBA deficiency was confirmed by absent protein expression on flow cytometry (Fig E2 in the online repository). Patients P5 and P6 are newly diagnosed Turkish brothers with LRBA deficiency due to a single bp deletion in exon 54 of *LRBA*, as confirmed by genomic analysis, leading to a frame shift and premature stop codon (c.7885delA, p.R2629fs). LRBA deficiency was confirmed by near absent protein expression on flow cytometry (Fig E3 in the online repository). Patients P7–P9 were found to have heterozygous mutations in *CTLA4* (Fig E4 in the online repository). Their clinical presentations are detailed in the Online Repository Text. A patient with IPEX due to an A384T amino acid substitution in FOXP3 was identified by exome analysis followed by Sanger sequencing of the mutation site in the *FOXP3* gene. Subject with the diagnosis of SLE was identified at the Rheumatology clinic at the Boston children's Hospital. Control subjects were age group-matched. All study participants were recruited using written informed consent approved by the local Institutional Review Boards. Studies at the Boston Children's Hospital were conducted under approved protocol #04-09-113R.

Antibodies and flow cytometry

Information on the antibodies employed is provided in the Online Repository Text. Whole blood was incubated with mAbs against surface markers for 30 min on ice. Intracellular staining with FOXP3 and CTLA4, was performed using eBioscience Fixation/ Permeabilization according to the manufacturer's instructions. Indirect intracellular staining for LRBA was performed on freshly isolated peripheral blood mononuclear cells (PBMCs) using BD Biosciences Fixation/Permeabilization buffer with polyclonal rabbit anti-LRBA antibodies (Sigma-Aldrich, St. Louis, MO) or Rabbit IgG XP (R) isotype control (cell signaling), followed by secondary detection with Brilliant Violet 421™ Donkey anti-Rabbit IgG (Biolegend). For chemokine receptor staining in cT_{FH} cells, PBMCs were isolated by Ficoll-Paque Plus gradient and surface staining for PD1, CXCR5, CXCR3 and CCR6 was performed for 30 min on ice. For cytokine detection among T_{FH} cells, PBMCs were stimulated *in vitro* for 4h in complete RPMI medium with Golgi plug (1/1000), PMA (50ng/mL) and ionomycin (500 ng/mL). Cells were washed and incubated with mAbs against surface markers for 30 min on ice, permeabilized using BD Biosciences Fixation/ Permeabilization buffer and intracellular staining with mAbs against cytokines was performed. For characterization of circulating B cell subsets, previously frozen PBMCs were

stained for surface antigens CD19, CD27 and IgD. Data were collected with a Fortessa cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar).

Whole exome sequencing (WES)

WES was performed on genomic DNA of the probands P3, P4, P5 and P9 as described in earlier reports [25, 29].

Sanger sequencing analysis

LRBA and *CTLA4* sequences were amplified from genomic and complimentary (c)DNA by the polymerase chain reaction and sequenced bidirectionally using dye-terminator chemistry [29]. Primers used in amplification reactions are available upon request.

Immunoblotting

Protein lysates derived from lymphocytes were resolved on SDS-PAGE gels and transferred to nitrocellulose filters as described. Immunoblots were carried out using polyclonal rabbit anti-LRBA antibody (Sigma-Aldrich, St. Louis, MO). The blots were reprobed with polyclonal rabbit anti-DOCK8 antibody (Sigma-Aldrich, St. Louis, MO).

T_{FH} and naïve B cell co-culture

cT_{FH} and naïve B cells were isolated from the peripheral blood of patient P3 and her human leukocyte antigen (HLA) fully matched healthy sister by fluorescent activated cell sorting (FACS). The cells were mixed as indicated and cultured for 12 days in the presence of endotoxin-reduced staphylococcal enterotoxin B (SEB) at 1μg/ml in RPMI 1640 complete medium supplemented with 10% heat-inactivated fetal bovine serum [32]. At the end of the incubation period, the IgM and IgG concentrations in the culture supernatants were measured by ELISA.

In vitro generation of induced T_{FH}

$CD4^+$ T cells were isolated from PBMCs by negative selection using magnetic beads (Miltenyi). Naïve $CD3^+CD4^+CCR7^+CD45RA^+$ cells were purified from $CD4^+$ T cells by cell sorting on a FACS ARIA (purity > 98%). Naïve CD4 T cells were seeded at a concentration of 5×10^4 per well of a 96-well plate and stimulated with recombinant human IL-12, 1L-23 and TGF-β1 in the presence or absence of T_{reg} cells, CTLA4-Ig and anti-CTLA4 [33]. The cells were cultured for 4 days, at the end of which they were stained for T_{FH} cell markers.

Autoantibody assays

For autoantibody detection, plasma aliquots from patient and control subjects were analyzed using microarrays spotted with 84 autoantigens (University of Texas Southwestern Medical Center, Genomic and Microarray Core Facility), as described [34]. Data was normalized to healthy controls.

Statistical Analysis

Comparison between groups was carried out using Student's unpaired two tailed *t* test and 2-way ANOVA with Bonferroni post-test analysis, as indicated. Differences in mean values were considered significant at a $p < .05$.

