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Antiviral and Regulatory T Cell Immunity in a Patient with Stromal Interaction Molecule 1 Deficiency

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Stromal interaction molecule 1 (STIM1) deficiency is a rare genetic disorder of store-operated calcium entry, associated with a complex syndrome including immunodeficiency and immune dysregulation. The link from the molecular defect to these clinical manifestations is incompletely understood. We report two patients with a homozygous R429C point mutation in STIM1 completely abolishing store-operated calcium entry in T cells. Immunological analysis of one patient revealed that despite the expected defect of T cell proliferation and cytokine production in vitro, significant antiviral T cell populations were generated in vivo. These T cells proliferated in response to viral Ags and showed normal antiviral cytotoxicity. However, antiviral immunity was insufficient to prevent chronic CMV and EBV infections with a possible contribution of impaired NK cell function and a lack of NKT cells. Furthermore, autoimmune cytopenia, eczema, and intermittent diarrhea suggested impaired immune regulation. FOXP3-positive regulatory T (Treg) cells were present but showed an abnormal phenotype. The suppressive function of STIM1-deficient Treg cells in vitro, however, was normal. Given these partial defects in cytotoxic and Treg cell function, impairment of other immune cell populations probably contributes more to the pathogenesis of immunodeficiency and autoimmunity in STIM1 deficiency than previously appreciated.

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Abbreviations used in this article: CRAC, calcium release-activated calcium; EBER, EBV-encoded RNA; ER, endoplasmic reticulum; LCL, lymphoblastoid cell line; PD-1, programmed cell death-1; SOCE, store-operated calcium entry; STIM1, stromal interaction molecule 1; TG, thapsigargin; Treg, regulatory T.

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cytotoxicity as well as cytokine production induced by target cell recognition was severely impaired (16). Although these results provide a framework for the explanation of the infection susceptibility, a number of questions remain unresolved. The spectrum of antimicrobial effector cells and effector mechanisms affected by the human CRAC channel disorders remains incompletely characterized. Moreover, the relative role of defects in T cell priming, proliferation, differentiation, and effector function in the failure of antiviral defense has not been defined. In particular, virus-specific T cell responses have not been analyzed.

Another important aspect of the phenotype of human CRAC channel disorders is an impairment of immune regulation that has predominantly been observed in patients with STIM1 deficiency. All four published patients showed autoimmune cytopenia, and several showed lymphoproliferation and prolonged diarrhea (10). Due to the pleotropic function of Ca2+ flux in lymphocytes, multiple checkpoints of autoimmunity are likely to be affected. In particular, a role for impaired T cell regulation has been postulated. Reduced Treg cell numbers have been reported in a single STIM1-deficient patient, and in mice with T cell-specific deletion of STIM1 and STIM2, adoptive transfer of wild-type Treg cells can suppress the lymphoproliferative phenotype (10, 17). However, a more detailed phenotypic analysis of human STIM1-deficient Treg cells has not been reported, and, due to the small number of patients, the phenotypic spectrum of impaired immune regulation in STIM1 deficiency remains incomplete.

In this study, we present the first detailed immunological analysis, to our knowledge, of human STIM1 deficiency in two new patients with a homozygous Arg429Cys point mutation in analysis, to our knowledge, of human STIM1 deficiency in two complete.

Due to the small number of patients, the phenotypic spectrum of impaired immune regulation in STIM1 deficiency remains incomplete. In this study, we present the first detailed immunological analysis, to our knowledge, of human STIM1 deficiency in two new patients with a homozygous Arg429Cys point mutation in STIM1 leading to absent Ca2+ flux in T cells. Surprisingly, we new patients with a homozygous Arg429Cys point mutation in analysis, to our knowledge, of human STIM1 deficiency in two complete.
Cytokine expression of T and NK cells

