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STAT3 mutations underlying autosomal dominant hyper-IgE syndrome impair human CD8+ T cell memory formation and function

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Abstract

Background—The capacity of $CD8^+$ T cells to control infections and mediate anti-tumor immunity requires the development and survival of effector and memory cells. IL-21 has emerged as a potent inducer of $CD8^+$ T cell effector function and memory development in mouse models of infectious disease. However, the role of IL-21 and associated signaling pathways in protective $CD8^+$ T cell immunity in humans is unknown.

Objective—To determine which signaling pathways mediate the effects of IL-21 on human $CD8^+$ T cells and whether defects in these pathways contribute to disease pathogenesis in primary immunodeficiencies caused by mutations in components of the IL-21 signaling cascade.

Methods—Human primary immunodeficiencies resulting from monogenic mutations provide a unique opportunity to assess the requirement for particular molecules in regulating human lymphocyte function. Lymphocytes from patients with loss-of-function mutations in *STAT1, STAT3* or *IL21R* were used to assess the respective roles of these genes in human $CD8^+$ T cell differentiation *in vivo* and *in vitro*.

Results—Mutations in *STAT3* and *IL21R*, but not *STAT1*, lead to a decrease in multiple memory $CD8^+$ T cell subsets *in vivo*, indicating that STAT3 signaling – possibly downstream of IL-21R - regulates the memory cell pool. Furthermore, STAT3 was important for inducing the lytic machinery in IL-21-stimulated naïve $CD8^+$ T cells. However, this defect was overcome by TCR engagement.

Conclusion—The IL-21R/STAT3 pathway is required for many aspects of human $CD8^+$ T cell behavior but in some cases can be compensated by other signals. This helps explain the relatively mild susceptibility to viral disease observed in STAT3 and IL-21R-deficient individuals.

Keywords

autosomal dominant hyper-IgE syndrome; STAT3; STAT1; IL-21; human $CD8^+$ T cells; memory; differentiation

INTRODUCTION

$CD8^+$ T cell responses are essential for the control of viruses and protection against some tumors. Common γ-chain family cytokines are important regulators of $CD8^+$ T cell behavior. Thus, IL-7 and IL-15 control lymphocyte homeostasis[1-3], while IL-2 regulates differentiation of naïve cells into effector or memory populations[4, 5]. IL-21 has also been reported to control $CD8^+$ T cell function. In vitro, IL-21 increases survival and proliferation of mouse[6-9] and human $CD8^+$ T cells[10-12], induces effector molecules such as IFN-γ, granzyme B and perforin 6, 13-15, and transcription factors BCL6 and EOMES that control their differentiation into effector and memory populations[16].

IL-21 is also implicated in controlling immune responses in vivo. Treatment of cancer patients with IL-21 resulted in upregulation of cytotoxic molecules such as granzyme B, perforin and IFN-γ in their $CD8^+$ T cells and NK cells[17]. In mice, IL-21 enhanced memory $CD8^+$ T cell responses during vaccinia infection[9, 18] and was required for $CD8^+$ T cell mediated control of chronic LCMV infection[19-21]. IL-21 alone, or together with IL-15, also increased the efficacy of anti-tumor responses by $CD8^+$ T cells[8, 9, 22-25]. Thus, IL-21 is a potent inducer of $CD8^+$ T cell effector function and memory with clinical relevance in both anti-viral and anti-tumor immunity.

IL-21 mediates its effects by activating JAK1 and JAK3[15, 26, 27] leading to phosphorylation of STAT1, STAT3 and STAT5[6, 15, 26]. IL-21 can also activate MAPK and Akt[6]. It is not clear, however, which of these pathways mediates the stimulatory effects of IL-21 on human $CD8^+$ T cells. Primary immunodeficiencies (PID) resulting from mutations in single genes provide a unique opportunity to address the role of individual molecules in regulating immune responses. Autosomal dominant hyper IgE syndrome (AD-HIES) is a PID characterized by chronic eczema, elevated serum IgE levels and recurrent infections of the skin, mucosa and lungs[28, 29]. Notably, some AD-HIES patients have impaired control of reactivation of infection with herpes viruses (HSV, VZV)[29, 30], and are predisposed to developing non Hodgkin's B-cell lymphoma[29-32]. The molecular lesion in AD-HIES is heterozygous mutations in *STAT3*, with mutant alleles working in a dominant negative manner[33, 34]. Mutations in *STAT1* also result in infectious susceptibility to particular pathogens. Thus, mono- or bi-allelic loss-of-function *STAT1* mutations severely compromise responses to IFN-γ. However, responses to IFN-α/β and IFN-λ are either intact (AD STAT1 deficiency; heterozygous mutations) or impaired (autosomal recessive [AR] STAT1 deficiency; bi-allelic mutations). Consequently, these mutations result in clinical disease caused by weakly virulent mycobacteria, and occasionally non-lethal viral infection[35, 36]. On the other hand, bi-allelic null mutations abolish STAT1-dependent cellular responses to IFN-γ, IFN-α/β and IFN-λ, thereby predisposing affected individuals to fatal infection with herpes viruses and mycobacteria[35, 36]. The importance of IL-21 signalling in humans was recently highlighted by the identification of four patients with *IL21R* mutations who develop recurrent respiratory and gastrointestinal infections, particularly with cryptosporidia resulting in chronic liver disease[37]. Two of these patients also exhibited ongoing infection with norovirus and rhinovirus, but immunity against herpes viruses and other pathogens that are commonly problematic for patients with combined immunodeficiencies (eg CMV, EBV) appeared to be intact[37].

Here, we used STAT3 mutant ($STAT3^{MUT}$), $STAT1^{MUT}$ and $IL21R^{MUT}$ patients to determine the requirement for STAT1 and STAT3 in regulating human $CD8^+$ T cell responses. IL-21 in combination with IL-15 induced proliferation of, and granzyme expression in, naïve $CD8^+$ T cells. Loss of STAT3 function impaired IL-21-induced granzyme B expression but did not affect its ability to induce proliferation. However, strong TCR/co-stimulatory signals could rescue granzyme expression in $STAT3^{MUT}$ T cells. Loss

of STAT1 function did not affect controlled the formation/maintenance of effector and memory $CD8^+$ T cell subsets in vivo, as proliferation or granzyme B production. We also found that STAT3, but not STAT1, evidenced by reduced frequencies of differentiated memory populations. We also observed some memory cell deficiencies in patients with *IL21R* mutations, implicating IL-21 as a potential STAT3-activating cytokine required for $CD8^+$ memory T cell homeostasis. These findings provide insight into some of the clinical features of AD-HIES and IL-21R deficiency, including impaired control of viral infection and susceptibility to B-cell lymphoma.

Materials and Methods

Human Samples

Buffy coats from normal donors were purchased from the Australia Red Cross Blood Service. Peripheral blood (PB) was collected from patients with mutations in *STAT3*, *STAT1* or *IL21R* (Refer to Table E1 in Online Repository for Patient details). All human experiments were approved by ethics committees in Canberra, Sydney, Melbourne, Brisbane and Perth, and institutional review boards of Necker Medical School, Rockefeller University and NIH.

T cell phenotyping & isolation

PB $CD8^+$ T cells were stained with mAbs to CD4, CD8, CCR7 and CD45RA. Subsets were defined as naïve ($CD8^+CD4^-CCR7^+CD45RA^+$), central memory (T_{CM}; $CD8^+CD4^-CCR7^+CD45RA^-$), effector memory (T_{EM}; $CD8^+CD4^-CCR7^-CD45RA^-$) or T_{EMRA} (revertant CD45RA effector memory; $CD8^+CD4^-CCR7^-CD45RA^+$). For experiments with $STAT3^{MUT}$ samples naïve cells were isolated from samples using a Positive Isolation Dynal Kit (Invitrogen) followed by sorting $CD8^+CCR7^+CD45RA^+$ cells (FACSAria: BD). Due to limiting numbers of cells in $STAT1^{MUT}$ and $IL\text{-}21R^{MUT}$ samples, naïve $CD8^+$T cells were isolated directly by sorting. For phenotyping cells were also stained for further cell surface markers (Table E2 in the Online Repository lists mAbs used)

Expression of phospho-STATs

Normal naïve $CD8^+$ T cells were cultured for four days with TAE beads (Miltenyi Biotech), rested for 2 hours in OPTI-mem (LifeTech) plus Normicin (InVivogen), and then stimulated in the absence or presence of IL-2 (50 U/ml), IL-15 (50 ng/ml) and/or IL-21 (50 ng/ml) for 30 min. Cells were fixed with 2% paraformaldehyde, permeabilized with 90% methanol and stained with anti-phospho-STAT1, STAT3 and STAT5 mAbs.

In Vitro Stimulation of Naïve $CD8^+$ T cells

Naïve $CD8^+$ T cells were labeled with CellTrace™ Violet (CTV; Invitrogen) and then cultured ($\sim 4 \times 10^4$ cells/200μl/well) with or without TAE beads (one bead/5 cells) for 4 or 10 d respectively; alone or together with 50 U/ml IL-2 (Millipore), 50 ng/ml IL-15 or 50 ng/ml IL-21 (PeproTech). Cells were thenharvested, permeabilized and stained with anti-perforin and anti-granzyme B mAb. Cell division and phenotype were determined using FlowJo software (Tree Star, Inc.).