Results

Three patients with definitive LRBA deficiency were studied for their response to CTLA4 therapy. Details of the clinical and immunological findings of the three patients are shown in Table E1 and Table E2, respectively, in the Online Repository. The three patients exhibited severe immune dysregulation with autoimmune cytopenias, chronic end organ inflammation and damage, especially affecting the lungs (P1, P3) and the gut (P1, P2). All patients showed marked clinical response to CTLA4-Ig therapy with decreased disease symptomatology, weight gain and resolution of their thrombocytopenia (Fig 1, *A* and *B*). Patients P1 and P2, who suffered from colitis, showed good clinical response with decreased diarrhea. Patient P2, who developed type 1 diabetes shortly before initiation of therapy, had resolution of her insulin dependence and normalization of her blood glucose (Fig 1, *C*). Patient P3, who suffered from severe lung disease and was oxygen dependent, responded very favorably to CTLA4-Ig therapy with marked improvement in her lung imaging and function and resolution of her oxygen dependency (Fig 1, *D* and *E*).

We have previously documented that patients with LRBA deficiency exhibit dysregulated cT_{FH}[29], a phenotype related to the profound deficiency of CTLA4 expression by LRBA-deficient T_{reg} cells [21, 22, 29]. Flow cytometric analysis of peripheral blood T lymphocyte cells of the three patients demonstrated a markedly increased number of $CD4^+FOXP3^-PD1^+CXCR5^+$ cT_{FH} cells (Fig 2, *A*). The frequency of cT_{FH} and CTLA4 expression of T_{reg} cells of three other patients with LRBA deficiency (P4–P6) were also analyzed. They also exhibited high frequency of cT_{FH} cells in association with low CTLA4 expression on their T_{reg} cells (Fig E2 and E3). Importantly, the frequency of cT_{FH} cells significantly declined after CTLA4-Ig therapy (Fig 2, *A* and *B*). CTLA4-Ig therapy also resulted in the decline of other markers of inflammation, including serum soluble CD25 (sCD25) (Fig 2, *C*). The frequency of circulating naïve $CD4^+$ T cells ($CD4^+CD45RA^+CCR7^+$) increased following CTLA4-Ig therapy, indicative of more effective immunoregulation (Fig 2, *D*). The decline in cT_{FH} cells upon CTLA4-Ig therapy correlated well with that of sCD25, validating the monitoring of these cells as a proxy for the immune dysregulatory status of the patients (Fig 2, *E*). In addition to their increased frequency, cT_{FH} cells of LRBA-deficient subjects also had increased expression of the cT_{FH} cell markers ICOS and PD1 as compared to those of control subjects, consistent with them being activated [11, 35]. The expression levels of both markers were normalized following therapy with CTLA4-Ig (Fig 2, *F* and *G*).

To further characterize the phenotype of cT_{FH} cells in LRBA-deficient patients and also the impact of CTLA4-Ig treatment, we evaluated the expression of chemokine receptors and the capacity of cT_{FH} to secrete cytokines. cT_{FH} cells of LRBA deficient patients highly expressed the T_H1-associated chemokine receptor CXCR3 and IFN-γ, markers that define T_H1-biased T_{FH} cells (or T_{FH}-1)[11, 35]. In contrast, expression of the T_H17-associated

chemokine receptor CCR6 and IL-17, markers that define T_H17-biased T_{FH} cells ($T_{FH}17$) [11, 35], was markedly decreased in patient as compared to control cT_{FH} cells (Fig 3, A–D). Some of these abnormalities, such as increased CXCR3 expression, persisted despite CTLA4-Ig therapy, and probably reflected either attributes intrinsic to LRBA-deficient T_{FH} cells or ongoing T_H1 cell inflammation in the hosts. In contrast, expression of IL-21 and IL-4 were similar in patient and control cT_{FH} cells and appeared unaffected by CTLA4-Ig therapy.

We have previously reported that patients with LRBA deficiency have profound T_{reg} cell abnormalities including decreased T_{reg} cell frequencies and reduced expression of several T_{reg} cell markers, including CTLA4 [29]. In view of this immune dysregulation, we analyzed the correlation of the magnitude of CTLA4 expression on T_{reg} cells and the frequency of cT_{FH} cells. To that end, we examined the frequency of cT_{FH} cells in three patients with heterozygous loss of function mutations in *CTLA4*. Their mutational analysis is shown in Fig E4 in the online Repository, and their clinical and immunological findings are detailed in Table E1 and Table E3, respectively, in the Online Repository. Consistent with the role of CTLA4 deficiency in dysregulated cT_{FH} cells in LRBA deficiency, patients with heterozygous loss of function mutations in *CTLA4* also manifested increased cT_{FH} cell frequencies (Fig 4, *A* and *B*). Overall, cT_{FH} cells frequencies were inversely correlated with the CTLA4 MFI on T_{reg} cells of LRBA and CTLA4-deficient subjects, reflecting the role of CTLA4 in controlling T_{FH} cell differentiation. Subjects with complete LRBA deficiency, associated with near complete absence of CTLA4 expression on T_{reg} cells, manifested the highest cT_{FH} frequencies, while those with heterozygous loss of function *CTLA4* mutations had a more moderate increase in cT_{FH} frequencies. (Fig 4, *C*). Similar to the case of CTLA4-Ig-treated LRBA-deficient subjects, treatment of a patient with CTLA4 deficiency with CTLA4-Ig resulted in the decline of cT_{FH} cells, consistent with the role of CTLA4 deficiency in cT_{FH} dysregulation in both disorders (Fig 4, *D* and *E*).