For T cell cytokine induction, 1 x 10^6 PBMC were stimulated with either PMA (50 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Sigma-Aldrich) for 4 h or with anti-CD3/CD28 beads (Invitrogen; two beads per cell) for 16 h in the presence of brefeldin A (GolgiPlug 1:1000; BD Biosciences). Cells were surface stained, fixed/permeabilized (Cytofix/Cytoperm plus Kit; BD Biosciences), and stained with the respective anti-cytokine Abs. For determination of cytokine production by NK cells, either 5 x 10^5 PBMC were coincubated with 2 x 10^5 K562 target cells for 5 or 5 x 10^5 MACS-purified NK cells were stimulated with IL-12 (10 ng/ml; R&D Systems) and IL-15 (30 ng/ml; PeproTech) for 16 h. Brefeldin A was added for the last 4 h. Cells were surface stained with anti-CD3 and anti-CD56 and with the respective anti-cytokine Abs as described for T cells.

Immunohistochemistry

Biopsies from the left lower lobe of the lung and hilar lymph nodes were fixed in 4% buffered formaldehyde. Three-micrometer-thick sections were deparaffinized in xylene and rehydrated through graded alcohols subjected to Ag retrieval in a pressure cooker using a retrieval buffer (DakoCyto- mation). Immunodetection was performed using the LSAB visualization system in an Autostainer plus (DakoCytomation). The primary Abs used are listed in Supplemental Table I. All sections were counterstained with Meyer’s hemalaun. Negative controls were obtained by omitting the primary Ab. Chromogen-based in situ hybridization for the detection of EBV-encoded RNAs (EBER1, -2) was performed using a biotin-labeled probe (ZytoFast EBV ISH, Biotin, protein T-1014-400; Zytomed Systems). Denaturation and hybridization steps were carried out in a slide processing system (StatSpin ThermoBrite; Abbott Molecular). To assess the immunophenotype of EBER-positive cells, chromogen-based in situ hybridization was combined with immunolabeling for CD20 or CD3 using the Dako REAL Detection System (number K5005; DakoCytoation).

Results

A novel homozygous point mutation in STIM1 causing absence of store-operated Ca^{2+} influx in T cells

Because the clinical and immunological phenotype of the two siblings resembled that of four previously reported patients with STIM1 deficiency, we sequenced STIM1 and identified in both patients a homozygous C > T exchange at cDNA position 1285 in exon 10 leading to substitution of an arginine by a cysteine at position 429. Western blot analysis revealed that the mutated STIM1^{Arg429Cys} protein was still expressed in LCL EBV blasts of P5 (Fig. 1G). Nevertheless, TCR-mediated calcium influx was completely abolished in T cells from P5 (Fig. 1E). Also, the depletion of ER calcium stores by TG failed to induce calcium influx in T cells (Fig. 1F). Both parents were heterozygous for the mutation (data not shown), and cells from the mother showed reduced calcium influx after TG-mediated store depletion (Fig. 1F).

Destructive EBV-positive lymphoproliferative disease in lung and lymph node

At the time of referral, the patient had been treated for a lung abscess following lobar pneumonia with bronchopleural fistulas developing after thoracoscopic debridement (Fig. 2A, 2B). Cervical lymphadenopathy and splenomegaly was noted. Histological analysis of a resected lymph node revealed that lymphoid follicles were present, but beset with massively expanded lymphocytes, which were distributed throughout the parenchyma and the follicles (Fig. 2C–E). The reticular meshwork was partially destroyed by the lymphocyte infiltrations, which predominantly consisted of CD3^{+} T cells accompanied by some CD20^{+} B cells (Fig. 2F, 2G). Focal accumulations of EBER^{+} lymphocytes were identified as B cell blasts, but EBER^{+} T cells were also found (Fig. 2H and data not shown). The resected lung tissue showed similar destructive infiltrations with activated, terminally differentiated programed cell death-1 (PD-1) and granzyme B-positive CD8^{+} T cells, rarely seen in other lymphoproliferative diseases including EBV-driven lymphomatoid granulomatosis (Fig. 2J–O). This aggressive pulmonary and lymphoid EBV-positive lymphoproliferative disease was successfully treated with rituximab, which led to complete virus elimination from the blood and significant regress of the lymphadenopathy.