Quantitative PCR analysis

RNA was isolated immediately after *ex vivo*isolation or after 4 or 10 days of culture using the QIAGEN RNeasy kit. For quantitative PCR, total RNA was reverse transcribed using oligo-dT. Expression of genes was determined by real-time PCR using the LightCycler 480 Probe Master Mix and System (Roche). All primers (Table E3 in the Online Repository) were from Integrated DNA Technologies. All reactions were standardized to *GAPDH*.

RESULTS

IL-21 activates STAT1, STAT3 and STAT5 in human CD8$^+$ T cells

IL-21 activates numerous intracellular signaling pathways including STAT1, STAT3, STAT5, MAPK and Akt[6, 15, 26]. We assessed which pathways were activated by IL-21 in human naïve CD8$^+$ T cells. IL-21 induced strong phosphorylation of STAT3, and a low level of STAT1 and STAT5 phosphorylation (Fig 1). We also analysed STAT activation induced by two other γc cytokines that are potent inducers of CD8$^+$ T cell proliferation and differentiation, namely IL-2 and IL-15. In contrast to IL-21, IL-2 and IL-15 did not result in phosphorylation of STAT1 or STAT3, but did induce STAT5 phosphorylation (Fig 1). The combination of IL-15 and IL-21 did not alter the level of STAT phosphorylation above that observed with these cytokines alone (Fig 1A-F). Therefore, of these cytokines, IL-21 uniquely activates STAT1 and STAT3 in human CD8$^+$ T cells.

STAT1 and STAT3 mutations do not impair proliferation of naïve CD8$^+$ T cells

IL-21 plays a pivotal role in inducing proliferation of CD8$^+$ T cells[10-12, 38]. However, since IL-21 activates multiple signaling pathways, it is not clear which of these underlies this proliferative effect. To address this we utilized naïve CD8$^+$ T cells from patients with mutations in *STAT1* (n=8), *STAT3* (n=15) or *IL21R* (n=3).

Homeostatic cytokines support survival of CD8$^+$ T cells and can induce proliferation and differentiation in the absence of extrinsic TCR stimulation. Thus, IL-15 mediates homeostatic proliferation of memory cells and combined with IL-21 drives naïve cells to effector phenotypes[9, 12, 38, 39]. *In vitro* culture of naïve CD8$^+$ T cells with IL-2 or IL-15 for 10 days significantly increased the recovery of viable cells (Fig 2A). In contrast, IL-21 alone did not increase survival above that seen with media alone. However, co-culture with IL-15 plus IL-21 induced significant proliferation as assessed by CTV dilution (Fig 2B). STAT3^{MUT} or STAT1^{MUT} CD8$^+$ T cells stimulated with IL-15 and IL-21 showed comparable proliferation to controls (Fig 2B-D), however proliferation of IL-21R^{MUT} CD8$^+$ T cells was strongly reduced, with the residual proliferation likely being induced by IL-15 (Fig 2C, 2D). Thus, IL-21's involvement in the homeostatic turnover requires a functional IL-21R but is unaffected by loss-of function mutations in *STAT3* or *STAT1*.

STAT3 is required for IL-21 induced expression of granzyme B

The capacity of CD8$^+$ T cells to produce the cytotoxic molecules granzyme B and perforin is important for their effector function[40]. IL-2, IL-15 and IL-21 can all induce expression of these molecules[7-9, 16, 41-43]. Therefore, we assessed the impact of *STAT1*, *STAT3* and *IL21R* mutations on the ability of these cytokines to induce granzyme B. Co-culture with IL-21 plus IL-15 induced higher granzyme B expression in normal naïve CD8$^+$ T cells than did IL-2, IL-15 or IL-21 alone (Fig 3A). However, both IL21R^{MUT} and STAT3^{MUT} CD8$^+$ T cells cultured with IL-15 and IL-21 failed to upregulate granzyme B ($p<0.001$) to the same level as control cells (Fig 3A-C). In contrast, STAT1^{MUT} CD8$^+$ T cells showed normal upregulation of granzyme B after stimulation with IL-15/IL-21 (Fig 3B, 3C). Thus, acquisition of the lytic machinery by naïve CD8$^+$ T cells stimulated with IL-21 in combination with IL-15 was dependent on STAT3 signaling downstream of a functional IL21R.

TCR/costimulation rescues defective IL-21 responses in STAT3^{MUT} CD8$^+$ T cells

During an immune response CD8$^+$ T cells also receive signals via the TCR and costimulatory molecules. Therefore, we examined naïve CD8$^+$ T cell responses following culture with cytokines and anti-CD3/anti-CD28/anti-CD2 stimulus (provided by TAE beads). Addition of IL-15 or IL-21/IL-15 to cells from healthy donors resulted in the

recovery of a significantly more $CD8^+$ T cells than stimulation with TAE beads alone, or TAE beads plus IL-2 (Fig 4A). Proliferation analysis revealed that treatment of naïve $CD8^+$ T cells with TAE beads plus IL-21 increased the total percentage of divided cells and the average number of divisions the cells had undergone compared to those cultured with TAE beads alone (Fig 4B,C). Interestingly, we detected no significant defect in the ability of TAE-activated naive $STAT3^{MUT}$ (Fig 4A-C) or $STAT1^{MUT}$ (Fig 4D) $CD8^+$ T cells to respond to IL-21 or IL-21/IL-15 co-stimulation. However, mutations in IL-21R decreased recovery of viable cells and progression through division in cultures containing IL-21 (Fig 4E). IL-7, another γc cytokine, also enhanced proliferation of normal naïve $CD8^+$ T cells that had been stimulated with TAE beads (not shown). Although previous studies have found that IL-7 can activate STAT3[44, 45], the ability of IL-7 to promote naïve $CD8^+$ T cell proliferation was unaffected by mutations in *STAT3* (not shown).

In cultures receiving cytokines alone, STAT3 mutations impaired the ability of IL-21 to upregulate granzyme B (Fig 3). We therefore determined whether signals provided by TCR/costimulation modulated this impairment. Addition of IL-2, IL-15, IL-21 or IL-21/IL-15 to TAE-stimulated cultures strongly (i.e. >20-fold) up-regulated granzyme B expression in normal naïve $CD8^+$ T cells (Fig 5A,D). In contrast to these cytokines, the effect of IL-7 on granzyme B induction was modest (ie <10% granzyme B^+ cells; ref[16] and not shown).Mutations in *STAT3* (Figure 5A,D) or *STAT1* (Figure 5B,D) did not impair the ability of TAE-stimulated T cells to upregulate granzyme B in response to any of the cytokines tested. However, $IL\text{-}21R^{MUT}$ naïve $CD8^+$ T cells were unable to upregulate granzyme B following IL-21 stimulation and this was partially recovered by IL-15 (Fig 5C,D). These results demonstrate that stimulation through TCR/costimulation alters the activation of the naïve $CD8^+$ T cells such that *STAT3* mutations no longer prevent IL-21-induced expression of the cytotoxic mediator granzyme B.

Mutations in *STAT3* and *IL21R* alter the frequencies of memory $CD8^+$ T cells

Since IL-21 signals through IL-21R to activate STAT1 and STAT3 and regulates effector function we speculated that impaired IL-21R, STAT1 or STAT3 function may also affect $CD8^+$ T cell differentiation *in vivo*. Therefore, we examined PB $CD8^+$ T cell populations and phenotypes in patients with mutations in these molecules. We found that $CD8^+$ T cells represented 21.0 ± 1.1% of PB lymphocytes in healthy donors. This did not differ for $STAT3^{MUT}$ (23.5 ± 1.5%), $STAT1^{MUT}$ (31.9 ± 3.8%) or $IL\text{-}21R^{MUT}$ patients (17.4 ± 2.6%; Fig 6A).

In humans the $CD8^+$ T cell population can be divided into subsets based on differential expression of CD45RA and CCR7[46]. The $CD8^+$ T cell compartment of normal donors thus comprises naïve (35.5 ± 2.3%), T_{CM} (8.9 ± 7.0%), T_{EM} (33.5 ± 1.8%) and T_{EMRA} (22.1 ± 2.1%) cells (Fig 6B). The distribution of these subsets in STAT1-deficient patients did not differ from normal controls (Fig 6B). However, the frequency of naïve $CD8^+$ T cells in STAT3-deficient patients was significantly increased (62.6 ± 5.7%, $p< 0.001$) compared to normal controls. This was associated with substantial decreases in T_{EM}, T_{EMRA} and T_{CM} cells in STAT3-deficient patients (19.9%± 3.8%; 14.0 ± 2.7% and 3.6 ± 0.8%, respectively; Fig 6B). Furthermore, analysis of $CD8^+$ T cell subsets from the three IL-21R-deficient patients suggested that memory in these patients may also be dysregulated, with increased naïve (57.5 ± 6.3%) and reduced T_{EMRA} (8.2 ± 5.0%) (Fig 6B). It was recently reported that populations of memory and effector CD8+ T cells reach adult levels by ~5-10 years of age[47, 48]. This is consistent with our finding that the proportions of memory and effector $CD8^+$ T cells in the cohort of STAT1-deficient patients, the average age of which was 13 years (Table E1), were normal (Fig 6B). Thus, it is unlikely that the decreased frequencies of non-naïve $CD8^+$ T cells in STAT3 and IL-21R-deficient individuals reflects the inclusion of some younger patients in these cohorts. These results suggest that STAT3, but not

STAT1, plays an important role in generating and/or maintaining memory $CD8^+$ T cells. Analysis of further IL-21R-deficient patients will be required to determine whether or not the required STAT3 activation is occurring downstream of IL-21 signaling or whether other cytokines, such as IL-10[49], are also involved.