To determine whether LRBA deficiency impaired T_{FH} differentiation, we tested the capacity of LRBA-sufficient and deficient naive $CD4^+$ T cells to differentiate into induced follicular helper (iT_{FH}) cells upon stimulation with IL12, IL23 and TGF-β1 in the presence or absence of CTLA4-Ig [33]. $CD4^+$ T cells from patients and control subjects were enriched by magnetic bead separation, and naive $CD4^+$ T cells were purified to greater than 99% purity by cell sorting (Fig E5 in the Online Repository). LRBA-sufficient and -deficient T cells were found to be equivalent in their capacity to differentiate into iT_{FH} cells that expressed similar levels of Inducible T cell Costimulator (ICOS) regardless of the CTLA4 status of naïve $CD4^+$ cells (Fig 5, *A*–*C*). However, iT_{FH} cells generated from LRBA-deficient naïve T cells exhibited higher expression of PD1, as compared to those from LRBA-sufficient naïve T cells (Fig 5, *D* and *E*). Both iT_{FH} differentiation and expression of ICOS and PD1 were partially inhibited upon treatment of the differentiating T cell cultures with CTLA4-Ig (Fig 5, *B*–*E*). Given the normal differentiation of LRBA deficient T cell into iT_{FH} cells, we further analyzed the capability of LRBA-sufficient and deficient T_{reg} cells to control iT_{FH} cells differentiation. T_{reg} cells were isolated from patient and healthy control by means of cell sorting of $CD4^+CD25^+CD127$low T_{reg} cells. Equal numbers of patient and control T_{reg} cells were added to an equal number of control naïve $CD4^+$cells that were stimulated with IL12, IL23 and TGF-β in the presence or absence of anti-CTLA4. LRBA-deficient T_{reg} cells failed

to suppress iT_{FH} differentiation compared with control T_{reg} cells, indicative of their impaired function (Fig 5, *F* and *G*).

Patients with LRBA deficiency frequently present with hypogammaglobulinemia and defective specific antibody titers, with the majority exhibiting B cell dysfunction and reduced class-switched memory B-cells [31, 36]. Nevertheless, most of these patients suffer from autoimmunity with increased circulating autoantibodies [29, 31, 36]. To determine whether the production of circulating autoantibodies responded to CTLA4-Ig therapy, we screened our patients for serum autoantibodies before and two time points after CTLA4-Ig therapy using an array of 84 auto-antigens [34]. Also included in the analysis were four healthy subjects whose sera were used as negative controls and two positive control sera including one patient with IPEX and another patient with SLE. LRBA patients exhibited a number of circulating IgG autoantibodies whose relative abundance significantly decreased following CTLA4-Ig therapy (Fig 6, *A*). We next employed in vitro co-culture studies to examine the functional capacity of LRBA-deficient cT_{FH} to support IgM and IgG antibody production by naïve B cells. $CD4^+CD25^-CD127^{High}PD1^+CXCR5^+$ cT_{FH} cells and $CD19^+IgD^+CD27^-$ naïve B cells were isolated from LRBA-deficient patient P3 and her fully HLA-matched healthy sister by cell sorting and co-cultured in the presence of staphylococcal enterotoxin B (SEB). Results showed that while cT_{FH} cells of both the LRBA-deficient patient and her healthy sibling supported *in vitro* IgM and IgG antibody production by the sibling's naïve LRBA-sufficient B cells (Fig 6, *B*), the LRBA-deficient cT_{FH} cells were more effective in that regard, consistent with their heightened activation state (Fig 2, *F* and *G*) [11, 35]. In contrast, the patient's cT_{FH} supported IgM but minimally IgG production by B cells, in agreement with the previous report of defective IgG isotype switching in LRBA-deficient B cells [26]. We also evaluated the impact of CTLA4-Ig treatment on B cell phenotype. Whereas LRBA-deficient patients exhibited increased frequencies of naïve (IgD^+CD27^{low}) and correspondingly decreased frequencies of switched memory (IgD^+CD27^{low}) B cells, CTLA4-Ig treatment did not change the ratio of naïve/memory B cells in circulation (Fig 6, *C*). Given that expansion of T_{FH} cells promotes auto-antibody production [5–10], these findings indicated that the dysregulated T_{FH} expansion in LRBA deficiency is functionally relevant to the excess auto-antibody production in this disorder.

Discussion

Patients with deleterious mutations in *LRBA* gene have dysregulated T_{FH} cell responses, as reflected by the high frequency of cT_{FH} cells, which may play a causative role in disease-related autoimmunity. In this report, we examined the mechanisms of T_{FH} dysregulation in LRBA deficiency and the usefulness of monitoring cT_{FH} cell frequencies as a measure of disease activity and response to therapy. We found that T_{FH} cell dysregulation involved failure of CTLA4-dependent T_{reg} cell to control T_{FH} cell differentiation. Furthermore, the elevated cT_{FH} cell frequencies in LRBA deficiency and the related CTLA4 deficiency dramatically declined following CTLA4-Ig therapy, in concordance with the decline in other markers of disease activity and improved clinical outcome. While cT_{FH} cell frequencies in LRBA-deficient subjects tightly correlated with other markers of immune dysregulation such as sCD25, the former offers the advantage of ease of its measurement by flow cytometry and its sensitive and fast response to CTLA4-Ig therapy. Thus, monitoring cT_{FH} cell frequencies

in LRBA and CTLA4 deficiencies maybe particularly useful in tracking disease activity and response to different therapies.

