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Infectious Complications Predict Premature CD8+ T-cell Senescence in CD40 Ligand-Deficient Patients

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Abstract

Purpose—CD40 ligand (CD40L)-deficient patients display increased susceptibilities to infections that can be mitigated with effective prophylactic strategies including immunoglobulin G (IgG) replacement and prophylactic antibiotics. $CD8^+$ T-cell senescence has been described in CD40L deficiency, but it is unclear if this is an intrinsic feature of the disease or secondary to

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Ethical Approval This study was approved by the Institutional Review Boards of all participating institutions with appropriate consents (see subjects in the "Methods" section).

Consent to Participate Appropriate consents were obtained and all authors agree to publish (see subjects in the "Methods" section).

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infectious exposures. To address this question, we assessed $CD8^+$ T-cell senescence and its relationship to clinical histories, including prophylaxis adherence and infections, in CD40L-deficient patients.

Methods—Peripheral $CD8^+$ T-cells from seven CD40L-deficient patients and healthy controls (HCs) were assessed for senescent features using T-cell receptor excision circle (TREC) analysis, flow cytometry, cytometry by time of flight (CyTOF) and in vitro functional determinations including CMV-specific proliferation and cytokine release assays.

Results—Three patients (5, 28, and 34 years old) who were poorly adherent to immunoglobulin G replacement and *Pneumocystis jirovecii* pneumonia (PJP) prophylaxis and/or experienced multiple childhood pneumonias (patient group 1) had an expansion of effector memory $CD8^+$ T-cells with the senescent phenotype when compared to HCs. Such changes were not observed in the patient group 2 (four patients, 16, 22, 24, and 33 years old) who were life-long adherents to prophylaxis and experienced few infectious complications. CyTOF analysis of $CD8^+$ T-cells from the 5-year-old patient and older adult HCs showed similar expression patterns of senescence-associated molecules.

Conclusions—Our findings support that recurrent infections and non-adherence to prophylaxis promote early $CD8^+$ T-cell senescence in CD40L deficiency. Premature senescence may increase malignant susceptibilities and further exacerbate infectious risk in CD40L-deficient patients.

Keywords

X-linked CD40 ligand deficiency; $CD8^+$ T-cell senescence; combined immunodeficiency; class switching recombination defect; immunoglobulin replacement therapy; *Pneumocystis jirovecii* pneumonia (PJP) prophylactic antibiotics

Introduction

CD40 ligand (CD40L, CD154), encoded by *CD40LG* on chromosome X, is a type II transmembrane protein which belongs to the TNF ligand superfamily (TNFSF) [1]. CD40L, expressed primarily on activated $CD4^+$ T-cells, regulates innate, humoral, and cellular arms of the immune system [2] by interacting with CD40 expressed on various immune and non-immune cells [3, 4]. Such an interaction with antigen-activated B-cells promotes humoral immunity by inducing B-cell clonal expansion, immunoglobulin class switching, and affinity maturation [5, 6]. Due to defective co-stimulation, it is not surprising that patients with the CD40L deficiency have a defect in antigen-induced T-cell proliferation and effector function [7–9], supporting the critical role of the CD40 and CD40L interaction in T-cell immunity.

In mouse models, it is well described that priming of naïve $CD8^+$ T-cells depends on $CD4^+$ T-cell-mediated dendritic cell licensing via CD40-CD40L interaction [10–12]. Additionally, rapid impairment of $CD8^+$ T-cell responsiveness and failure to permanently control viral replication in CD40L-deficient mice were reported [13]. The loss of $CD8^+$ T-cell memory was shown to be prevented by passive transfer of virus-specific antibodies into infected mice, indicating that long-term $CD8^+$ T-cell memory and virus control are promoted by virus-specific IgG responses via the CD40-CD40L interaction [14]. The importance of the CD40L-dependent $CD4^+$ T-cell help in maintaining $CD8^+$ T-cell homeostasis is further

supported by the observation that CD40L-deficient mice have decreased generation of effector memory (EM) $CD8^+$ T-cells expressing IL-7Rα, an essential molecule for memory T-cell homeostasis [15]. IL-$7R\alpha^{low}$ EM $CD8^+$ T-cells with senescent characteristics were shown to expand with aging, chronic and/or recurrent infection, systemic lupus erythematous, and malignancies in humans [16–19]. Collectively, these data support the critical role of the CD40-CD40L interaction and immunoglobulins in maintaining $CD8^+$ T-cell homeostasis.

Indeed, patients with pathogenic variants in *CD40LG* develop an X-linked combined immunodeficiency, also known as X-linked hyper-IgM syndrome with infectious and autoimmune manifestations, and a predisposition to developing lymphomas and gastrointestinal/biliary malignancies [3, 4]. The prognosis of CD40L deficiency is poor, with an average survival rate of 20% by the age of 25 years [20]. Patients with CD40L deficiency are treated with IgG replacement to control infections. Though hematopoietic cell transplant (HCT) can be potentially curative, these patients can still develop liver disease and malignancy after the 2nd and 3rd decades of life, contributing to mortality [20, 21].

Although the results of animal studies support the critical role of the CD40-CD40L interaction in maintaining $CD8^+$ T-cell homeostasis, the effect of CD40L deficiency in human $CD8^+$ T-cell immunity is largely unexplored. A recent case report of a 32-year-old patient with CD40L deficiency showed an expansion of memory $CD8^+$ T-cells with increased expression of the senescence marker CD57, suggesting that CD40L may play a role in $CD8^+$ T-cell senescence [22]. However, little is known about the mechanisms of how CD40L deficiency drives expansion of senescent $CD8^+$ T-cells. To address this question, we assessed $CD8^+$ T-cell senescence in CD40L-deficient patients with and without histories of significant multiple childhood infections and/or adherence to prophylaxis including IgG replacement and *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis, based on the hypothesis that CD40L deficiency-associated $CD8^+$ T-cell senescence occurs as an intrinsic feature of CD40L deficiency or secondary to repetitive infections and prophylaxis non-adherence. The results of our study indicate that $CD8^+$ T-cell senescence in CD40L deficiency can develop and persist, even during early childhood, in the setting of significant childhood infections including multiple pneumonias and poor adherence to prophylaxis, supporting the importance of early diagnosis, prompt initiation of and compliance with prophylaxis to avoid multiple infections and long-term and irreversible derangement of T-cell immunity.

Methods

Subjects and Peripheral Blood Mononuclear Cell Purification

The 5-year-old male CD40L deficiency patient was recruited from the Yale Immunodeficiency Clinic. Also, a total of 12 male healthy controls (HCs, four pediatric HCs (age 3–18), four young adult HCs (age 19–40), and four older adult HCs (age 41–62)) were recruited as previously described [18]. After obtaining informed consent, peripheral blood was collected and subjected to Ficoll peripheral blood mononuclear cell (PBMCs) purification [16]. We obtained frozen PBMCs, clinical histories, and immunological data of six de-identified male patients with CD40L deficiency from the Children's Hospital of

Philadelphia (CHOP), the Nationwide Children's Hospital, and the National Institute of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID) (see details in Fig. 1 and Table 1). This study was approved by the Institutional Review Boards of all institutions.