To further understand the decrease in memory $CD8^+$ T cell in STAT3-deficient patients we analyzed expression of genes that control their differentiation and survival (Fig E1). *BCL2* was significantly higher in $STAT3^{MUT}$ naïve compared to normal naïve $CD8^+$ T cells (Fig E1). This suggests Bcl-2 may play a role in the survival, and thus increased frequency, of naïve $CD8^+$ T cells in STAT3-deficient patients. However, we observed no other ifferences in expression of pro- or anti-apoptotic molecules between patients and controls (Fig E1). Similarly, although transcription factors responsible for $CD8^+$ T cell function and differentiation were differentially expressed across $CD8^+$ T cell populations, we observed no significant differences between normal and $STAT3^{MUT}$ $CD8^+$ T cells (Fig E1).

STAT3-deficient T_{EM} and T_{EMRA} $CD8^+$ T cells have a phenotype suggestive of sustained activation

$CD8^+$ T cell subsets were further assessed for expression of a range of molecules that change during differentiation from naïve to effector cells[50, 51]. $STAT3^{MUT}$ cells showed dysregulated expression of many of these molecules (Fig 7). For example, 2B4 was highest on normal $CD8^+$ T_{EM} and T_{EMRA} cell, but was increased 2-4 fold on $STAT3^{MUT}$ T_{EM} cells ($p<0.001$). In contrast, 2B4 was expressed at normal levels on $STAT1^{MUT}$ $CD8^+$ T cells (Fig 7, E2A, E3A). CD57 expression is associated with poorly proliferative terminally differentiated T cells[51, 52]. Consistent with this, the greatest frequency of $CD57^+$ cells was found in the TEMRA population. However, proportions of $CD57^+$ cells in T_{EM} and T_{EMRA} populations of $STAT3^{MUT}$ $CD8^+$ T cells were significantly increased relative to normal and $STAT1^{MUT}$ cells (Fig 7). CD127 (IL-7Rα) is highly expressed on naïve and T_{CM} cells, and downregulated on T_{EM} and T_{EMRA} cells. However, in $STAT3^{MUT}$ patients all memory populations displayed significantly decreased levels of CD127 compared to healthy donors (Fig 7). Not all activation molecules displayed altered expression however as CD95 was not altered on $CD8^+$ T cells from either the $STAT1^{MUT}$ or $STAT3^{MUT}$ patients (Fig 7, E2A, E3A).

Chemokine receptors and adhesion molecules are important for regulating migration to secondary lymphoid organs and inflamed tissues. CX3CR1, CD11a and CD11b are highest on T_{EMRA} and T_{EM} cells from normal donors. However, their expression on $STAT3^{MUT}$, but not $STAT1^{MUT}$, T_{EM} and T_{EMRA} cells was significantly (2-3-fold) higher than on normal cells (Fig 7, E2B, E3B). Interestingly, $STAT3^{MUT}$ T_{EM} and T_{EMRA} $CD8^+$ T cells had significantly higher expression of granzyme B but not perforin. The altered expression of these molecules by $STAT3^{MUT}$ $CD8^+$ T cells suggests they have undergone aberrant differentiation *in vivo* and the residual effector memory populations exhibit a more senescent/exhausted phenotype.

DISCUSSION

To establish protective anti-viral and anti-tumor immunity, naïve $CD8^+$ T cells must proliferate and acquire effector function, processes believed to be regulated by IL-21. Indeed, IL-21 has been found to increase *in vitro* proliferation and survival of mouse and human $CD8^+$ T cells[6-12, 38], as well as induce effector molecules - IFN-γ, granzyme B, perforin - and enhance their overall cytotoxicity[7-10, 16, 42, 43]. Furthermore, *in vivo* delivery of IL-21 to cancer patients upregulated granzyme B and perforin in $CD8^+$ T cells[17]. Consistent with previous studies in multiple cell types[6, 15, 17, 26], IL-21 induced phosphorylation of STAT1, STAT3 and STAT5 in human $CD8^+$ T cells. Yet, the

contribution of individual STATs and cytokines to the development and effector function of human $CD8^+$ T cells has not previously been defined. To clarify the requirements for these molecules in human $CD8^+$ T cell function, we analyzed $CD8^+$ T cell differentiation *in vivo*, and IL-21 signaling *in vitro*, in individuals with loss-of function mutations in *IL21R*, *STAT1* or *STAT3*.

The ability of IL-21 to promote proliferation of naïve $CD8^+$ T cells was unaffected by mutations in *STAT1* or *STAT3*. In contrast, impaired STAT3 signaling abolished upregulation of granzyme B in response to IL-15/IL-21 where no exogenous TCR stimulus was provided. This paralleled our observations from cells unable to signal through the IL-21R, thereby suggesting that intact signaling through the IL-21R/STAT3 axis is required for *GZMB* transcription and subsequent cytotoxicity in human $CD8^+$ T cells stimulated with IL-15/IL-21. These findings are physiologically relevant because they infer that the increase in expression in granzyme B observed in CD8+ T cells of cancer patients who were administered IL-21 in the absence of specific T-cell activation[17] was STAT3-dependent. This is supported by work showing that IL-21-induced phosphoSTAT3 binds upstream of *Gzmb* in murine $CD4^+$ T cells[53]. The inability of STAT3-deficient $CD8^+$ T cells to upregulate granzyme B in response to IL-15/IL-21 would be further compounded by impaired production of IL-21 by STAT3-deficient $CD4^+$ T cells[54]. Surprisingly, we observed that when naïve $STAT3^{MUT}$ $CD8^+$ T cells were provided with extrinsic TCR stimulus and IL-21 they were capable of upregulating granzyme B to similar levels as controls. IL-21-induced granzyme B upregulation was also intact in STAT1-deficient naïve $CD8^+$ T cells suggesting that TCR/costimulation did not result in a switch from a STAT3 to a STAT1 pathway downstream of IL-21R for granzyme B regulation. Rather TCR/costimulatory signaling is likely to alter the sensitivity of cells to STAT3 such that residual STAT3 activity in $STAT3^{MUT}$ $CD8^+$ T cells is sufficient to induce a cytotoxic response.

Studies in mice have suggested that IL-21 is important for controlling some viral infections, such as vaccina[18] and chronic LCMV[19-21]. AD-HIES patients generally do not exhibit heightened susceptibility to primary viral infection, but some (<20%) do have an impaired ability to control reactivation of herpes viruses (HSV, EBV, VZV)[29, 30]. On the other hand, the few IL-21R-deficient individuals documented to date appear capable of mounting protective responses to these viruses, yet experience ongoing infection with norovirus and rhinovirus[37]. Both of these PIDs though are associated with increased susceptibility to infection with bacterial and fungal pathogens (*Staphylococcus aureus*, *Candida albicans*, *Pneumocystis*, cryptosporidia)[28, 29, 37, 55, 56]. Our findings actually provide an explanation for the relatively mild susceptibility of $STAT3^{MUT}$ and $IL\text{-}21R^{MUT}$ individuals to primary viral infection, despite predictions from mouse models[18-21, 49]: first, delivery of strong TCR and costimulatory signals during viral infection would facilitate normal induction of granzyme B in IL-21-stimulated $STAT3^{MUT}$ cells. Second, granzyme B induction by IL-2 and IL-15 is intact in $STAT3^{MUT}$ and $IL\text{-}21R^{MUT}$ $CD8^+$ T cells. Thus, during most viral infections, combined signals by TCR/costimulation, IL-2, IL-15 and/or IL-21 in naïve $CD8^+$ T cells would be sufficient to generate a protective cytotoxic thereby circumventing the dependency on signaling through IL-21R or STAT3.

An interesting feature of AD-HIES is predisposition to B-lymphoma[29, 31, 32, 57]. It is possible that during an anti-tumor response immune activation is not as strong as during viral infection; thus the relative contribution of IL-21R/STAT3 signaling in regulating granzyme B may be greater. Consequently, *STAT3* mutations may contribute to impaired $CD8^+$ T cell immune surveillance against B-cell malignancies. This is reminiscent of the susceptibility of perforin-deficient individuals to hematological neoplasms, including B-lymphoma[58].