Recent studies identified the critical role of CTLA4 in T_{reg} cells in controlling humoral immunity. *In vivo* T_{reg}/T_{FR} cells depletion or selective deletion or blockage of CTLA4 in T_{reg} compartment resulted in unrestrained T_{FH} cell differentiation and profoundly increased auto-antibody production [21, 22]. In concordance with these studies, we demonstrated that LRBA-deficient naïve $CD4^+$ T cells effectively differentiate into T_{FH} cells. However, LRBA-deficient T_{reg} cells, like normal T_{reg} cells treated with anti-CTLA4 mAb, failed to suppress the expansion of *in vitro* differentiated T_{FH} cells, pointing to the reduced CTLA4 expression on LRBA-deficient T_{reg} cells as a key mechanism underlying the dysregulated T_{FH} response in LRBA deficiency.

Many patients with LRBA deficiency present with hypogammaglobulinemia and low numbers of switched memory B cells [31, 36]. LRBA deficient naïve B cells have an intrinsic defect in their capacity to differentiate into IgM and IgG producing plasma cells [26]. Despite their B cell switch defect, LRBA-deficient subjects mount an intense autoantibody response. cT_{FH} cells of LRBA-deficient subjects were activated, as evidenced by their heightened expression of ICOS and PD1, a phenotype that was normalized upon CTLA4-Ig therapy. They were more effective in supporting immunoglobulin production by LRBA-sufficient B cells as compared to control cT_{FH} cells, indicative of their augmented functional capacity. In vitro differentiated iT_{FH} cells also exhibited increased PD1 expression that was reversed by CTLA4-Ig treatment, reflecting the role of CTLA4 deficiency in dysregulating LRBA-deficient T_{FH} cells both *in vitro* and *in vivo*. We suggest that the profound dysregulation of the T_{FH} cell compartment in LRBA deficient subjects, precipitated by T_{FR} cell depletion and virtually absent CTLA4 expression, coupled with defective B cell isotype switching and its associated somatic hypermutation, may propel humoral autoimmunity by limiting the pruning of auto-reactive antibodies normally achieved with somatic hypermutation [37]. Such a mechanism would be consistent with the reversal of T_{FH} dysregulation and down-regulation of auto-antibody production by CTLA4-Ig therapy. Because CD80 signaling favors B cell isotype switching and immunoglobulin production, we cannot exclude an additional direct effect of CTLA4-Ig treatment on B cells resulting in the reduction of auto-antibody production in LRBA-deficient patients [38]. Further studies would be required to validate these predictions.

In summary, these findings support the monitoring of cT_{FH} cells as a sensitive marker for monitoring the response of LRBA and CTLA4-deficient patients to CTLA4-Ig therapy. Its use may help optimize the management of LRBA-deficient subjects to reach sustained clinical improvement and optimal preparation for hematopoietic stem cell transplantation. More broadly, monitoring the T_{FH}/T_{FR} axis may also aid in the diagnosis and treatment of primary immune dysregulatory disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CTLA4	cytotoxic T lymphocyte associated antigen 4
CVID	Common variable immunodeficiency
ELISA	enzyme-linked immunosorbant assay
FACS	fluorescence-activated cell sorting
HLA	human leukocyte antigens
IPEX	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked
LRBA	LPS-responsive beige-like anchor
MFI	mean fluorescence intensity
SLE	systemic lupus erythematosus
SEB	staphylococcal enterotoxin B
T_{reg}	T regulatory
T_{FH}	T Follicular Helper
T_{FR}	T Follicular Regulatory
T_H1	T helper type 1
T_H17	T helper Type 17
WES	Whole exome sequencing

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Key Messages

- LRBA and CTLA4 deficiencies result in highly elevated frequencies of circulating T follicular helper (cT_{FH}) cells.
- cT_{FH} dysregulation in LRBA deficiency reflects impaired CTLA4-dependent suppression of T_{FH} cell differentiation by T regulatory (T_{reg}) cells.
- Monitoring cT_{FH} frequencies in LRBA and CTLA4 deficiencies is useful in gauging the clinical response to CTLA4-Ig therapy.