Flow Cytometric Analysis

PBMCs were stained with relevant antibodies (Supplement table 1A). Cells were analyzed using an LSRII flow cytometer (BD) and FlowJo® software (BD).

Measurement of T-cell Receptor Excision Circles

Genomic DNA was isolated from PBMCs using a DNeasy® blood and tissue kit (Qiagen, Hilden, Germany). The quantitative PCR (qPCR) for T-cell receptor excision circle (TREC) enumeration was performed using MyTRECKit® (Genenplus, Oak Creek, WI).

CyTOF Analysis

All mass cytometry reagents were purchased from Fluidigm, Inc. (South San Francisco, CA). PBMCs (2×10^6) were stained with a panel of metal-tagged antibodies (supplement Table 1B) and processed as previously described [23]. All FCS files were normalized and analyzed using the analytic tool CYT and FlowJo™ software (BD) as described [23]. *t*-SNE and PhenoGraph® were performed on gated cells (3000 $CD3^+CD8^+$ T-cells). Heatmap shows the results of an unbiased hierarchical clustering analysis of molecule and studied subjects based on the *z*-scores of geometric mean metal expression intensities (GMMI) of individual molecules.

Analysis of EM $CD8^+$ T-cell Proliferation and Cytomegalovirus-Specific $CD8^+$ T-cells

Analyses for EM $CD8^+$ T-cell proliferation and cytomegalovirus (CMV)-specific $CD8^+$ T-cells producing cytokines were performed using flow cytometry as previously described [24, 25]. Briefly, EM $CD8^+$ T-cells were purified from PBMCs by negative selection (EasySep™ Kit, STEMCELL technologies, Cambridge, MA), stained with carboxyfluorescein diacetate (CFSE), and incubated for 72 h with or without T-cell receptor stimulation (beads coated with anti-CD3 and -CD28 antibodies) (Thermo Scientific, Waltham, MA). For the analysis of CMV-specific $CD8^+$ T-cells, PBMCs were incubated for 6 h with or without (unstimulated control) overlapping CMV peptides (Proimmune, Sarasota, FL) in the presence of GolgiPlug® (BD) during the last 5 h of stimulation. Cells were fixed, permeabilized, and stained with antibodies to TNF-α and IFN-γ for flow cytometric analysis of intracellular TNF-α and IFN-γ as described above.

Statistical analysis

Data were analyzed by the ANOVA with the Dunnett's test for multiple comparisons using Prism 7 (GraphPad Software, Inc, San Diego, Calif). p values < 0.05 were considered statistically significant. Principal component analysis (PCA) was done based on the frequencies of naïve, CM, EM, IL-7Rα^{high}, $CD28^+$, and $CD57^+$ EM $CD8^+$ T-cells using R software.

Results

The Clinical Characteristics of CD40 Ligand Deficient Patients

We enrolled seven CD40L-deficient subjects from five unrelated families whose ages ranged from 5 to 34 years (Fig. 1 and Table 1). Three subjects (patient group 1, A1, B1, and C2) had histories of non-adherence to IgG replacement, PJP prophylactic antibiotics, and/or multiple significant infections such as pneumonia in childhood. The other four subjects (patient group 2, C1, D1, D2 and E1) were life-long adherents to both IgG replacement and PJP prophylactic antibiotics and had no multiple childhood pneumonia. Additional infection histories of the studied subjects are provided in Table 1. Serum immunoglobulin levels and absolute cell counts of lymphocyte subsets analyzed at the diagnosis of CD40L deficiency were available for subject A1 but not for others (Table 2). Subject A1 had elevated levels of IgM and reduced levels of IgG at the diagnosis, which decreased and increased, respectively, after the initiation of IgG replacement. Of note, subjects A1, C1, C2, and E1 had undetectable IgA (Table 2). All patients showed reduced or undetectable switched memory B cells ($CD19^{+}IgD^{-}IgM^{-}$) (Table 2).

$CD8^{+}$ T-cell Senescence Correlates with Early Childhood Infection Histories and Prophylaxis Non-adherence in Patients with CD40L Deficiency

Using flow cytometry, we analyzed subjects' $CD8^{+}$ T-cells for features of physiologic aging, including the frequencies of naïve, central memory (CM), and effector memory (EM) $CD8^{+}$ T-cells (Fig. 2A–B). Compared to pediatric and young adult HCs, CD40L-deficient patients who had histories of non-adherence to IgG replacement, PJP prophylactic antibiotics, and/or multiple significant infections such as pneumonia in childhood (patient group 1, patients A1, B1, and C2, Table 1) displayed a decreased mean frequency (%) of naïve ($CD45RA^{+}CCR7^{+}$) $CD8^{+}$ T-cells (25.03 ± 7.65 vs 65.55 ± 10.40, $p = 0.0002$, and 25.03 ± 7.65 vs 53.13 ± 8.53, $p = 0.003$, respectively) and an increased frequency of EM CD8+ T-cells ($CCR7^{-}CD45RA^{+/-}$; 63.47 ± 7.14 vs 14.20 ± 4.61, $p < 0.0001$, 63.47 ± 7.14 vs 23.68 ± 7.05, $p < 0.0001$, respectively) (Fig. 2A–B). In contrast, the frequency of naïve $CD8^{+}$ T-cells in the patients with strict adherence to both IgG replacement and PJP prophylactic antibiotics (patient group 2, Table 1) was comparable to that in young adult HCs with similar ages (Fig. 2A–B). The frequency of CM $CD8^{+}$ T-cells was not different among all patients and HCs (Fig. 2A–B). In addition, the frequencies of naïve, CM, and EM $CD4^{+}$ T-cells were similar in all patients and HCs (Supplement Figure 1A).

The decreased naïve T-cell frequency in our CD40L-deficient patients could be secondary to reduced thymic output or excessive cell replication. To address this point, we measured thymus output via TREC analysis of PBMCs. In all CD40L-deficient subjects, TREC copy numbers were similar to age-matched HCs (Supplement Figure 1B), emphasizing the T-cell intrinsic nature of the disorder which does not appreciably affect thymic function.

To further assess $CD8^{+}$ T-cell senescence, we measured cell surface expression of IL-7Rα, CD28, and CD57 by EM $CD8^{+}$ T-cells (Fig. 2A and C). The T-cell homeostasis cytokine receptor IL-7Rα and the costimulatory molecule CD28 on EM $CD8^{+}$ T-cells decrease with aging while the senescence-associated molecule CD57 increases on the same cells.