The reported role of IL-21 in memory cell development[12, 18, 49] prompted us to investigate the phenotype of $CD8^+$ T cells from STAT1- and STAT3-deficient patients. Recent studies reported reduced frequencies of T_{CM} cells in AD-HIES patients[30] thereby implicating STAT3 in the development of „central memory' $CD8^+$ T cells[30, 49]. We found significant decreases not only in T_{CM} but also in T_{EM} cells in STAT3-deficient, but not STAT1-deficient, individuals. These changes were not due to alterations in the total percentages of $CD8^+$ T cells or age-related variations in the number of naïve versus memory cells (Fig E4A-D). T_{EM} and TEMRA populations from STAT3-deficient patients also displayed a phenotype of exaggerated differentiation often associated with increased/sustained exposure to antigen[50, 51, 59]. Assessment of IL-21R-deficient patients suggested IL-21 may contribute to establishing some of the memory populations in STAT3-deficient individuals. However, to determine whether IL-21 is the STAT3-activating cytokine required for maintaining $CD8^+$ T cell memory analysis of additional IL-21R-deficient patients will be required.

Several explanations can be proposed for the memory $CD8^+$ T cell deficiency in AD-HIES. First, *STAT3* mutations may affect $CD8^+$ T cell homeostasis or differentiation. However, proliferation of $STAT3^{MUT}$ naïve $CD8^+$ T cells in response to IL-7 or IL-21/IL-15, which regulate homeostasis of $CD8^+$ T cells[2, 9, 38, 42, 60-64], was normal. Thus, there is no evidence that the homeostatic proliferation and survival of naïve T cells induced by these cytokines requires STAT3, implying that the memory cell deficit is unlikely to be caused by impaired proliferation. Second, impaired STAT3 function may alter $CD8^+$ memory T cell numbers through effects on differentiation. $CD8^+$ T cell differentiation is regulated by multiple transcription factors that control opposing fates: Eomes and Bcl-6 favor "central memory" $CD8^+$ T cell development, whereas T-bet and Blimp-1 promote differentiation to "effector" $CD8^+$ T cells[13, 65-69]. The observations that *Socs3*, *Tbx21*, *Bcl6* and *Prdm1* are direct targets of STAT3[53], together with reduced expression of these genes in *Stat3*$^{-/-}$ murine $CD8^+$ T cells post-infection with LCMV[49], and of *SOCS3* and *BCL6* in *ex vivo* isolated STAT3-deficient human naïve $CD8^+$ T cells[30], suggest that STAT3 regulates $CD8^+$ T cell differentiation by controlling expression of transcription factors. Consistent with this we observed decreased *SOCS3* expression in IL-21-stimulated STAT3-deficient naïve $CD8^+$ T cells in vitro. Thus, as proposed previously[49], decreased SOCS3 expression may contribute to aberrant differentiation resulting in the enhanced activated phenotype we observed in T_{EM} and T_{EMRA} cells in $STAT3^{MUT}$ patients. However, we saw no significant differences in the levels of other transcription factors between normal and STAT3-deficient $CD8^+$ T cells either directly *ex vivo* (Fig E1) or following IL-21 stimulation (Fig E5). This suggests that the IL-21/STAT3 axis proposed to drive Bcl-6 expression and $CD8^+$ T_{CM} cell generation may be an oversimplification. Instead, STAT3 appears to have broader effects, being required for the generation or maintenance of not only T_{CM} cells, but also T_{EM} and T_{EMRA} populations.

Lastly, reduced memory $CD8^+$ T cell frequencies in $STAT3^{MUT}$ individuals may reflect reduced signaling through IL-7R (CD127). IL-7 is another homeostatic cytokine important for memory T cell maintenance[39, 61, 63, 64]. Interestingly, CD127 was significantly decreased on all memory populations from STAT3-deficient patients compared to normal donors consistent with a more activated phenotype. Thus, reduced expression of CD127 on STAT3-deficient $CD8^+$ T cells may limit their responsiveness to IL-7 signals, thereby compromising the pro-survival effects of IL-7 and compounding any decrease in memory cell numbers caused by reduced memory cell differentiation.

These findings reveal STAT3, but not STAT1, as an important downstream component of IL-21 signaling that mediates induction of effector function in $CD8^+$ T cells. However, a high level of redundancy for the induction of cytotoxic function seems to exist suggesting that in most circumstances a functional level of killing would still be generated. This is

consistent with mild susceptibility to viral infections in either AD-HIES or IL-21R-deficiency. In contrast, STAT3 signals, possibly initiated by IL-21, are critical for regulating the pool of all memory $CD8^+$ T cell subsets in humans. Collectively, these insights significantly add to our understanding of the function of IL-21 and STAT3 in human $CD8^+$ T cell development and behavior, and disease pathogenesis in individuals with mutations in *STAT3* and *IL21R*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AD-HIES	autosomal dominant hyper-IgE syndrome
AR	autosomal recessive
IL-21R	IL-21 receptor
BCL-2	B cell lymphoma 2
EOMES	Eomesodermin
LCMV	Lymphocytic choriomeningitis virus
PID	primary immune deficiency
TCR	T cell receptor
$\mathbf{T_{CM}}$	central memory T cells
$\mathbf{T_{EM}}$	effector memory T cells
$\mathbf{T_{EMRA}}$	effector memory T cells expressing CD45RA
TAE	T cell activation and expansion beads
EBV	Epstein Barr Virus
CMV	cytomegalovirus
VZV	varicella zoster virus
CTV	Cell Trace Violet

References cited

1. Becker TC. Interleukin 15 Is Required for Proliferative Renewal of Virus-specific Memory CD8 T Cells. J Exp Med. 2002; 195:1541–8. [PubMed: 12070282]
2. Lodolce JP, Burkett PR, Koka RM, Boone DL, Ma A. Regulation of lymphoid homeostasis by interleukin-15. Cytokine Growth Factor Rev. 2002; 13:429–39. [PubMed: 12401478]
3. Surh CD, Sprent J. Homeostasis of Naive and Memory T Cells. Immunity. 2008; 29:848–62. [PubMed: 19100699]

4. Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A. Interleukin-2 and Inflammation Induce Distinct Transcriptional Programs that Promote the Differentiation of Effector Cytolytic T Cells. Immunity. 2010; 32:79–90. [PubMed: 20096607]
5. Kalia V, Sarkar S, Subramaniam S, Haining WN, Smith KA, Ahmed R. Prolonged Interleukin-2Rα Expression on Virus-Specific CD8+ T Cells Favors Terminal-Effector Differentiation In Vivo. Immunity. 2010; 32:91–103. [PubMed: 20096608]
6. Zeng R, Spolski R, Casas E, Zhu W, Levy DE, Leonard WJ. The molecular basis of IL-21-mediated proliferation. Blood. 2007; 109:4135–42. [PubMed: 17234735]
7. Casey KA, Mescher MF. IL-21 Promotes Differentiation of Naive CD8 T Cells to a Unique Effector Phenotype. J Immunol. 2007; 178:7640–8. [PubMed: 17548600]
8. Kasaian MT, Whitters MJ, Carter LL, Lowe LD, Jussif JM, Deng B, et al. IL-21 Limits NK Cell Responses and Promotes Antigen-Specific T Cell Activation: A Mediator of the Transition from Innate to Adaptive Immunity. Immunity. 2002; 16:559–69. [PubMed: 11970879]
9. Zeng R, Spolski R, Finkelstein SE, Oh S, Kovanen PE, Hinrichs CS, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med. 2005; 201:139–48. [PubMed: 15630141]
10. Barker BR, Parvani JG, Meyer D, Hey AS, Skak K, Letvin NL. IL-21 Induces Apoptosis of Antigen-Specific CD8+ T Lymphocytes. J Immunol. 2007; 179:3596–603. [PubMed: 17785794]
11. Li Y, Bleakley M, Yee C. IL-21 Influences the Frequency, Phenotype, and Affinity of the Antigen-Specific CD8 T Cell Response. J Immunol. 2005; 175:2261–9. [PubMed: 16081794]
12. Nguyen H, Weng Np. IL-21 preferentially enhances IL-15-mediated homeostatic proliferation of human CD28+ CD8 memory T cells throughout the adult age span. J Leukoc Biol. 2010; 87:43–9. [PubMed: 19797296]
13. Ichii H, Sakamoto A, Hatano M, Okada S, Toyama H, Taki S, et al. Role for Bcl-6 in the generation and maintenance of memory CD8+ T cells. Nat Immunol. 2002; 3:558–63. [PubMed: 12021781]
14. Ostiguy V, Allard EL, Marquis M, Leignadier J, Labrecque N. IL-21 promotes T lymphocyte survival by activating the phosphatidylinositol-3 kinase signaling cascade. J Leukoc Biol. 2007; 82:645–56. [PubMed: 17554014]
15. Strengell M, Sareneva T, Foster D, Julkunen I, Matikainen S. IL-21 Up-Regulates the Expression of Genes Associated with Innate Immunity and Th1 Response. J Immunol. 2002; 169:3600–5. [PubMed: 12244150]
16. Parmigiani A, Pallin MF, Schmidtmayerova H, Lichtenheld MG, Pahwa S. Interleukin-21 and cellular activation concurrently induce potent cytotoxic function and promote antiviral activity in human CD8 T cells. Hum Immunol. 2011; 72:115–23. [PubMed: 20977918]
17. Frederiksen KS, Lundsgaard D, Freeman JA, Hughes SD, Holm TL, Skrumsager BK, et al. IL-21 induces in vivo immune activation of NK cells and CD8(+) T cells in patients with metastatic melanoma and renal cell carcinoma. Cancer Immunol Immunother. 2008; 57:1439–49. [PubMed: 18286285]
18. Novy P, Huang X, Leonard WJ, Yang Y. Intrinsic IL-21 Signaling Is Critical for CD8 T Cell Survival and Memory Formation in Response to Vaccinia Viral Infection. J Immunol. 2011; 186:2729–38. [PubMed: 21257966]
19. Elsaesser H, Sauer K, Brooks DG. IL-21 Is Required to Control Chronic Viral Infection. Science. 2009; 324:1569–72. [PubMed: 19423777]
20. Frohlich A, Kisielow J, Schmitz I, Freigang S, Shamshiev AT, Weber J, et al. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science. 2009; 324:1576–80. [PubMed: 19478140]
21. Yi JS, Du M, Zajac AJ. A vital role for interleukin-21 in the control of a chronic viral infection. Science. 2009; 324:1572–6. [PubMed: 19443735]
22. Ma HL, Whitters MJ, Konz RF, Senices M, Young DA, Grusby MJ, et al. IL-21 activates both innate and adaptive immunity to generate potent antitumor responses that require perforin but are independent of IFN-gamma. J Immunol. 2003; 171:608–15. [PubMed: 12847225]