Polyclonal T cell repertoire dominated by terminally differentiated CD8^{+} T cells with an exhausted phenotype

Analysis of the T cell compartment in P5 revealed that most of the analyzed TCR Vβ-chains were expressed in normal frequencies on CD4^{+} T cells, whereas there was some alteration in the Vβ repertoire of CD8^{+} T cells, probably related to the active EBV infection (Fig. 3A). Overall, the elevated T cell numbers with a polyclonal repertoire suggested normal T cell development. The differentiation state of CD8^{+} T cells was analyzed using the markers CD27, CCR7, and CD45RA, which allow classification of CD8^{+} T cells from naive to terminally differentiated CTL into five subgroups (20). P5 had very few naive CD8^{+} T cells, whereas the proportion of terminally differentiated CD8^{+} T cells was increased compared with healthy donors (Fig. 3B). Moreover, inhibitory receptors associated with T cell exhaustion were strongly (2B4 and PD-1) or moderately (Tim-3) expressed on patient’s CD8^{+}T cells but not on CTL of the healthy control, whereas the expression of killer cell lectin-like receptor G1 on CD8^{+} T cells was comparable between patient and control (Fig. 3C). The accumulation of terminally differentiated CD8^{+} T cells was not due to impaired CD95-mediated (10 and data not shown) or anti-CD3–induced activation-induced cell death (data not shown). Thus, the CD8^{+} T cell compartment was highly activated, but high levels of exhaustion markers suggested poor functionality in the context of the viral infection.

STIM1 deficiency impairs T cell proliferation and cytokine production

Because STIM1 is important for TCR-mediated, Ca^{2+}-dependent T cell activation, the predominance of a highly activated, terminally differentiated CD8^{+} T cell population was not expected. We therefore analyzed T cell activation in more detail. Upregulation of CD69 after stimulation with anti-CD3/CD28–coated beads was normal, whereas the expression of CD25 on resting CD4^{+} T cells and activated CD4^{+} and CD8^{+} T cells was lower than in T cells from a healthy control (Fig. 4A, 4B). Analysis of T cell proliferation by CFSE dilution revealed a severe defect in the proliferative response of STIM1-deficient CD4^{+} T cells following stimulation with PHA or anti-CD3, whereas the proliferation of CD8^{+} T cells was partially impaired. Addition of IL-2 was able to partially restore the proliferation of both CD4^{+} and CD8^{+} T cells (Fig. 4C). To analyze T cell effector functions, we determined cytokine production and cytotoxicity. After stimulation with PMA/ ionomycin, the percentage of IFN–γ–producing CD4^{+} cells was markedly reduced, whereas the percentage of IL–4–producing cells was within the normal range (Fig. 4D). T cells with IL-17 production were nearly absent (Fig. 4E). As expected (17), the expression of IL-2 upon T cell activation was highly deficient in STIM1-deficient CD4^{+} T cells (Fig. 4F). In contrast to the reduced cytokine production, T cell cytotoxicity measured by anti-CD3 redirected lysis of L210 target cells was normal (Fig. 4G). These data indicated a partial deficiency in T cell activation and effector functions.