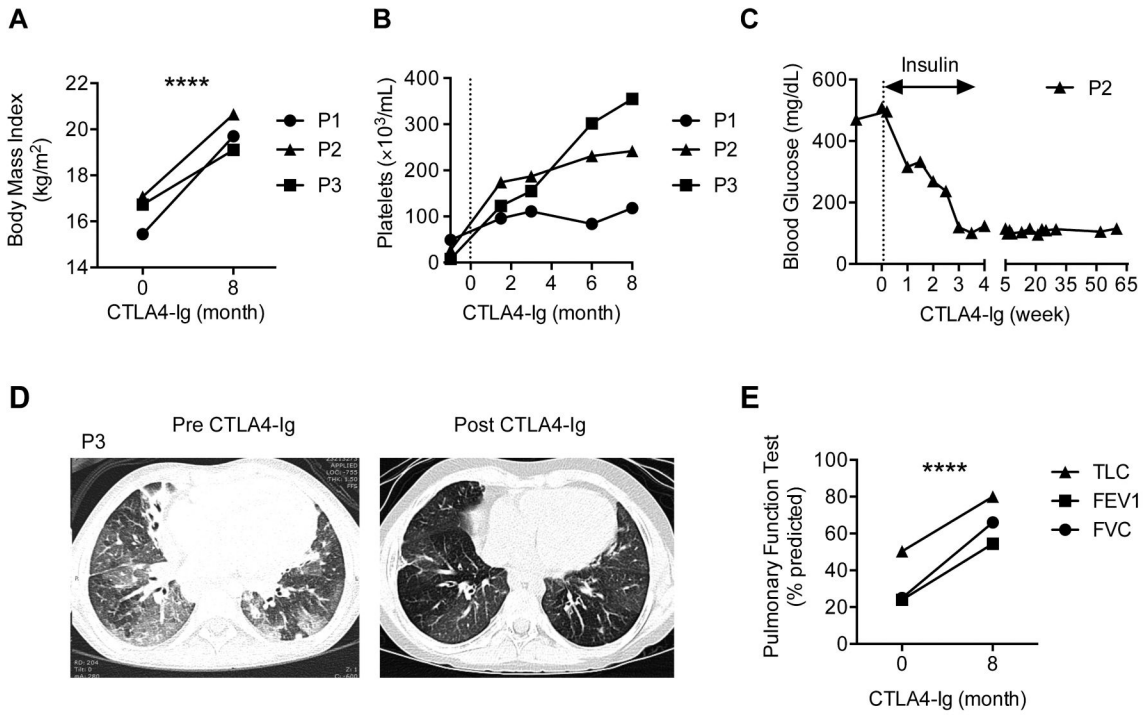

Figure 1. Clinical response of LRBA-deficient subjects to CTLA4-Ig therapy

A and B, Body mass index (Fig 1, *A*) and platelet counts (Fig 1, *B*) of LRBA deficient subjects pre- and post-treatment with CTLA4-Ig. **C,** Serum glucose levels and insulin use in patient P2 status post CTLA4-Ig therapy. **D and E,** High-resolution chest CT scans (Fig 1, *D*) and Pulmonary function tests (Fig 1, *E*) of patient P3 pre and post treatment with CTLA4-Ig; FVC, forced vital capacity; FEV1, forced expiratory volume; TLC, total lung capacity. ****P < .0001, 2-way ANOVA with post-test analysis.

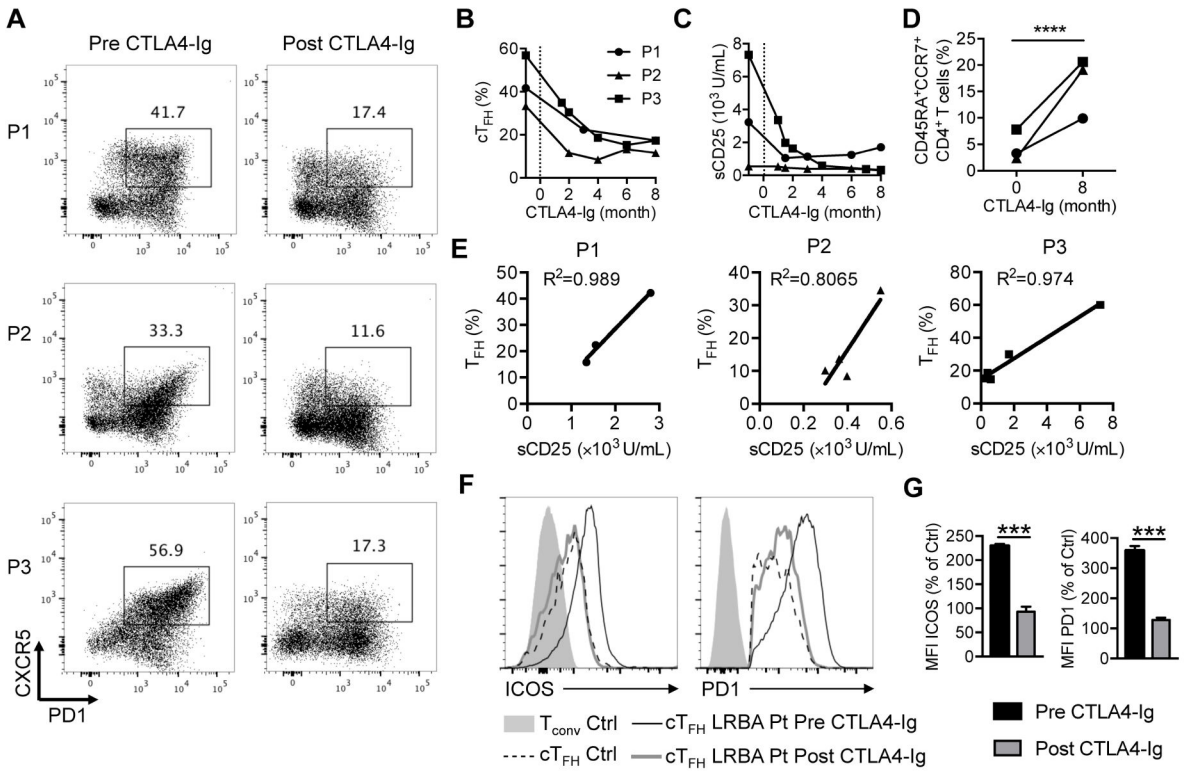

Figure 2. T_{FH} cell frequency in LRBA-deficient patients correlates with other markers of immune dysregulation