23. Di Carlo E, Comes A, Orengo AM, Rosso O, Meazza R, Musiani P, et al. IL-21 induces tumor rejection by specific CTL and IFN-gamma-dependent CXC chemokines in syngeneic mice. J Immunol. 2004; 172:1540–7. [PubMed: 14734732]
24. Moroz A, Eppolito C, Li Q, Tao J, Clegg CH, Shrikant PA. IL-21 enhances and sustains CD8+ T cell responses to achieve durable tumor immunity: comparative evaluation of IL-2, IL-15, and IL-21. J Immunol. 2004; 173:900–9. [PubMed: 15240677]
25. Markley JC, Sadelain M. IL-7 and IL-21 are superior to IL-2 and IL-15 in promoting human T cell-mediated rejection of systemic lymphoma in immunodeficient mice. Blood. 2010; 115:3508–19. [PubMed: 20190192]
26. Asao H, Okuyama C, Kumaki S, Ishii N, Tsuchiya S, Foster D, et al. Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol. 2001; 167:1–5. [PubMed: 11418623]
27. Habib T, Senadheera S, Weinberg K, Kaushansky K. The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. Biochemistry. 2002; 41:8725–31. [PubMed: 12093291]
28. Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. Immunol Rev. 2005; 203:244–50. [PubMed: 15661034]
29. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L, et al. Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. Medicine (Baltimore). 2012; 91:e1–19. [PubMed: 22751495]
30. Siegel A, Heimall J, Freeman A, Hsu A, Brittain E, Brenchley J, et al. A Critical Role for STAT3 Transcription Factor Signaling in the Development and Maintenance of Human T Cell Memory. Immunity. 2011; 35:806–18. [PubMed: 22118528]
31. Kumánovics A, Perkins S, Gilbert H, Cessna M, Augustine N, Hill H. Diffuse Large B Cell Lymphoma in Hyper-IgE Syndrome Due To STAT3 Mutation. J Clin Immunol. 2010; 30:886–93. [PubMed: 20859667]
32. Jiao H, Toth B, Erdos M, Fransson I, Rakoczi E, Balogh I, et al. Novel and recurrent STAT3 mutations in hyper-IgE syndrome patients from different ethnic groups. Mol Immunol. 2008; 46:202–6. [PubMed: 18706697]
33. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med. 2007; 357:1608–19. [PubMed: 17881745]
34. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature. 2007; 448:1058–62. [PubMed: 17676033]
35. Boisson-Dupuis S, Kong XF, Okada S, Cypowyj S, Puel A, Abel L, et al. Inborn errors of human STAT1: allelic heterogeneity governs the diversity of immunological and infectious phenotypes. Curr Opin Immunol. 2012; 24:364–78. [PubMed: 22651901]
36. Casanova JL, Holland Steven M, Notarangelo Luigi D. Inborn Errors of Human JAKs and STATs. Immunity. 2012; 36:515–28. [PubMed: 22520845]
37. Kotlarz D, Zietara N, Uzel G, Weidemann T, Braun CJ, Diestelhorst J, et al. Loss-of-function mutations in the IL-21 receptor gene cause a primary immunodeficiency syndrome. J Exp Med. 2013; 210:433–43. [PubMed: 23440042]
38. Alves NL, Arosa FA, van Lier RAW. IL-21 Sustains CD28 Expression on IL-15-Activated Human Naive CD8+ T Cells. J Immunol. 2005; 175:755–62. [PubMed: 16002671]
39. Melchionda F, Fry TJ, Milliron MJ, McKirdy MA, Tagaya Y, Mackall CL. Adjuvant IL-7 or IL-15 overcomes immunodominance and improves survival of the CD8+ memory cell pool. J Clin Inves. 2005; 115:1177–87.
40. Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nat Rev Immunol. 2002; 2:735–47. [PubMed: 12360212]
41. Tamand DL, Redelman D, Alves BN, Vollger L, Bethley C, Hudig D. Induction of granzyme B and T cell cytotoxic capacity by IL-2 or IL-15 without antigens: Multiclonal responses that are extremely lytic if triggered and short-lived after cytokine withdrawal. Cytokine. 2006; 36:148–59. [PubMed: 17188506]

42. White L, Krishnan S, Strbo N, Liu H, Kolber MA, Lichtenheld MG, et al. Differential effects of IL-21 and IL-15 on perforin expression, lysosomal degranulation, and proliferation in CD8 T cells of patients with human immunodeficiency virus-1 (HIV). Blood. 2007; 109:3873–80. [PubMed: 17192392]
43. Strengell M, Matikainen S, Sirén J, Lehtonen A, Foster D, Julkunen I, et al. IL-21 in Synergy with IL-15 or IL-18 Enhances IFN-γ Production in Human NK and T Cells. J Immunol. 2003; 170:5464–9. [PubMed: 12759422]
44. Lin JX, Migone TS, Tsang M, Friedmann M, Weatherbee JA, Zhou L, et al. The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity. 1995; 2:331–9. [PubMed: 7719938]
45. Yu CR, Young HA, Ortaldo JR. Characterization of cytokine differential induction of STAT complexes in primary human T and NK cells. J Leukoc Biol. 1998; 64:245–58. [PubMed: 9715265]
46. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999
47. van Gent R, van Tilburg CM, Nibbelke EE, Otto SA, Gaiser JF, Janssens-Korpela PL, et al. Refined characterization and reference values of the pediatric T- and B-cell compartments. Clin Immunol. 2009; 133:95–107. [PubMed: 19586803]
48. Schatorje EJ, Gemen EF, Driessen GJ, Leuvenink J, van Hout RW, de Vries E. Paediatric reference values for the peripheral T cell compartment. Scand J Immunol. 2012; 75:436–44. [PubMed: 22420532]
49. Cui W, Liu Y, Weinstein Jason S, Craft J, Kaech Susan M. An Interleukin-21- Interleukin-10-STAT3 Pathway Is Critical for Functional Maturation of Memory CD8+ T Cells. Immunity. 2011; 35:792–805. [PubMed: 22118527]
50. Appay V, van Lier RAW, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: Consensus and issues. Cytometry A. 2008; 73A:975–83. [PubMed: 18785267]
51. Focosi D, Bestagno M, Burrone O, Petrini M. CD57+ T lymphocytes and functional immune deficiency. J Leukoc Biol. 2010; 87:107–16. [PubMed: 19880576]
52. 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; 101:2711–20. [PubMed: 12433688]
53. Kwon H, Thierry-Mieg D, Thierry-Mieg J, Kim HP, Oh J, Tunyaplin C, et al. Analysis of Interleukin-21-Induced Prdm1 Gene Regulation Reveals Functional Cooperation of STAT3 and IRF4 Transcription Factors. Immunity. 2009; 31:941–52. [PubMed: 20064451]
54. Ma CS, Avery DT, Chan A, Batten M, Bustamante J, Boisson-Dupuis S, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. Blood. 2012; 119:3997–4008. [PubMed: 22403255]
55. Minegishi Y. Hyper-IgE syndrome. Curr Opin Immunol. 2009; 21:487–92. [PubMed: 19717292]
56. Kotlarz D, Zietara N, Uzel G, Weidemann T, Braun CJ, Diestelhorst J, et al. Loss-of-function mutations in the IL-21 receptor gene cause a primary immunodeficiency syndrome. J Exp Med. 201310.1084/jem.20111229
57. Heimall J, Freeman A, Holland S. Pathogenesis of Hyper IgE Syndrome. Clinic Rev Allerg Immunol. 2010; 38:32–8.
58. Brennan AJ, Chia J, Trapani JA, Voskoboinik I. Perforin deficiency and susceptibility to cancer. Cell Death Differ. 2010; 17:607–15. [PubMed: 20075937]
59. 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; 208:2305–20. [PubMed: 22006977]
60. Berard M, Brandt K, Paus SB, Tough DF. IL-15 Promotes the Survival of Naive and Memory Phenotype CD8+ T Cells. J Immunol. 2003; 170:5018–26. [PubMed: 12734346]
61. Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol. 2000; 1:426–32. [PubMed: 11062503]
62. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and Selective Stimulation of Memory Phenotype CD8+ T Cells In Vivo by IL-15. Immunity. 1998; 8:591–9. [PubMed: 9620680]

63. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol. 2003; 4:1191–8. [PubMed: 14625547]

64. Maraskovsky E, Teepe M, Morrissey P, Braddy S, Miller R, Lynch D, et al. Impaired survival and proliferation in IL-7 receptor-deficient peripheral T cells. J Immunol. 1996; 157:5315–23. [PubMed: 8955178]

65. Banerjee A, Gordon SM, Intlekofer AM, Paley MA, Mooney EC, Lindsten T, et al. Cutting Edge: The Transcription Factor Eomesodermin Enables CD8+ T Cells To Compete for the Memory Cell Niche. J Immunol. 2010; 185:4988–92. [PubMed: 20935204]

66. Ichii H, Sakamoto A, Arima M, Hatano M, Kuroda Y, Tokuhisa T. Bcl6 is essential for the generation of long-term memory CD4+ T cells. Int Immuno. 2007; 19:427–33.