Compared to pediatric and young adult HCs, the patient group 1 had lower frequencies of IL-7Rα^{high} EM CD8$^+$ T-cells (28.67 ± 19.14 vs 87.78 ± 6.32, $p = 0.001$, 28.67 ± 19.14 vs 79.80 ± 12.52, $p = 0.003$, respectively) and CD28$^+$ EM CD8$^+$ T-cells (19.33 ± 9.02 vs 89.40 ± 4.88, $p < 0.0001$, 19.33 ± 9.02 vs 77.95 ± 14.65, $p < 0.0001$, respectively), and a higher frequency of CD57$^+$ EM CD8$^+$ T-cells (53.33 ± 4.16 vs 3.47 ± 3.21, $p = 0.001$, 53.33 ± 4.16 vs 21.59 ± 21.67, $p = 0.02$, respectively). These alterations were not observed in the patient group 2. We previously reported that human naïve CD8$^+$ T-cells express high levels of IL-7Rα which can be downregulated by common gamma chain cytokines including IL-2, IL-7, and IL-15 [26, 27]. The frequencies of IL-7Rα^{high} naïve CD8$^+$ T-cells were similar in patient groups 1 and 2 (Supplement Figure 2), indicating that the decreased expression of IL-7Rα by memory CD8$^+$ T-cells in group 1 was not a global phenomenon driven by such cytokines. PCA with variables including naïve, CM, EM, IL-7Rα^{high}, CD28$^+$, and CD57$^+$ EM CD8$^+$ T-cells showed that the patients with histories of non-adherence to prophylaxis and/or multiple childhood pneumonias (ages 5, 30, and 34 years) were clustered with older HCs (ages, 43–62 years) whereas the patients without such histories (ages, 16–33 years) were grouped with pediatric (ages, 3–7 years) and young (ages, 19–35 years) HCs (Fig. 2D).

In-depth, multi-dimensional CyTOF analysis of CD8$^+$ T-cells from the 5-year-old CD40L-deficient subject A1 and older HCs revealed similar expression patterns of molecules related to senescence including CD57, T-bet, granzyme B, and perforin (Fig. 3a). The geometric mean metal expression intensities (GMMI) for CD62L, CD27, CD57, granzyme B, perforin, and T-bet were similar in EM CD8$^+$ T-cells of the 5-year-old patient and older adult HCs (Fig. 3b). An unbiased hierarchical analysis was performed based on the *z*-scores of GMMI of individual molecules on or in EM CD8$^+$ T-cells. These analyses demonstrated that the CD8$^+$ T-cell profile of patient A1 is consistent with that observed in older adult HCs (Fig. 3c).

CD8$^+$ T-cells with Senescence Phenotypes in CD40L Deficiency Were Unresponsive and Durably Senescent

To determine if EM CD8$^+$ T-cells of subject A1 were less responsive, we analyzed proliferation of these cells in response to anti-CD3/CD28 antibodies, which showed poor proliferation (Fig. 4a). As subject A1 was CMV infected, we analyzed IFN-γ and TNF-α in CMV peptide stimulated CD8$^+$ T-cells. CMV-specific CD8$^+$ T-cells producing IFN-γ and TNF-α were modest (< 3%) in subject A1 indicating that expanded memory CD8$^+$ T-cells were either CMV-unresponsive and/or CMV-unspecific despite a prior history of CMV infection (Fig. 4b). Hence, CD8$^+$ T-cells from a treatment non-adherent CD40L-deficient subject appear and behave senescent.

After 5 years of poor adherence and a second PJP, CD40L-deficient patient A1 received IgG replacement and did not have active infection over 650 days; however, the senescence changes in CD8$^+$ T-cells persisted for > 600 days (Table 3). Also, the senescence changes in CD8$^+$ T-cells remained the same over 210 days in the 34-year-old subject C2 who had been adherent to IgG replacement since age 4 but started PJP prophylactic antibiotics at age 21 with two incidences of pneumonia as a teenager (Table 3), whereas the 33-year-old subject C1 with excellent prophylaxis adherence since infancy continued to have high frequencies of

naïve and IL-7Rα^{high} CD8^{+} EM cells (Table 3). The persistently altered frequencies of naïve, EM, and IL-7Rα^{low} EM CD8^{+} T-cells suggest that the immune alterations related to non-adherence to IVIG replacement and PJP prophylaxis in CD40L deficiency are durable and may persist for years.

Discussion

Here, we hypothesized that CD40L deficiency-associated CD8^{+} T-cell senescence occurs as an intrinsic feature of CD40L deficiency or secondary to repetitive infections and prophylaxis non-adherence. We tested this hypothesis by investigating the relationship of CD8^{+} T-cell senescence with clinical histories, including infections and prophylaxis compliance, in seven patients with CD40L deficiency aged 5 to 34 years and healthy controls aged 3 to 62 years. Three of the seven patients with histories of non-adherence to IgG replacement, PJP prophylactic antibiotics, and/or multiple significant infections such as pneumonia in childhood had the senescent CD8^{+} T-cell phenotype characterized by EM (CD45RA$^{+/-}$CCR7^{-}) CD8^{+} T-cell expansion with decreased IL-7Rα and CD28 expression and increased CD57 expression. In the 5-year-old patient, such senescent changes in CD8^{+} T-cells persisted for > 600 days even in the absence of clinically apparent infection and EM CD8^{+} T-cells had impaired proliferation in response to anti-CD3 and -CD28 antibodies. Also, the 34-year-old patient who started PJP prophylactic antibiotics at age 21 and two pneumonias during teenage years had persistent senescent changes in CD8^{+} T-cells over 210 days. The results of our study indicate that CD8^{+} T-cell senescence in CD40L deficiency can develop and persist, even during early childhood, in the setting of poor adherence to prophylaxis, supporting the importance of early diagnosis, prompt initiation of, and compliance with prophylaxis to avoid infectious complications and long-term and irreversible derangement of T-cell immunity which may further the risks of infections and malignancies.

CD8^{+} T-cell senescence can result from repetitive T-cell stimulation, which leads to a loss of the replicative capacity of antigen-specific T-cell population [28]. Physiologically, alternations in T-cell immunity are observed with aging [16, 18]. The most noticeable change is likely the expansion of memory CD8^{+} T-cells which express low and high levels of CD28 and CD57, respectively [29, 30]. Also, the frequency of IL-7Rα^{low} EM CD8^{+} T-cells possessing senescence characteristics have been shown to increase in the context of aging, chronic, and/or recurrent immune stimulation from infections and tumors [16, 17, 31]. CD57 is primarily expressed by senescent CD8^{+} T-cells while PD-1 is associated with T-cell exhaustion [32]. Although both senescent and exhausted T-cells have impaired cell proliferation, only senescent T-cells produce cytokines upon activation [32]. In our analysis, the expression levels of CD57 and PD-1 by CD8^{+} T-cells of our patient A1 were high and low, respectively, suggesting the senescent characteristics of these cells. Indeed, CD8^{+} cells of this patient had intact cytokine production but impaired proliferation. CD40L-deficient patients with their associated hypogammaglobulinemia are highly susceptible to infections, especially opportunistic infections [2–4, 8] and such susceptibility could account for the senescent changes in CD8^{+} T-cells. This idea is supported by our observation of different degrees of CD8^{+} T-cell senescence in two separately raised siblings with CD40L deficiency who carried distinct histories of infections and adherence to the prophylaxis.