STIM1 deficiency allows the generation of virus-specific T cell responses

To address the question whether these defects still allowed the generation of antiviral T cell responses, we quantified CMV- and EBV-specific T cells isolated from P5 by tetramer staining ex vivo.
FIGURE 1. Clinical presentation of two new patients with STIM1 deficiency. A, Clinical presentation and basic immunological data of the two patients described in this report (P5 and P6) in comparison with data from the four previously published patients. B, Eczematous skin lesions of P5. C, Dental enamel defect of P5. D, Nail dysplasia of P6. E and F, Calcium flux in MACS-purified T cells from P5, the heterozygous mother, and a healthy control in response to stimulation with anti-CD3 (5 \( \mu \text{g/ml} \)) and F(ab')\( _2 \) fragment (E) or TG (F). The results were obtained in two independent experiments, collecting \( \sim 600 \) events/s during FACS analysis. The measurement was started in Ca\( ^{2+} \)-free medium, and calcium was added at the indicated time point. G, Western blot analysis of lysates from EBV LCL of P5, the heterozygous mother, and a healthy donor incubated with anti-STIM1 (upper panel) and anti-actin Abs as a loading control (lower panel). Specific bands are designated by an asterisk. Representative blot from three independent experiments. *(10), †(9). AIHA, autoimmune hemolytic anemia; HHV, human herpes virus; HiB, hemophilus influenzae type B; HSCT, hematopoietic stem cell transplantation; ITP, idiopathic thrombocytopenic purpura; LN, lymph node; MM, measles and mumps; PN, pneumococcus; rub, rubella; tet, tetanus; VZV, varicella zoster virus.
Interestingly, we could demonstrate a significant population of CMV-specific CD8+ T cells (Fig. 5A), indicating that antiviral T cells can be primed and proliferate in human STIM1 deficiency. Healthy CMV-seroconverted donors show values between 1 and 30% of epitope-specific CD8 T cells, with high percentages observed in elderly patients (21). Despite the highly active EBV infection, there were only few EBV LMP-2 and BMLF-1–specific CD8+ T cells (Fig. 5A). However, LMP2-specific cells could be amplified after peptide stimulation in vitro (Fig. 5B). To further explore this issue, we stimulated CFSE-labeled PBMC isolated from P5 with autologous EBV-LCL, which induced cell division in both CD4+ and CD8+ T cells (Fig. 5C). Also, CMV-specific CTL could be amplified by peptide stimulation in vitro (Fig. 5D, inset). When these cells were tested for their lytic activity on autologous EBV-LCL loaded with CMV-specific Ag, significant viral epitope-specific target cell lysis could be observed (Fig. 5D). In contrast, epitope-specific IFN-γ production was significantly reduced (data not shown). Thus, STIM1 deficiency allowed the generation of antiviral T cells, which showed impaired cytokine production but normal virus-specific cytotoxicity in vitro.

**Impaired NK cell function and lack of NKT cells in STIM1 deficiency**

NK cells and NKT cells can also be relevant for the control of viral infections (22, 23), and we have previously demonstrated defects in degranulation and cytotoxicity of human ORAI1- or STIM1-deficient NK cells (16). In extension of these findings, we found that impaired NK cell degranulation as assessed by expression of CD107a after stimulation with NK-sensitive K562 cells could only partly be restored by IL-2 prestimulation (Fig. 6A, 6B). NK cytotoxicity was severely reduced in the absence of STIM1 (Fig. 6C). Similar to ORAI1-deficient NK cells (16), NK cells from P5 with a mutation in STIM1 showed poor production of IFN-γ and partially impaired production of MIP-1β after stimulation with K562 cells, whereas this response was normal after stimulation with IL-12 and IL-15 (Fig. 6D). Thus, the specific impairment of target cell-induced degranulation and cytokine production with preservation of activation pathways mediated by cytokine receptors is comparable in ORAI1- and STIM1-deficient NK cells. Interestingly, there was a complete absence of circulating Va24+Vb11+ NKT cells in the STIM1-deficient patient.
contrast, the numbers of NKT cells in peripheral blood of both heterozygous parents were unusually high (0.56 and 0.61% of CD3+ T cells for the father and the mother, respectively) compared with NKT cell counts in healthy controls with a median of 0.045% and a range of 0.008–0.76% in healthy donors (Fig. 6 and data not shown).

Abnormal phenotype but normal suppressive function of STIM1-deficient Treg cells

One possible explanation for the autoimmunity observed in the STIM1-deficient patients is the absence of FOXP3+ Treg cells as has been described in one STIM1-deficient patient (10). In P5, the frequency of CD25+FOXP3+ Treg cells was 2.5% of all CD4+
T cells and therefore at the lower end of the normal range (Fig. 7A). We also observed an unusual population of CD25+CD4+ T cells expressing FOXP3. FOXP3+ T cells were also found in the pulmonary lymphoproliferative lesions (Fig. 7B). Expression of CD39 has been described to correlate with the suppressive activity of Treg cells (24). Whereas 70% of CD25+FOXP3+ Treg cells from healthy controls expressed CD39, only 30% of CD25+FOXP3+ and 8% of CD25−FOXP3+ Treg cells from the patient were positive for CD39 (Fig. 7D). Nearly all CD4+CD25+ T cells of the patient were CD45RA− and CD45RO+, and most cells were CD127 negative, indicating a high activation status of the patient Treg cells (Fig. 7C). To analyze Treg function in vitro, we labeled allogeneic CD4+CD25+ responder cells from a healthy donor with CFSE and stimulated these cells with irradiated allogeneic PBMC and anti-CD3 Ab. Treg cells from either the same donor or from the patient were then added to the culture, and CFSE dilution was