67. Ichii H, Sakamoto A, Kuroda Y, Tokuhisa T. Bcl6 Acts as an Amplifier for the Generation and Proliferative Capacity of Central Memory CD8 + T Cells. J Immunol. 2004; 173:883–91. [PubMed: 15240675]

68. Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat Immunol. 2005; 6:1236–44. [PubMed: 16273099]

69. Rutishauser RL, Martins GA, Kalachikov S, Chandele A, Parish IA, Meffre E, et al. Transcriptional Repressor Blimp-1 Promotes CD8+ T Cell Terminal Differentiation and Represses the Acquisition of Central Memory T Cell Properties. Immunity. 2009; 31:296–308. [PubMed: 19664941]

Key Messages

- loss-of function mutations in *STAT3*, causing autosomal dominant hyper-IgE syndrome, and *IL21R*, compromise differentiation of human CD8+ T cells to memory and effector cells
- mutations in *STAT3*, but not STAT1, abrogate the ability of human $CD8^+$ T cells to differentiate into granzyme B-expressing effector cells in response to IL-21

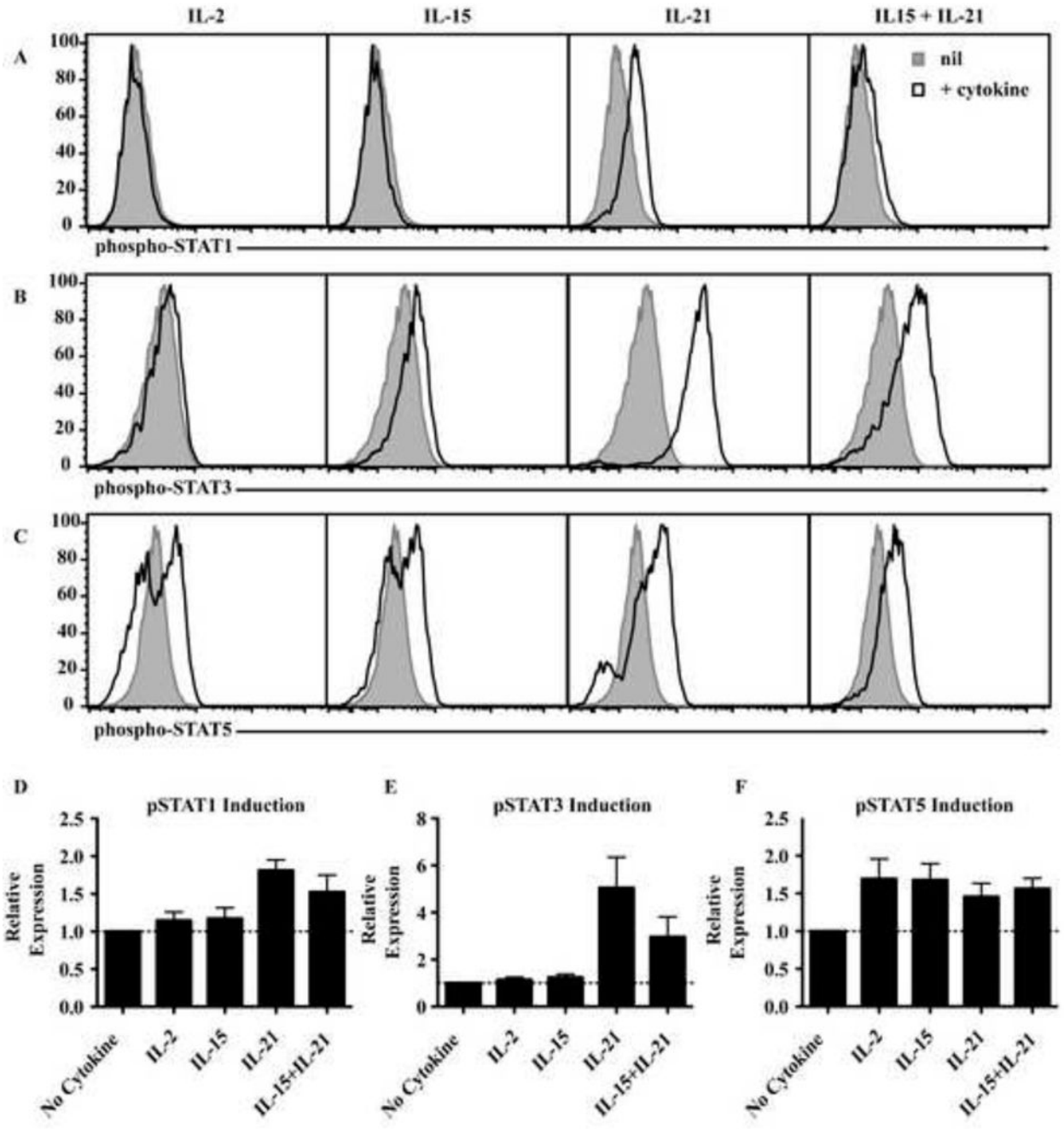

Figure 1. IL-21 predominately activates STAT1, STAT3 and STAT5 in human CD8$^+$T cells

(A-C) Naïve CD8$^+$T cells were activated for 4 days with TAE beads and recultured with cytokines for 30 min to determine phosphorylation of STAT1, 3 and 5. Histograms show nil or cytokine cultures and are representative of 4 experiments. (D-F) Graphs represent fold increase in MFI (mean ± SEM; n=4) of cells stimulated with cytokine over nil cultures. The dashed lines indicate a fold-change of 1 i.e. no change.

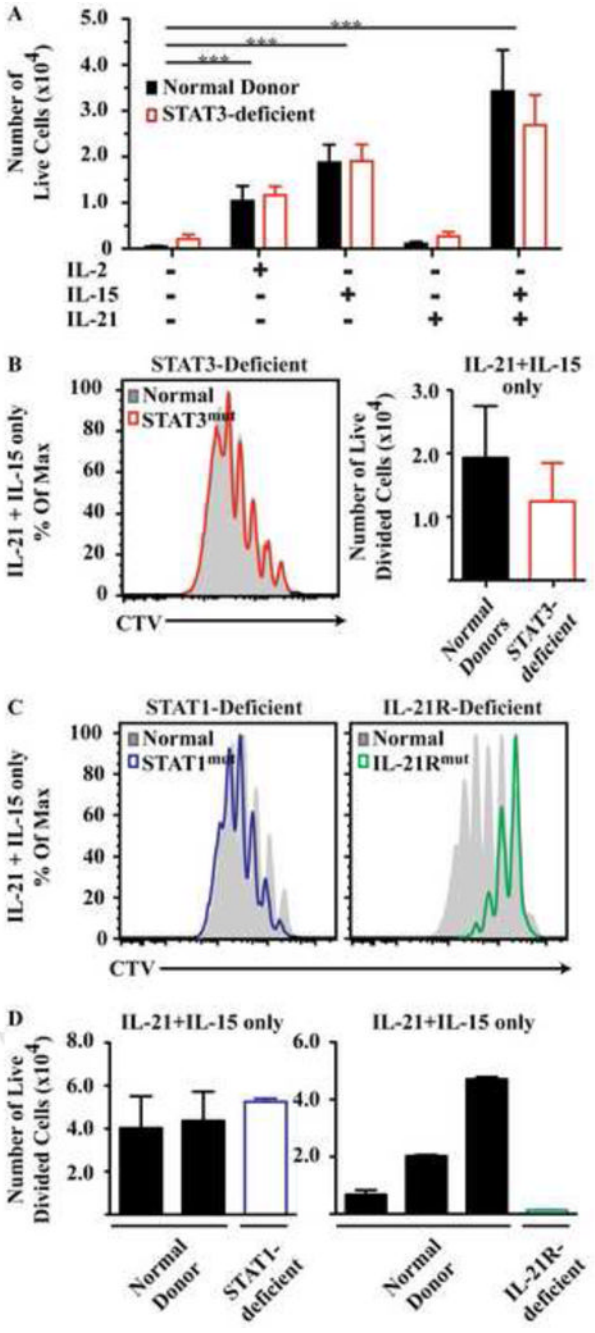

Figure 2. Cytokine-induced proliferation is impaired in IL-21R-deficient, but not STAT1- or STAT3-deficient, naïve CD8$^+$ T cells

Naïve CD8$^+$ T cells were cultures with cytokines only and (A) the numbers of live cells were determined (mean ± SEM, n=5). (B, C) Histograms show representative CTV profiles of cells stimulated with IL-21/IL-15. Graph in (B) shows the number of divided cells in IL-21/IL-15 cultures (mean ± SEM; n=5). (D) Each bar represents an individual patient or normal donor for experiments using cells from STAT1- and IL-21R-deficient patients. * P<0.05; **, P<0.001; *** P<0.001.