CD40L may directly regulate the development of long-term memory $CD8^+$ T-cells expressing the T-cell homeostasis cytokine receptor IL-7Rα as shown in CD40L-deficient mice. These mice had impaired generation of memory $CD8^+$ T-cells expressing IL-7Rα upon *Listeria monocytogenes* infection [15]. Two previous studies analyzed $CD8^+$ T-cells in a total of seven patients with CD40L deficiency. In the study by Jain et al., six patients with CD40L deficiency were assessed for the expression of CD45RA and CD45RO on $CD8^+$ T-cells as markers for naïve and memory cells, respectively [9]. Although this study found decreased and increased $CD45RO^+$ and $CD45RA^+$ T-cell counts, respectively [9], $CD45RA^+$ $CCR7^-$ EM T-cells (TEMRA) were likely incorrectly assigned as $CD45RA^+$ naïve T-cells [33]. In contrast, a case report of a 32-year-old CD40L-deficient patient observed an increased frequency of EM $CD8^+$ T-cells expressing the senescence marker CD57 and defective T-cell proliferation in response to mitogens [22]. Of note, this patient was initially diagnosed with common variable immunodeficiency (CVID) at age 10. Despite the initiation of IgG replacement, the patient continued to have infections [22]. Further immunological work up led to the diagnosis of CD40L deficiency at age 28 [22]. This patient's history of adherence to prophylaxis is unknown; however, it is conceivable that a delayed diagnosis and subsequent management of his immunodeficiency were a significant factor leading to premature $CD8^+$ T-cell senescence. The role of IgG replacement in CD40L-deficient patients could be more than a replenishment of immunoglobulins. The role of IgG replacement in preventing loss of virus-specific $CD8^+$ T-cell memory was shown in a CD40L-deficient mouse model [14]. High-dose immunoglobulin therapy (1 g/kg) is frequently used as an immune modulating therapy for autoimmune and inflammatory disorders [34]. Although this dose is typically higher than the replacement/reconstitution dose of 400–600 mg/kg for patients with primary immune deficiencies, the lower dose of IgG replacement may also modulate T-cell immunity. In a study where the effect of immunoglobulin replacement (400 mg/kg) was evaluated in patients with CVID, increased $CD8^+$ T-cell activation was suppressed by IgG replacement [35]. Thus, it is possible that exogeneous immunoglobulins positively alter the cellular immune response in CD40L-deficient patients.

T-cell senescence is reported in patients with other types of primary immunodeficiency diseases (PIDs) that occur secondary to germline mutations. Patients with activated PI3 kinase delta syndrome (APDS) that have lymphoproliferation, autoimmunity, and recurrent infections develop the expansion of effector memory $CD8^+$ T-cells, including those expressing the senescent marker CD57, with a decreased frequency of naïve $CD8^+$ T-cells [36–38]. Although recurrent infections could contribute to such senescent changes in these patients, the restoration of the balance of naïve and EM T-cells by the mTOR inhibitor rapamycin and the expansion of CM and EM $CD8^+$ T-cells with decreased naïve $CD8^+$ T-cells in a mouse model of APDS, even in the absence of infection, suggest that this phenomenon of T-cell senescence likely stems from a cell intrinsic alteration of Akt and mTOR activation which is regulated by PI3 kinase [36, 38]. Similarly, the expansion of $CD45RA^+CCR7^-$memory $CD8^+$ T-cells with increased CD57 expression was found in patients with dedicator of cytokinesis 8 (DOCK8) immunodeficiency syndrome characterized by severe cutaneous viral infections [39]. GATA binding protein 2 (GATA2) deficiency is a rare disorder that has clinical manifestations of bone marrow failure, acute

myeloid leukemia, deafness, severe viral, and bacterial infections. In a small number of GATA2-deficient patients with history of severe infections, the expansion of memory $CD8^+$ T-cells was reported [40]. These findings, together with the results of our study, support that $CD8^+$ T-cell senescence can occur in patients with PIDs, especially those who have suffered from significant infections.

To the best of our knowledge, this is the first report of $CD8^+$ T-cell senescence in a small cohort of CD40L-deficient patients including both pediatric and adult patients. Our data suggest that prompt diagnosis of CD40L deficiency coupled with early initiation of and strict adherence to prophylaxis including IgG replacement and prophylactic antibiotics may prevent the premature onset $CD8^+$ T-cell senescence. Given the critical role of $CD8^+$ T-cells in host defense, it is tempting to speculate that $CD8^+$ T-cell senescence may contribute to the poor prognosis of CD40L deficiency by further increasing the risks of infection and malignancy. This hypothesis warrants further investigations into the mechanism of how $CD8^+$ T-cell senescence contributes to the mortality and morbidity of CD40L-deficient patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

The authors confirm that the de-identified data supporting the findings of this study are available within the article and its supplementary materials.

References

1. Kawabe T, Matsushima M, Hashimoto N, Imaizumi K, Hasegawa Y. CD40/CD40 ligand interactions in immune responses and pulmonary immunity. Nagoya J Med Sci. 2011;73(3–4):69–78. [PubMed: 21928689]
2. Notarangelo LD, Lanzi G, Peron S, Durandy A. Defects of class-switch recombination. J Allergy Clin Immunol. 2006;117(4):855–64. [PubMed: 16630945]
3. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science. 1993;259(5097):990–3. [PubMed: 7679801]
4. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G. CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. Nature. 1993;361(6412):541–3. [PubMed: 8094231]
5. Notarangelo LD, Duse M, Ugazio AG. Immunodeficiency with hyper-IgM (HIM). Immunodefic Rev. 1992;3(2):101–21. [PubMed: 1554497]
6. Fuleihan R, Ramesh N, Geha RS. Role of CD40-CD40-ligand interaction in Ig-isotype switching. Curr Opin Immunol. 1993;5(6): 963–7. [PubMed: 7507684]
7. Cabral-Marques O, Ramos RN, Schimke LF, Khan TA, Amaral EP, Barbosa Bomfim CC, et al. Human CD40 ligand deficiency dysregulates the macrophage transcriptome causing functional

defects that are improved by exogenous IFN-gamma. J Allergy Clin Immunol. 2017;139(3):900–12 e7. [PubMed: 27554817]