**FIGURE 4.** Partial impairment of T cell activation and T cell effector functions. Expression of CD69 (A) and CD25 (B) on CD4+ and CD8+ T cells after 24-h incubation of PBMC in medium alone or supplemented with anti-CD3/CD28 beads. Dot plots are gated on CD3+ T cells. Results are representative of two independent experiments. C, CFSE proliferation assay using PBMC from the patient and a healthy donor after stimulation with PHA, anti-CD3, or anti-CD3 + IL-2 for 6 d. The histograms are gated on CD3+CD4+ T cells (left panels) or CD3+CD8+ T cells (right panels). All lymphocytes (line) including blasted T cells and T cell blasts with a high forward/side scatter profile (shaded gray) were analyzed separately. Three independent experiments were carried out. D and E, Cytokine production of CD4+ T cells after stimulation of PBMC with PMA and ionomycin for 4 h in the presence of brefeldin A. Representative results for two measurements. F, IL-2 production of CD4+ T cells after stimulation of PBMC with anti-CD3/antiCD-28 beads for 16 h in the presence of brefeldin A. Results obtained from two experiments. G, Cytotoxicity of PHA/IL-2 T cell blasts against 51Cr-labeled L1210 target cells loaded with different concentrations of anti-CD3. Two different experiments showed similar results.
measured 3 d later (Fig. 7E, 7F). The CD25⁺ Treg cells from the patient showed a suppressive activity similar to control cells. The patient Treg cells also suppressed autologous responder T cells, but due to their intrinsic proliferation defect, overall proliferation was lower than in control responders (data not shown). These findings suggest that the suppressive function of CD25⁺ Treg cells was lower than in control responders (data not shown). These in vitro stimulated cells also showed suppressive function despite the fact that Ca²⁺ influx after treatment with TG or anti-CD3 plus F(ab')₂ fragment was absent (data not shown).

Discussion

The severe primary immunodeficiencies caused by defects in STIM1 or ORAI1 impressively illustrate the key role for SOCE in human lymphocyte function. Although ORAI1 deficiency mainly manifests with severely increased susceptibility to infection (12, 25, 26), the immunological phenotype of STIM1 deficiency is more complex. Our description of a third kind now allows the evaluation of six affected patients (9, 10) and reveals a consistent phenotype of a combined immunodeficiency that is less severe than in ORAI1-deficient patients and is associated with important features of impaired immune regulation. These include early-onset autoimmune cytopenias in all six patients and lymphoproliferative disease, eczema, chronic diarrhea, and arthritis in some of them (9, 10). SOCE is required for activation, differentiation, and effector functions of most lymphocyte populations (27, 28). Impairment of antimicrobial effector lymphocytes could readily explain impaired infection control, but lymphoproliferation and autoimmune immunity are consequences of enhanced lymphocyte activation and therefore more difficult to explain. Because the defects also affect regulatory lymphocytes, this may imply that the consequences of STIM1 deficiency have greater impact on tolerogenic than on immunogenic effector functions. Our studies were therefore designed to more precisely define the impairment of Ag-specific effector and Treg immunity in human STIM1 deficiency.