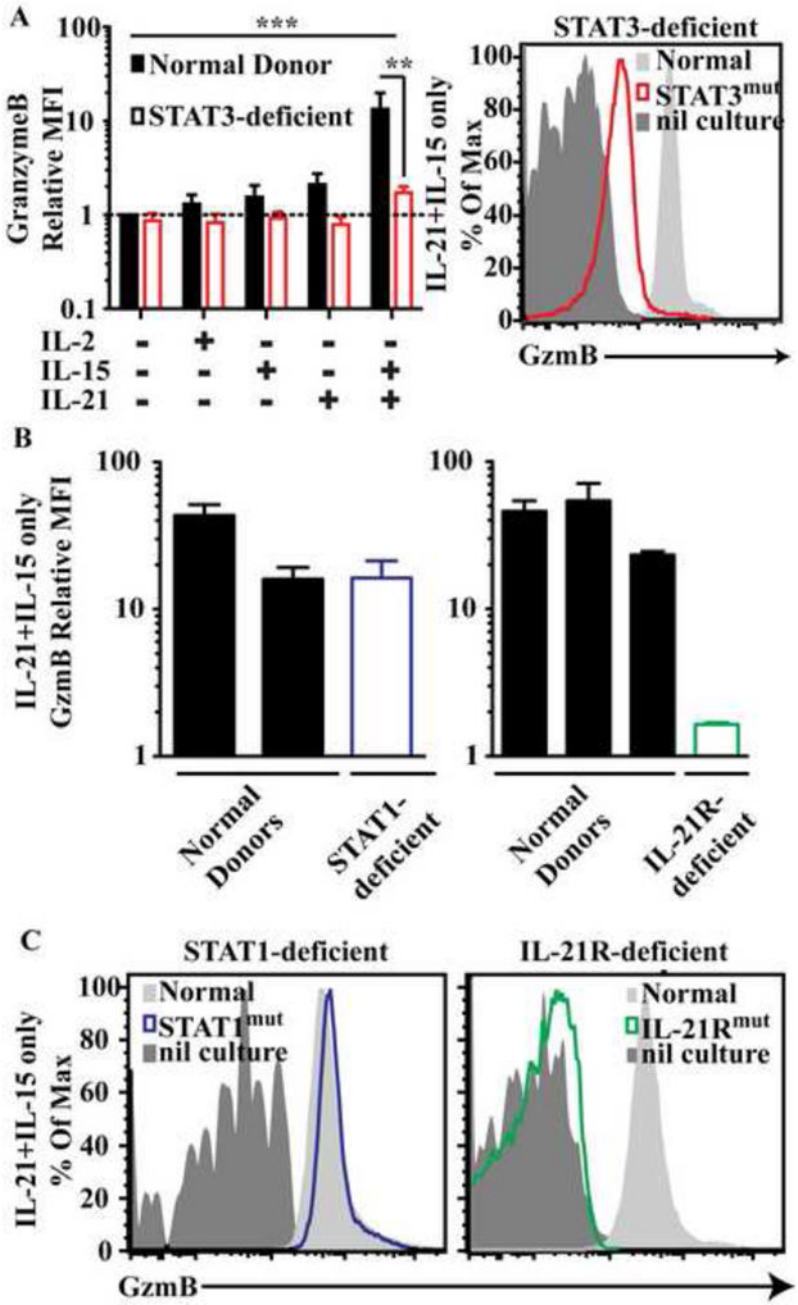

Figure 3. Cytokine-induced expression of granzyme B is impaired in IL-21R-deficient or STAT3-deficient naïve CD8$^+$ T cells

Naïve CD8$^+$ T cells were cultured with cyokines only and (A and B) graphs depict fold increase (mean ± SEM; n=5) in MFI of granzyme B over normal cells cultured with media alone (A). Each bar graph in (B) represents mean and range of duplicate cultures from an individual patient or normal donor for experiments using cells from STAT1- and IL-21R deficient patients. (A) and (C) are representative plots of cells from normal donors or the indicated patients stimulated with IL-21/IL-15 and normal donor cells from unstimulated cultures. ** P<0.001; *** P<0.001.

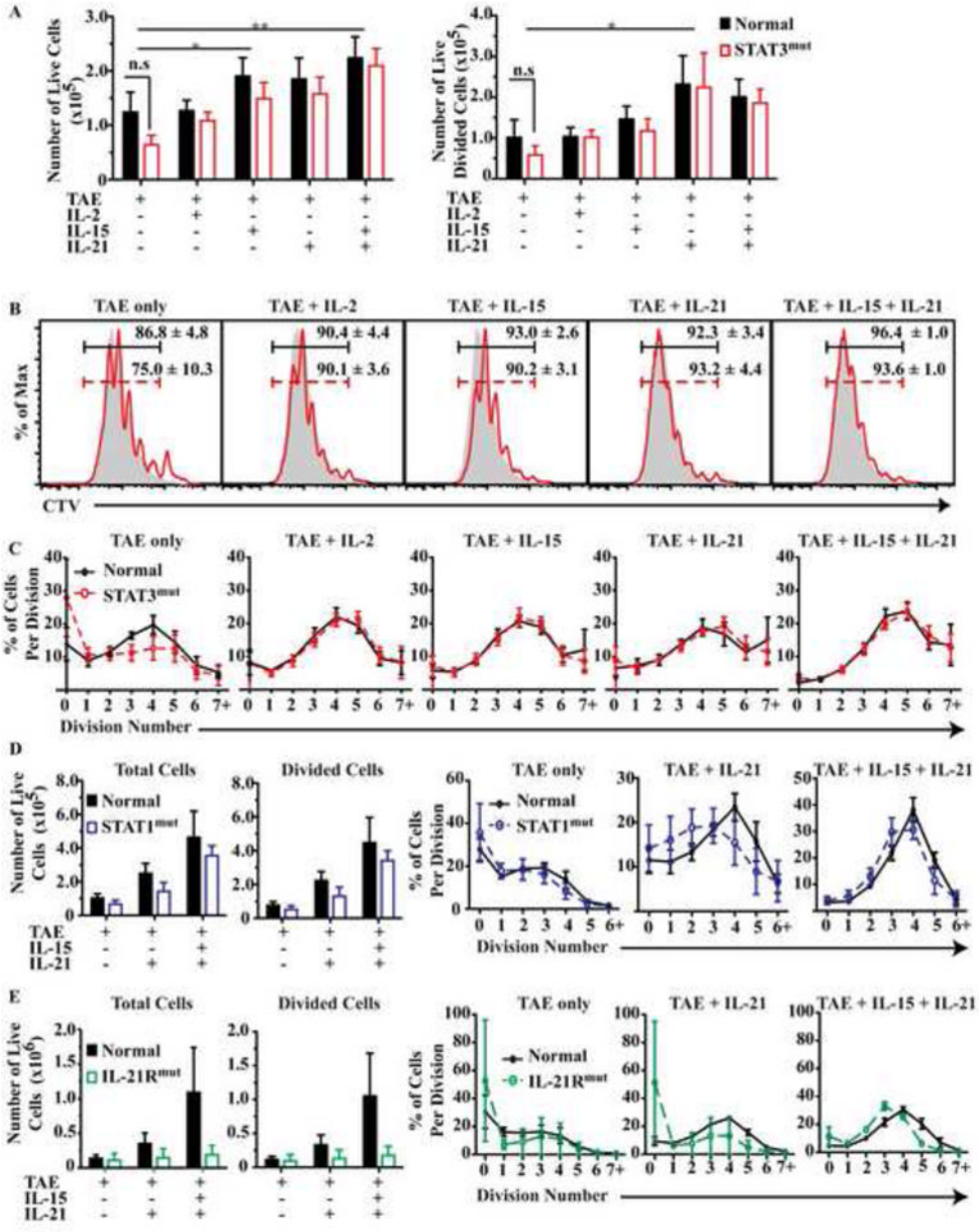

Figure 4. STAT3-deficient naïve CD8$^+$ T cells proliferate normally in response to TCR engagement and activating cytokines