8. Samten B, Thomas EK, Gong J, Barnes PF. Depressed CD40 ligand expression contributes to reduced gamma interferon production in human tuberculosis. Infect Immun. 2000;68(5):3002–6. [PubMed: 10769003]
9. Jain A, Atkinson TP, Lipsky PE, Slater JE, Nelson DL, Strober W. Defects of T-cell effector function and post-thymic maturation in X-linked hyper-IgM syndrome. J Clin Invest. 1999;103(8):1151–8. [PubMed: 10207167]
10. Grewal IS, Xu J, Flavell RA. Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. Nature. 1995;378(6557): 617–20. [PubMed: 8524395]
11. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature. 1998;393(6684):480–3. [PubMed: 9624005]
12. Lefrancois L, Olson S, Masopust D. A critical role for CD40-CD40 ligand interactions in amplification of the mucosal CD8 T cell response. J Exp Med. 1999;190(9):1275–84. [PubMed: 10544199]
13. Thomsen AR, Nansen A, Christensen JP, Andreasen SO, Marker O. CD40 ligand is pivotal to efficient control of virus replication in mice infected with lymphocytic choriomeningitis virus. J Immunol. 1998;161(9):4583–90. [PubMed: 9794385]
14. Bachmann MF, Hunziker L, Zinkernagel RM, Storni T, Kopf M. Maintenance of memory CTL responses by T helper cells and CD40-CD40 ligand: antibodies provide the key. Eur J Immunol. 2004;34(2):317–26. [PubMed: 14768036]
15. Huster KM, Busch V, Schiemann M, Linkemann K, Kerksiek KM, Wagner H, et al. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8+ memory T cell subsets. Proc Natl Acad Sci U S A. 2004;101(15): 5610–5. [PubMed: 15044705]
16. Kim HR, Hong MS, Dan JM, Kang I. Altered IL-7Ralpha expression with aging and the potential implications of IL-7 therapy on CD8+ T-cell immune responses. Blood. 2006;107(7):2855–62. [PubMed: 16357322]
17. Pellegrini M, Calzascia T, Toe JG, Preston SP, Lin AE, Elford AR, et al. IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. Cell. 2011;144(4):601–13. [PubMed: 21295337]
18. Park HJ, Shin MS, Kim M, Bilsborrow JB, Mohanty S, Montgomery RR, et al. Transcriptomic analysis of human IL-7 receptor alpha (low) and (high) effector memory CD8(+) T cells reveals an age-associated signature linked to influenza vaccine response in older adults. Aging Cell. 2019;18(4):e12960. [PubMed: 31044512]
19. Kim JS, Cho BA, Sim JH, Shah K, Woo CM, Lee EB, et al. IL-7Ralphalow memory CD8+ T cells are significantly elevated in patients with systemic lupus erythematosus. Rheumatology (Oxford). 2012;51(9):1587–94. [PubMed: 22661557]
20. de la Morena MT, Leonard D, Torgerson TR, Cabral-Marques O, Slatter M, Aghamohammadi A, et al. Long-term outcomes of 176 patients with X-linked hyper-IgM syndrome treated with or without hematopoietic cell transplantation. J Allergy Clin Immunol. 2017;139(4):1282–92. [PubMed: 27697500]
21. Ferrua F, Galimberti S, Courteille V, Slatter MA, Booth C, Moshous D, et al. Hematopoietic stem cell transplantation for CD40 ligand deficiency: results from an EBMT/ESID-IEWPSCETIDE-PIDTC study. J Allergy Clin Immunol. 2019;143(6): 2238–53. [PubMed: 30660643]
22. Lougaris V, Lanzi G, Baronio M, Gazzurelli L, Vairo D, Lorenzini T, et al. Progressive severe B cell and NK cell deficiency with T cell senescence in adult CD40L deficiency. Clin Immunol. 2018;190: 11–4. [PubMed: 29476811]
23. Shin MS, Yim K, Moon K, Park HJ, Mohanty S, Kim JW, et al. Dissecting alterations in human CD8+ T cells with aging by high-dimensional single cell mass cytometry. Clin Immunol. 2019;200: 24–30. [PubMed: 30659916]

24. Shin MS, Lee JS, Lee N, Lee WW, Kim SH, Kang I. Maintenance of CMV-specific CD8+ T cell responses and the relationship of IL-27 to IFN-gamma levels with aging. Cytokine. 2013;61(2):485–90. [PubMed: 23280240]
25. Lee N, You S, Shin MS, Lee WW, Kang KS, Kim SH, et al. IL-6 receptor alpha defines effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma. Am J Respir Crit Care Med. 2014;190(12):1383–94. [PubMed: 25390970]
26. Kim HR, Hong MS, Dan JM, Kang I. Altered IL-7R{alpha} expression with aging and the potential implications of IL-7 therapy on CD8+ T-cell immune responses. Blood. 2006;107(7):2855–62. [PubMed: 16357322]
27. Kim HR, Hwang KA, Park SH, Kang I. IL-7 and IL-15: biology and roles in T-cell immunity in health and disease. Crit Rev Immunol. 2008;28(4):325–39. [PubMed: 19166383]
28. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? Nat Rev Immunol. 2011;11(4):289–95. [PubMed: 21436838]
29. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood. 2003;101(7):2711–20. [PubMed: 12433688]
30. Weng NP, Akbar AN, Goronzy J. CD28(−) T cells: their role in the age-associated decline of immune function. Trends Immunol. 2009;30(7):306–12. [PubMed: 19540809]
31. Johnson CB, Riesenberg BP, May BR, Gilreath SC, Li G, Staveley-O'Carroll KF, et al. Effector CD8+ T-cell engraftment and antitumor immunity in lymphodepleted hosts is IL7Ralpha dependent. Cancer Immunol Res. 2015;3(12):1364–74. [PubMed: 26297711]
32. Xu W, Larbi A. Markers of T cell senescence in humans. Int J Mol Sci. 2017;18(8).
33. Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood. 2003;101(11):4260–6. [PubMed: 12576317]
34. Ballow M Mechanisms of immune regulation by IVIG. Curr Opin Allergy Clin Immunol. 2014;14(6):509–15. [PubMed: 25337683]
35. Paquin-Proulx D, Santos BA, Carvalho KI, Toledo-Barros M, Barreto de Oliveira AK, Kokron CM, et al. IVIg immune reconstitution treatment alleviates the state of persistent immune activation and suppressed CD4 T cell counts in CVID. PLoS One. 2013;8(10):e75199. [PubMed: 24130688]
36. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. Nature Immunology. 2014;15(1):88–97. [PubMed: 24165795]
37. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. J Allergy Clin Immunol. 2017;139(2):597–606 e4. [PubMed: 27555459]
38. Edwards ESJ, Bier J, Cole TS, Wong M, Hsu P, Berglund LJ, et al. Activating PIK3CD mutations impair human cytotoxic lymphocyte differentiation and function and EBV immunity. J Allergy Clin Immunol. 2019;143(1):276–91 e6. [PubMed: 29800648]
39. Randall KL, Chan SS, Ma CS, Fung I, Mei Y, Yabas M, et al. DOCK8 deficiency impairs CD8 T cell survival and function in humans and mice. J Exp Med. 2011;208(11):2305–20. [PubMed: 22006977]
40. Ruiz-Garcia R, Rodriguez-Vigil C, Marco FM, Gallego-Bustos F, Castro-Panete MJ, Diez-Alonso L, et al. Acquired senescent T-cell phenotype correlates with clinical severity in GATA binding protein 2-deficient patients. Front Immunol. 2017;8:802. [PubMed: 28747912]