The homozygous Arg429Cys STIM1 point mutation detected in the two reported siblings differs from the previously described E128RfsX9 frame shift (10) and 1538-IG > A splice site mutations (9) in that STIM1 protein expression was largely unaffected in our patients. Arg429 is located within a cytoplasmic STIM1 domain [alternatively named CRAC activation domain (29), STIM1 Orai-activating region (30), ORAI1-activating small fragment (31), or Coiled-coil fragment b9 (32)] that binds directly to the N and C termini of ORAI1 and that is sufficient for CRAC channel opening (29). The mutation completely abolished Ca²⁺ influx in purified CD3⁺ T cells and in vitro-expanded Treg cells (data not shown) similar to the observations in EBV-transformed B cell lines and fibroblasts of the previously reported STIM1-deficient patients (9, 10). Moreover, the clinical presentation including the dental enamel defect, anhidrosis, mild muscular hypotonia, and nonreactive pupils as well as the onset and severity of immunodeficiency and immune dysregulation was comparable in all six STIM1-deficient patients (9, 10). Interestingly, the clinically asymptomatic heterozygous mother showed a partial impairment of Ca²⁺ influx, which is similar to the Arg91Trp mutation in ORAI1 (26), but not to other loss-of-function mutations in that protein (25). It is tempting to speculate that the Arg429Cys mutation exerts a dominant-negative effect on CRAC channel function by interfering either with the multimerization of STIM1 or the interaction of STIM1 with ORAI1. This will have to be clarified in future experiments.

A key feature of STIM1 deficiency is a broad susceptibility to viral infections that includes enterovirus and several herpesviruses such as EBV, CMV, varicella zoster virus, HSV, and human herpes virus-8. However, antiviral immune responses have so far not been analyzed in humans or mice with CRAC channel deficiencies. Both of our patients had persistent EBV and CMV infection and suffered from recurrent HSV1 reactivation. This was not associated with a failure to mount virus-specific IgG Ab responses, which were detectable against all three viruses. This ability to mount at least some Ag-specific Ab responses is consistent with findings in STIM1-deficient bone marrow chimeric mice (33) and...
supports the notion that STIM1-dependent SOCE is dispensable for the differentiation of CD4+ T cells into Th cells and that of B cells into Ab-secreting plasma cells in vivo. More unexpectedly, we could demonstrate significant antiviral CD8+ T cell populations specific for CMV and to a lesser extent for EBV by MHC-peptide tetramer staining. Previous experiments on Ag-specific T cell responses in a mouse model of experimental autoimmune encephalitis yielded conflicting results on the requirement of STIM1 for the generation of neuroantigen-specific T cell responses in vivo (34, 35). The virus-specific T cells in P5 were reactive to Ag, because they could be further expanded by stimulation with their cognate peptide in vitro. The predominance of terminally differentiated CD8+ T cells in the context of chronic viral infections further suggested that their cellular differentiation program was not dependent on STIM1. Overall, these data indicated that the priming phase of antiviral CD8+ T cell responses was not dependent on STIM1.

Despite the presence of these important elements of antiviral immunity, the patients failed to control their infections. It is likely that one important factor was the reduced proliferative response of STIM1-deficient T cells (10, 17, 33), which could only partially be restored by the addition of IL-2. In the context of a viral infection, such a delay in the amplification of antiviral T cells is probably critical for virus control. Another relevant factor was the partial impairment of T cell effector functions. As expected from murine studies, the impairment in the production of cytokines was most obvious (17, 33–35). In particular, we observed a dramatic reduction in the ability of T cells to produce IL-2, IFN-γ, and IL-17. In contrast, the production of IL-4 was largely retained. This may be explained by the fact that the Ca2+-dependent transcription factor NFAT, which is essential for cytokine production by T cells, also mediates a negative-feedback loop that inhibits IL-4 transcription, leading to a strong Th2 bias in NFAT1-deficient mice (36, 37). Expression of high levels of PD-1 and 2B4, receptors associated with a poorly functional, exhausted CTL phenotype (19, 38), may also have contributed to the poor antiviral T cell activity.

In contrast to the severe impairment of cytokine production, the patient CTL showed normal antiviral cytotoxicity in vitro. This was unexpected, because we have recently demonstrated (16) and confirmed in this study that both ORAI1- and STIM1-deficient human NK cells have a severe cytotoxicity defect due to impaired lytic granule mobilization. The fact that NK cells were analyzed directly ex vivo or after short-term IL-2 stimulation, whereas CTL were analyzed after prolonged culture with PHA or viral peptide and IL-2 could explain these differences. However, different dependence of cytotoxicity on STIM1 in the two cell types remains another possibility. Overall, these data show that the effector phase of T cell responses is only partially affected by STIM1 deficiency. This conclusion is supported by the observation in mice that STIM1-deficient CD4+ T cells are capable of inducing acute graft-versus-host disease upon transfer into allogeneic recipients, albeit with delayed or reduced severity of disease and with lower levels of inflammatory cytokines (33).