A, Flow cytometric analyses of CXCR5 and PD1 expression in $CD4^+$ T cells in LRBA-deficient subjects pre- and post-treatment with CTLA4-Ig. **B–D,** cT_{FH} cell frequencies (Fig 2, *B*), serum sCD25 levels (Fig 2, *C*) and naïve $CD4^+CD45RA^+CCR7^+$ T cell frequencies (Fig 2, *D*) in LRBA deficient patients pre and post-treatment with CTLA4-Ig. **E,** Correlation between cT_{FH} cell frequencies and sCD25 levels in LRBA-deficient patients. **F**, Flow cytometric analysis of ICOS and PD1 expression on control cT_{FH} cells and on patient cT_{FH} cells before and after CTLA4-Ig therapy. **G**, Relative mean fluorescence intensity of ICOS and PD1 expression on patient versus control cT_{FH} cells. ***P < .001 and ****P < .0001, by student's two tailed t test.

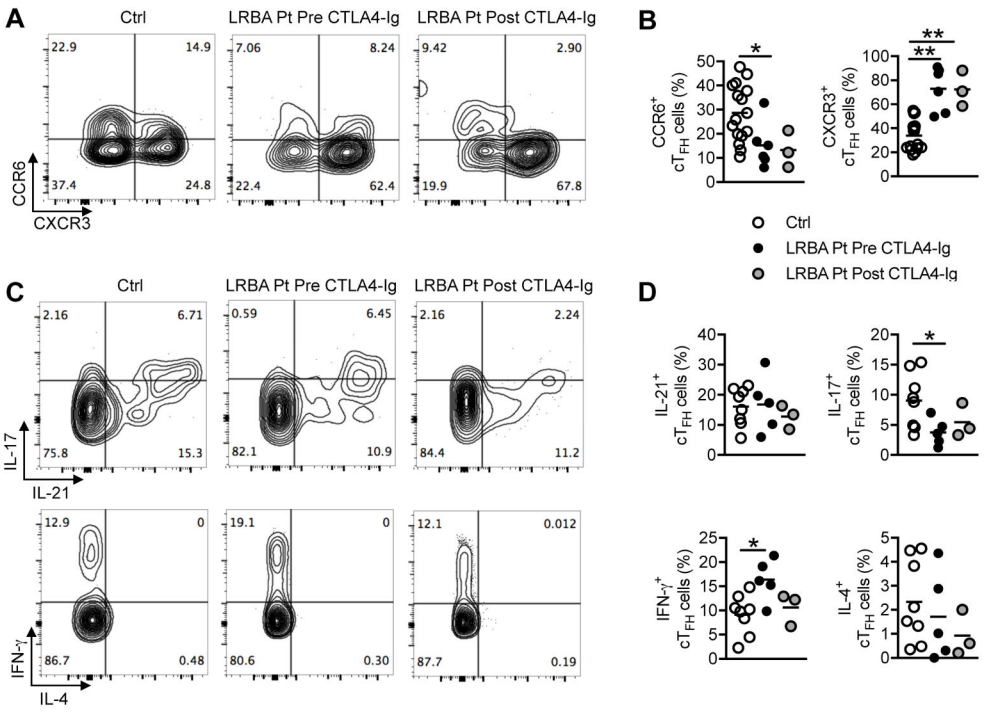

Figure 3. cT_{FH} cells of LRBA-deficient patients are skewed towards a T_H1-like cell phenotype
A and B, Flow cytometric analyses of CXCR3 and CCR6 expression in cT_{FH} cells of LRBA-sufficient and LRBA- deficient subjects pre- and post-treatment with CTLA4-Ig. **C and D,** Flow cytometric analyses of IL-17 and IL-21 expression (Fig 3, *C*, upper panels) or IFN-γ and IL-4 (Fig 3, *C*, lower panels) and the respective scatter plot representation (Fig 3, *D*) in cT_{FH} cells of LRBA-sufficient and LRBA- deficient subjects pre- and post-treatment with CTLA4-Ig. *P < .05, **P < .01 by 1-way ANOVA with post-test analysis.