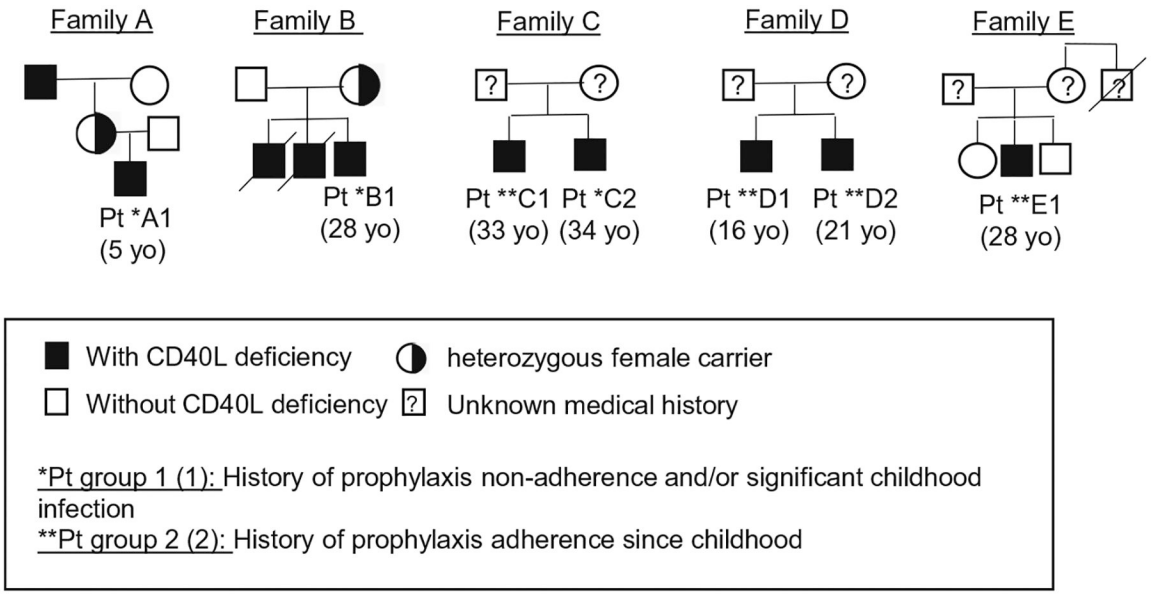

Fig. 1.

Pedigrees of studied patients with CD40L deficiency. The pedigrees of five families with CD40L pathogenic variants are depicted (filled squares, hemizygous males with CD40L deficiency; half-filled circles, heterozygous female carriers). For families C, D, and E, parental history was unavailable

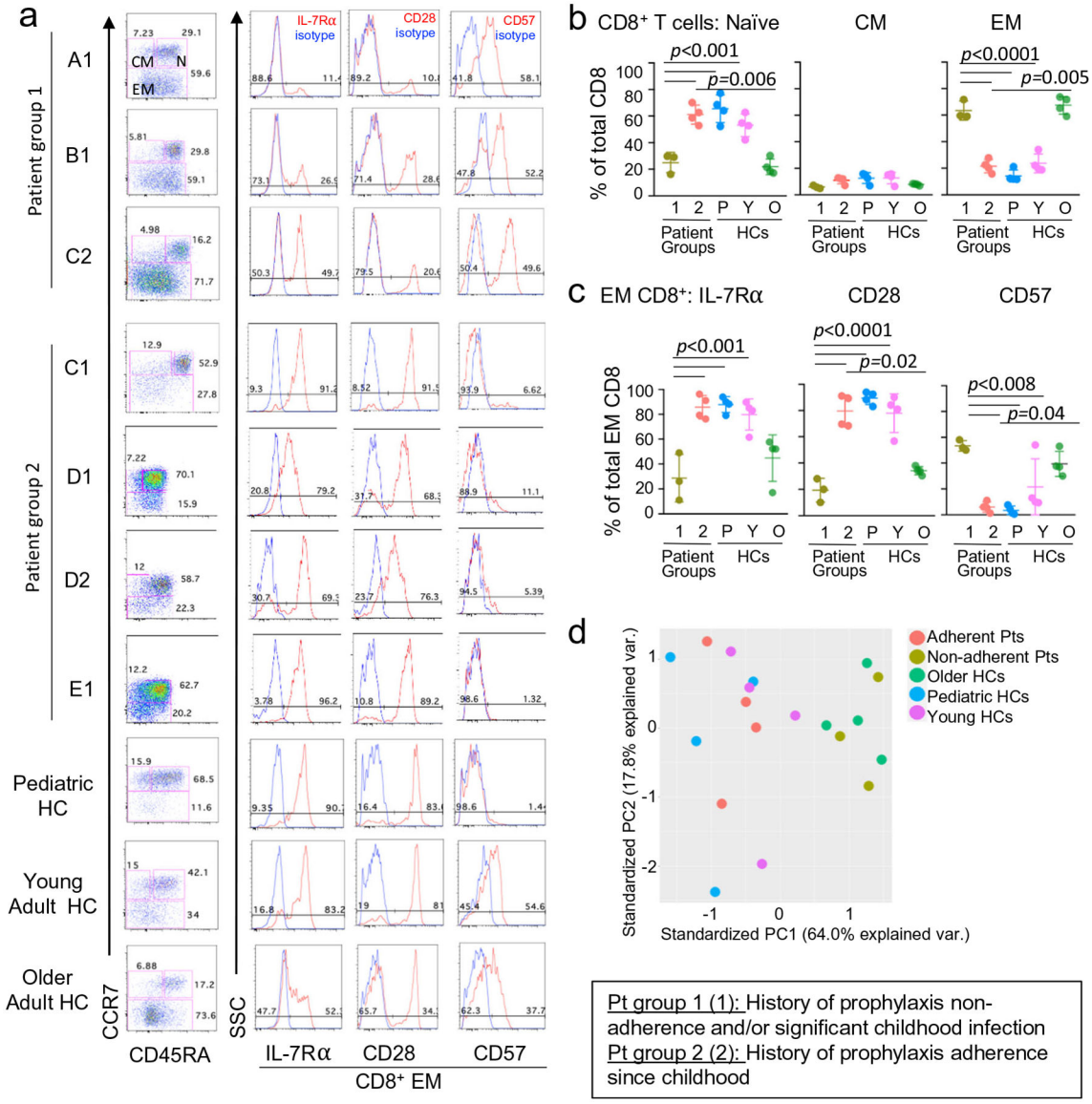

Fig. 2.