The other key clinical feature of STIM1 deficiency is impaired immune regulation, most consistently manifesting as autoimmune
cytopenia and lymphoproliferation. Both of our patients and three of the four previously reported STIM1-deficient patients had splenomegaly and lymphoadenopathy. In addition, P5 developed EBV-associated lymphoproliferation in the lung, which was unusually severe and destructive. The excellent clinical response to rituximab treatment may indicate that the disease was at least in part driven by the EBV infection. However, lymphoid infiltrations in the absence of infection have also been observed in the lung, liver, spleen, and lymph nodes of STIM1-deficient mice (33) and in mice lacking STIM1 and STIM2 exclusively in T cells (17). In the latter mice, the infiltrates showed a high proportion of myelomonocytic cells, whereas activated CD8+ T cells predominated in our patient. Another relevant observation in this context is the moderate T cell lymphocytosis that was documented in three of the six STIM1-deficient patients. Again, viral infections could contribute, but lymphocytosis has also been observed in the mouse models in the absence of infection (17). T cell lymphoproliferative disease in an immunodeficiency associated with impaired T cell proliferation in vitro is counterintuitive, but one potential explanation could be the increased proliferation of STIM1-deficient T cells toward homeostatic stimuli in vivo (33). Another possibility would be impaired apoptosis, but CD95- or anti-CD3–induced apoptosis was normal in our and one previously reported patient (10 and data not shown).

Because Ca2+ flux and NFAT activation are crucial for Treg cell differentiation and function (17, 39, 40), a role for Treg cells in the phenotype of immune dysregulation in STIM1 deficiency has been postulated. In fact, the clinical phenotype of lymphoproliferation with autoimmune cytopenia, eczema, and bowel inflammation is in part reminiscent of FOXP3 deficiency. In support of this notion, mice lacking STIM1 and STIM2 in T cells have reduced numbers of Treg cells, and their myelo- and lymphoproliferative phenotype could be prevented by transfer of wild-type CD4+CD25+ Treg cells. Moreover, the numbers of circulating Treg cells were reduced in one of the previously published STIM1-deficient patients (10). By contrast, the numbers of circulating FOXP3+ T cells were only slightly reduced in our patient, and FOXP3-expressing cells were abundant in his lymph nodes and lung. The patient’s Treg cells did, however, show some important phenotypic differences compared with control Treg cells, as their expression of CD25 and CD39 was significantly reduced. STIM1-deficient CD4+CD25+ T cells (defined as Treg cells) isolated from the patient showed good suppressive function in coincubation assays in vitro. These data are consistent with the observation of normal numbers and function of STIM1-deficient Treg cells in mice (17, 33). Although our data show that STIM1-mediated SOCE in human Treg is not required for their suppressive function in vitro, this may not reflect the situation in vivo. In particular, Treg function in vivo is dependent on IL-2 (41–44), the secretion of which was severely impaired in our patient. Overall, the contribution of impaired Treg cell differentiation and function to the immune dysregulation in STIM1 deficiency remains incompletely defined.

In summary, this comprehensive analysis of the immune system in a STIM1-deficient patient revealed that the complex clinical phenotype of human STIM1 deficiency cannot be easily explained by absent SOCE in T cells alone leading to impaired peripheral Treg and effector T cell function. Intrinsic defects in other immune cell populations such as NK cells, NKT cells, and B cells (45, 46)
are likely to contribute to the observed phenotype of immunodeficiency and immune dysregulation.

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Disclosures

The authors have no financial conflicts of interest.

References

**A) Antibodies used for flow cytometry**

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<th>Antibody</th>
<th>Clone</th>
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**B) Antibodies used for immunohistochemistry**

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**Supplementary table 1:** Antibodies used for flowcytometry and immunohistochemistry.