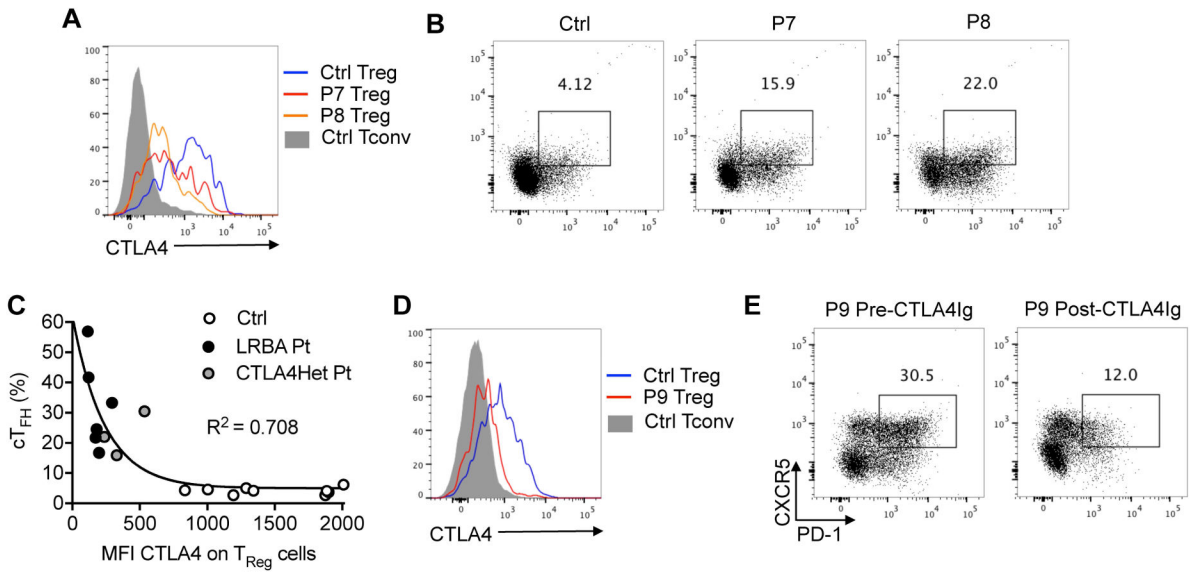

Figure 4. T_{FH} cell frequency in CTLA4 deficiency and its response to CTLA4-Ig therapy

A and B, Flow cytometric analysis of CTLA4 expression on $CD4^+FOXP3^+$ T_{reg} cells (Fig 3, *A*) and cT_{FH} (Fig 3, *B*) of control (Ctrl) subjects and patients P7 and P8 with a heterozygous CTLA4 mutation. **C,** Correlation between cT_{FH} cell frequencies and the mean fluorescence intensity (MFI) of CTLA4 expression in T_{reg} cells of Ctrl (n=8), LRBA-deficient (n=6) and heterozygous CTLA4-mutant subjects (n=3). **D,** Flow cytometric analysis of CTLA4 expression on $CD4^+FOXP3^+$ cells in patient P9 with a heterozygous CTLA4 mutation. **E**, cT_{FH} cells in patient P9 before and after CTLA4-Ig therapy.

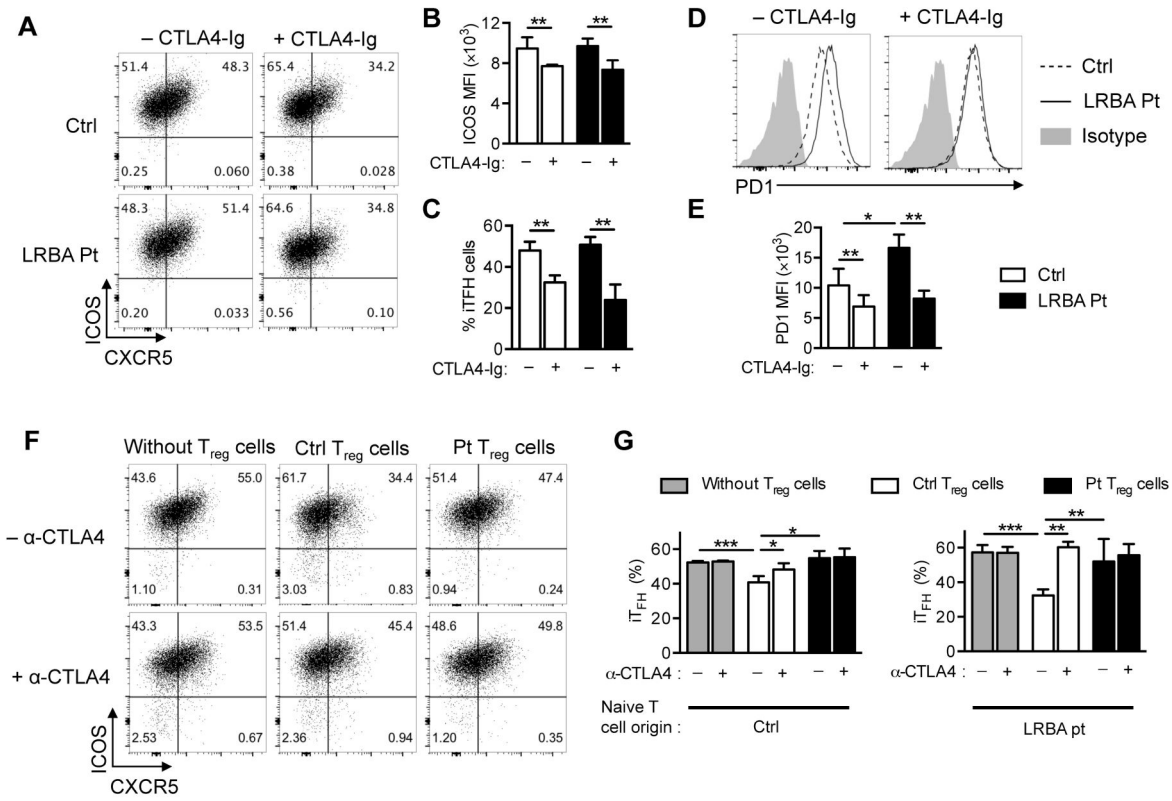

Figure 5. Ineffective T_{reg} cells control of T_{FH} Cell differentiation in LRBA deficient subjects