EM CD8$^+$ T-cells expressing senescence molecules expand in CD40L-deficient patients with history of prophylaxis non-adherence and/or multiple significant childhood infections.(A–C) Flow cytometric analysis of peripheral CD8$^+$ T-cell subsets and expression of surface molecules, including IL-7Rα, CD28 and CD57, in CD40L-deficient patients, pediatric (P, ages 3–7), young (Y, ages 18–35), and older (O, ages 43–62) adult healthy controls (HCs). Naïve (N, CD45RA$^+$CCR7$^+$), central memory (CM, CD45RA$^-$CCR7$^+$), and effector memory (EM, CCR7$^-$) CD8$^+$ T-cells are identified based on the expression of CD45RA and CCR7. (A) Histograms showing the expression of individual molecules. (B–C) Scatter graphs show the frequencies of naïve, CM, EM, IL-7Rα^{high}, CD28^{high}, and CD57^{high} EM CD8$^+$ T-cells. (D) PCA with the variables measured in A

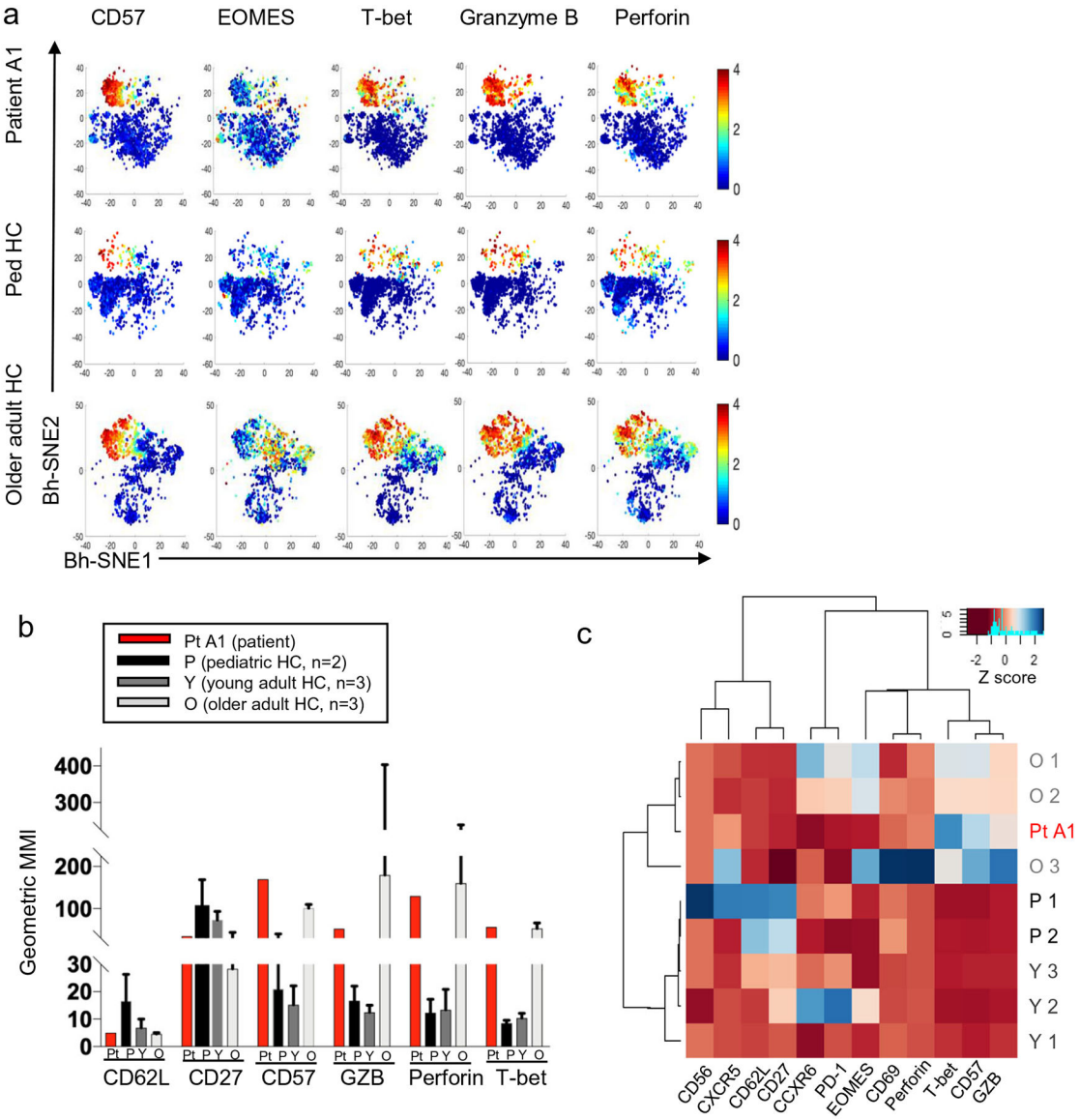

Fig. 3.

CyTOF analysis shows senescence profile of CD8$^+$ T-cells in patient A1. PBMCs of the patient and healthy controls (pediatric (P), young (Y), and older (O) adult controls) were stained with a panel of relevant antibodies. **a** *t*-SNE plots showing senescence-related molecules expressed by CD8$^+$ T-cells. **b** Geometric mean metal expression intensities (GMMI) of individual molecules by effector memory (EM, CCR7$^-$) CD8$^+$ T-cells. Bars ± error bars indicate mean ± standard error of mean (SEM), respectively. **c** Heatmap showing an unbiased hierarchical clustering analysis of molecules and subjects based on the *z*-scores of GMMI of individual molecules

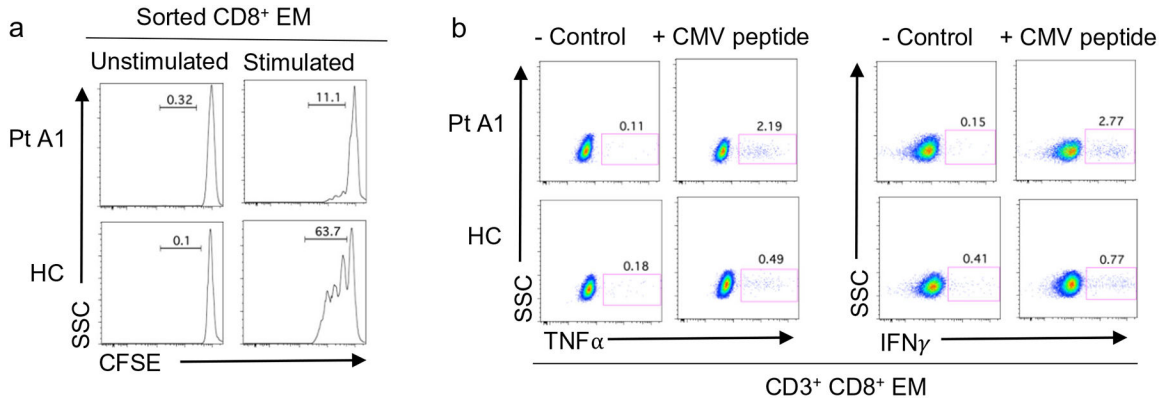

Fig. 4.

Patient A1 has impaired proliferation of EM CD8$^+$ T-cells but intact cytokine production by CMV-specific EM CD8$^+$ T-cells. The histograms (**a**) and dot plots (**b**) represent two independent experiments of assessing the frequency of proliferating cells stimulated with and without anti-CD3/CD28 antibodies and cytokine production after incubation with and without CMV overlapping peptides, respectively, in patient A1 and HC. Representative data of two independent experiments

Table 1

Clinical history of patients with X-linked CD40L deficiency

	Patient *A1 (Yale)	Patient *B1 (CHOP)	Patient **C1 (nationwide)	Patient *C2 (nationwide)	Patient **D1 (NIAID/NIH)	Patient **D2 (NIAID/NIH)	Patient **E (NIAID/NIH)
Pathogenic variant	c.75delG	c.455InsG	c.374_375 delAT	c.374_375 delAT	c.580delG	c.580delG	c.425_430del6
	p.M25Ifs	p.S128fs	p.H125fs	p.H125fs	p.A187fs	p.A187fs	p.E142_K143del
Age at diagnosis	6 months	2 months	5 months	2 years	4 months	2.5 years	5 months
Current age	5 years	30 years (28 at sample acquisition)	33 years	34 years	16 years	21 years	28 years
Type of first infection	PJP, CMV	Pneumonia (PNA), sepsis (multiple occasions)	PJP	Acute otitis media/ sinusitis	Acute bronchitis	Recurrent acute otitis media/ sinusitis	PJP
Age of first infection	6 months	unknown	5 months	Unknown	4 months	Unknown	5 months
PJP diagnosis	Confirmed at 6 month	unknown	Confirmed at 5 month	Negative	Negative	Negative	Confirmed at 5 months
Age of initiation of IgG replacement	6 months	6 months	5 months	2 years	4 months	2.5 years	5 months
Age of initiation of PJP prophylaxis	6 months	6 months	5 months	21 years	4 months	2.5 years	5 months
Adherence to IgGRT	Poor	Poor	Excellent since age of 5 months	Excellent since age of 4 years	Excellent since age 4 months	Excellent since age 2.5 years	Excellent since age 5 months
Adherence to PJP prophylaxis	Poor	Poor	Excellent since age of 5 months	Excellent since age of 21 years	Excellent since age 4 months	Excellent since age 2.5 years	Excellent since age 5 months
CMV PCR	Positive from 6 month to 2 years	Not tested	Not tested	Not tested	Negative	Negative	Negative
EBV serology/PCR	Negative, PCR negative	Not tested	Not tested	Not tested	IgG positive, PCR negative	Not tested, PCR negative	Not tested, PCR negative
Cryptosporidium	Negative	Not tested	Negative	Negative	Negative	Negative	Negative
Other bacterial infection	No other cultures done	No other cultures done	No other cultures done	No other cultures done	No other cultures done	No other cultures done	No other cultures done
Active infection on the day of sample collection	None	None	None	None	None	None	None
Number of infections since CD40L-deficiency diagnosis	Another PNA at age 5	Two more episodes of PNA	None	2 PNA during teenager years	2–3 URIs/year, marked neurologic decline w/ negative infectious CSF workup other than anelloviridae on CSF HTS	7–8 URIs/year	H1N1 PNA in 2019, asymptomatic norovirus in stool (2019), 4–5 Winter URIs/year
PJP prophylaxis	Bactrim	None	Atovaquone	Bactrim	Bactrim	Bactrim	Bactrim

CHOP, The Children's Hospital of Philadelphia; *NATIONWIDE*, Nationwide Children's Hospital; *NIAID/NIH*, National Institution of Health/Nation alI nstitution of Allergy and Infectious Diseases; *PJP*, *Pneumocystis jiroveci* pneumonia; *CMV*, cytomegalo virus; *EBV*, Epstein-Barrvirus; *PNA*, pneumonia; *URI*, upper respiratory infection

* Pt group 1 (1): History of prophylaxis non-adherence and/or significant childhood infection

** Pt group 2 (2): History of prophylaxis adherence since childhood

Table 2

Immunological data of patients with X-linked CD40L deficiency

Patient	*A1		*B1		**C1		*C2		**D1		**D2		**E1	
	At diagnosis	Current	At diagnosis	Current	At diagnosis	Current	At diagnosis	Current	At diagnosis	Current	At diagnosis	Current	At diagnosis	Current
Age	5 months	5 years	2 months	30 years	5 months	33 years	12 years	34 years	4 months	16 years	2.5 years	21 years	5 months	28 years
IgM (mg/dL)	503 (H)[1]	320	N/A	196	N/A	366	N/A	32	N/A	186	N/A	227	N/A	149
IgG (mg/dL)	109 (L)[2]	506	N/A	497	N/A	950	N/A	926	N/A	1791 (H)	N/A	1610 (H)	N/A	849
IgA (mg/dL)	14	< 7 (L)[2]	N/A	339	N/A	< 7 (L)[2]	N/A	< 7 (L)[2]	N/A	59	N/A	30 (L)	N/A	< 5 (L)
$CD3^+$ (cells/μL)	5146	2443	N/A	2321	N/A	1972	N/A	1052	N/A	1752	N/A	2197	N/A	1470
$CD3^+CD4^+$ (cells/μL)	4406	1526	N/A	1379	N/A	1516	N/A	600	N/A	797	N/A	1443	N/A	789
$CD3^+CD8^+$ (cells/μL)	752	564	N/A	813	N/A	369	N/A	398	N/A	730	N/A	618	N/A	439
$CD19^+$ (cells/μL)	5533	802	N/A	57 (L)[2]	N/A	183	N/A	74	N/A	1040 (H)	N/A	410 (H)	N/A	215
$CD19^+CD27^+IgD^-IgM^-$ (%)	0	0.4 (L)[2]	N/A	0.6 (L)[2]	N/A	0.06 (L)[2]	N/A	0 (L)[2]	N/A	0 (L)	N/A	0 (L)	N/A	0 (L)
$CD3^-CD16^+CD56^+$ (cells/μL)	388	193	N/A	147	N/A	121	N/A	49 (L)[2]	N/A	149	N/A	155	N/A	181

[1] Higher level than the normal range (H)

[2] Lower level than the normal range (L)

* Pt group 1 (1): History of prophylaxis non-adherence and/or significant childhood infection

** Pt group 2 (2): History of prophylaxis adherence since childhood

Table 3

Kinetic changes in frequencies of $CD8^+$ T-cell subsets in relation to active infection history for patients A1, C1, and C2

Patient *A1	**Day 0**	**Day 30**	**Day 131**	**Day 232**	**Day 652**
Infection history	**Suspected PJP**	**No active infection**	**No active infection**	**No active infection**	**No active infection**
WBC (4–15 × 1000/μL)	27.7	N/A	N/A	5.9	5.9
IgG (409–1509 mg/dL)	53.0	N/A	476.0	417.0	268
CD8+ naïve (%)	N/A	29.1	17.7	27.9	31.2
CD8+ CM (%)	N/A	6.5	4.7	3.0	6.2
CD8+ EM (%)	N/A	59.6	74.5	64.4	62.5
CD8+ EM IL-7Rα^{Low}	N/A	88.6	N/A	91.5	87.4
Patient **C1	**Day 0**	**Day 210**	**Patient *C2**	**Day 0**	**Day 210**
Infection history	**None**	**None**	**Infection history**	**None**	**None**
CD8+ naïve (%)	52.9	46.6	CD8+ naïve (%)	16.2	13.5
CD8+ CM (%)	12.9	14.3	CD8+ CM (%)	4.98	9.41
CD8+ EM (%)	27.8	30.9	CD8+ EM (%)	71.7	75.2
CD8+ EM IL-7Rα^{Low}	9.3	17.4	CD8+ EM IL-7αR ^{Low}	50.3	67.3

* Pt group 1 (1): History of prophylaxis non-adherence and/or significant childhood infection

** Pt group 2 (2): History of prophylaxis adherence since childhood