A, Flow cytometric analysis of CXCR5 and ICOS expression in *in vitro*-differentiated induced (i)T_{FH}-like cells of control and LRBA-deficient subjects. **B and C**, MFI of ICOS expression in iT_{FH}-like cells (Fig 5, *B*), and frequencies of *in vitro*-differentiated iT_{FH}-like cells (Fig 5, *C*) derived from control and LRBA-deficient naïve $CD4^+$ T cells in the absence or presence of CTLA4-Ig. **D and E,** Flow cytometric analysis of PD1 expression (Fig 5, *D*) and bar graph representation (Fig 5, *E*) of PD1 expression in iT_{FH} cells of control and LRBA-deficient subjects. **F and G,** Flow cytometric analysis (Fig 5, *F*) and frequencies (Fig 5, *G*) of *in vitro*-differentiated iT_{FH}-like cells derived from control and LRBA-deficient naïve $CD4^+$ T cells in the absence or presence of anti-CTLA4 mAb and/or patient or control T_{reg} cells. Results are representative of 2 independent experiments. *P < .05, **P < .01 and ***P < .001 2-way ANOVA with post-test analysis.

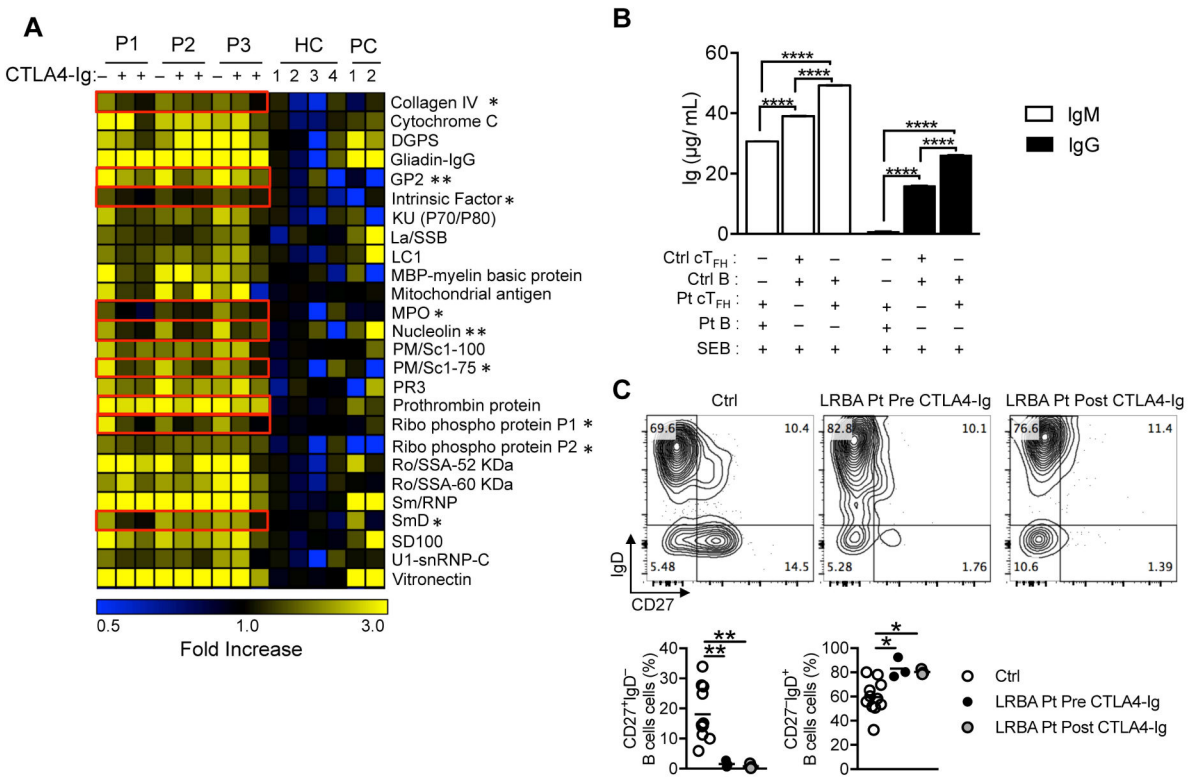

Figure 6. Autoantibody production and cT$_{FH}$ function in LRBA-deficient subjects

A, Autoantibody production in LRBA deficient patients before and after CTLA4-Ig therapy. Heat map showing IgG autoantibodies against self-antigens in sera of LRBA-deficient patients, healthy control subjects, patient with IPEX, and a patient with SLE. A value of 1 (black) is equal to the control average + 1 SD. Autoantibody responses affected by CTLA4-Ig therapy are boxed in red. **B,** IgM and IgG production in co-cultures of cell-sorted cT$_{FH}$ and naïve B cells derived from patient P3 and her HLA fully matched sister in the presence of Staphylococcal enterotoxin B (SEB). **C.** Flow cytometric analysis (Fig 6, *C*, upper panels) and scatter plot representation (Fig 6, *C*, lower panels) of CD27 and IgD expression on circulating B cells of control subjects and LRBA deficient subjects before and after treatment with CTLA4-Ig. *p<0.05 and **p<0.01, Student unpaired 2-tailed *t* test; ****P < . 0001 by 1-way ANOVA with post-test analysis.