Naive CD8$^+$ T cells from healthy donors (n=6-8 [A-C], 3 [D], 5 [E]), STAT3-deficient (A-C; n=6-8), STAT1-deficient (D; n=3); or IL-21R-deficient (E; n=2) patients, were cultured with TAE beads alone or together with cytokines. Total numbers of live cells that had entered division (A, D and E) from each culture were determined (mean ± SEM). (B) Histograms show representative CTV. Numbers give % of divided cells (mean ± SEM). (C-E) Percentage of CD8$^+$ T cells in each division was determined. * P<0.05; ** P<0.001; *** P<0.001

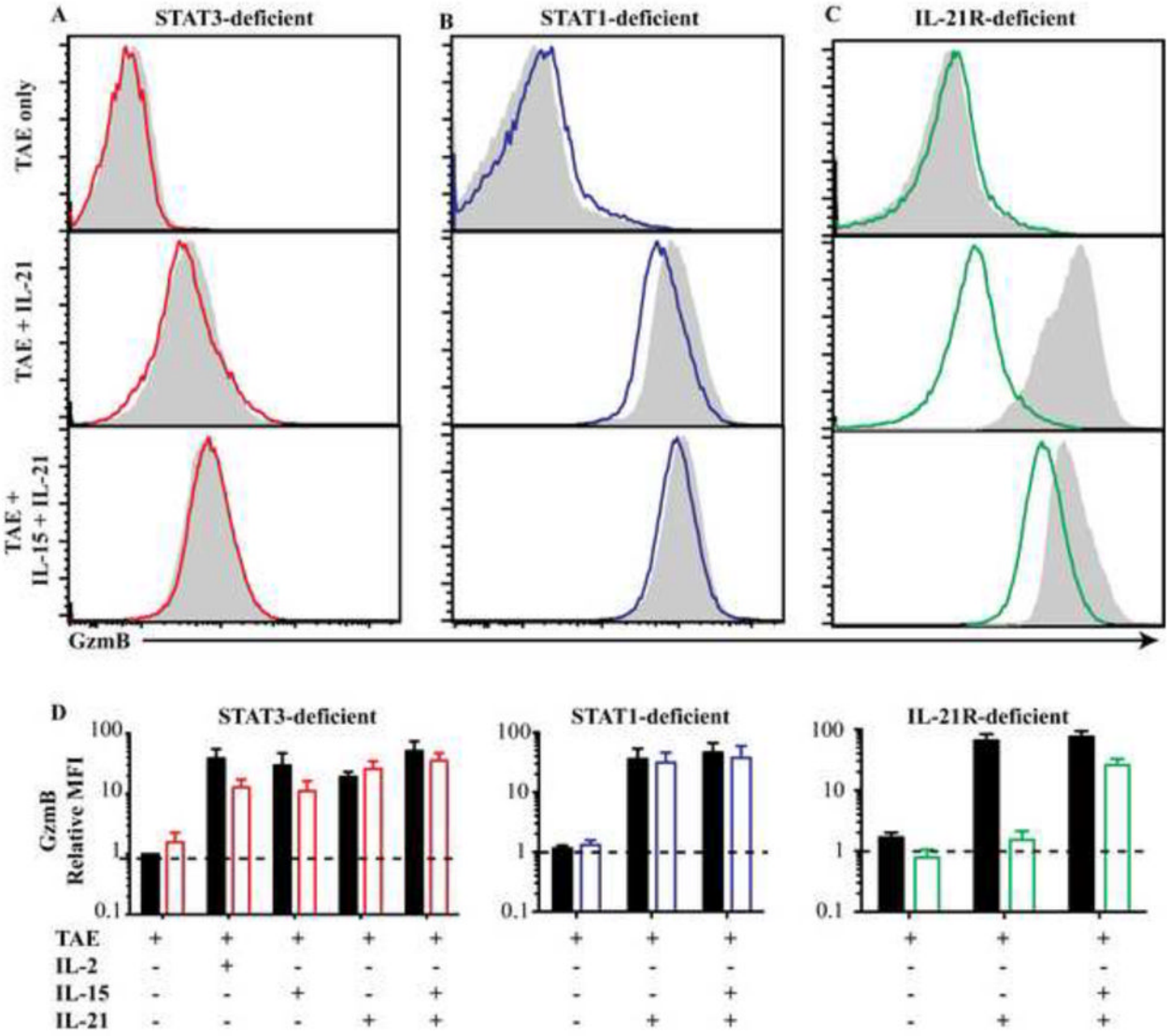

Figure 5. IL-21 induced granzyme B production is intact in TCR-stimulated STAT3-deficient CD8$^+$ T cells

Naive CD8$^+$ T cells from (A) STAT3-deficient (n=6-8), (B) STAT1-deficient (n=3), (C) IL-21R-deficient (n=2) patients or healthy donors (n=6-8), were cultured with TAE beads alone or together with cytokines. Representative histograms of granzyme B expression (filled – normal, coloured lines – patients) are depicted in (A-C). Graphs in (D) show fold-increase (mean ± SEM) in MFI of granzyme B expression by cytokine-stimulated normal and patient cells over those cultured with TAE beads alone.

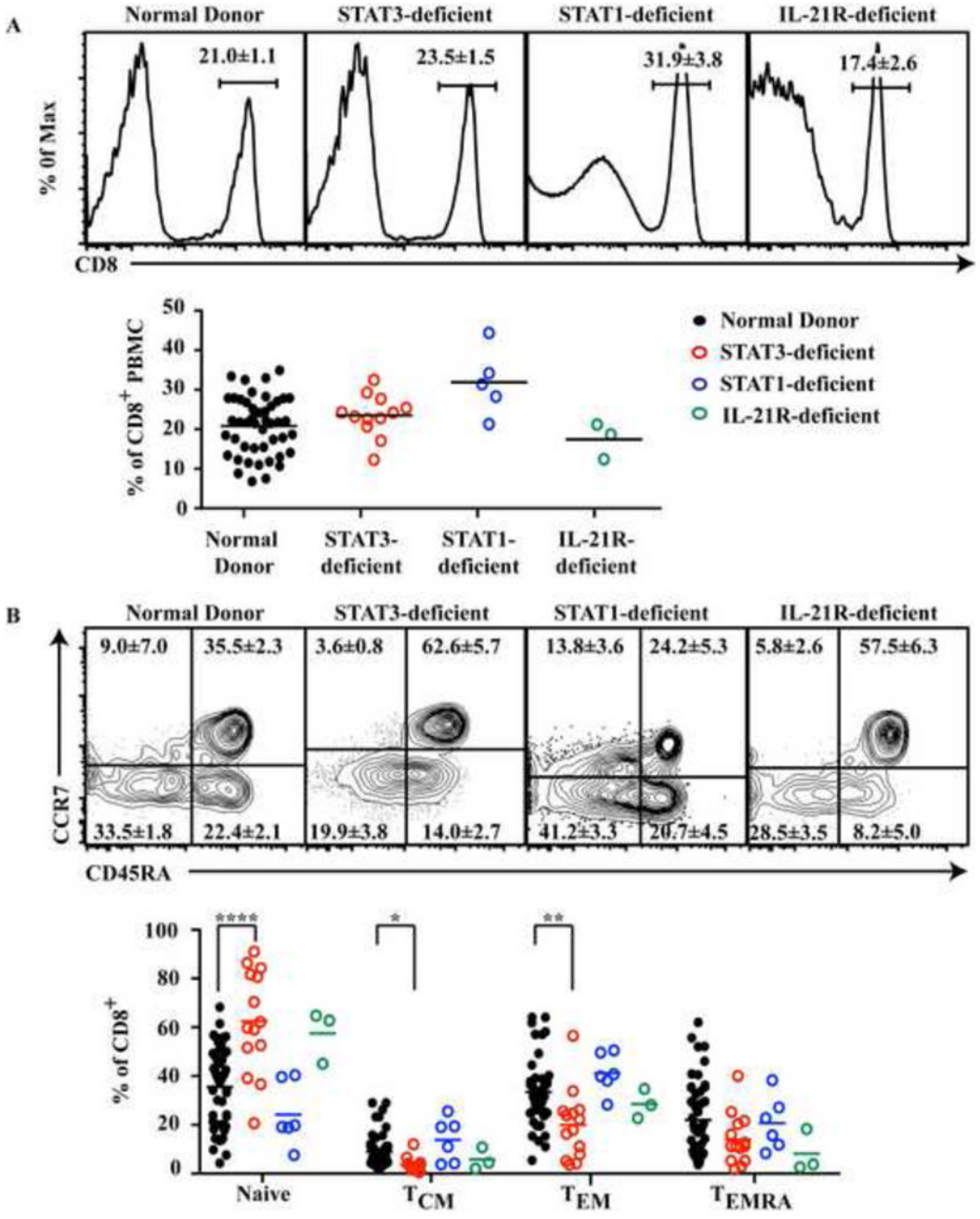

Figure 6. Mutations in *STAT3* but not *STAT1* impair generation of effector/memory CD8$^+$ T cells in vivo

PB from healthy donors (n=46 or 51), STAT3-deficient (n=13), STAT1-deficient (n=5 or 6) or IL-21R-deficient (n=3) patients were assessed for the percentage of (A) total CD8$^+$ T cells or (B) naïve, T_{CM}, T_{EM} and TEMRA cells. Each symbol corresponds to an individual donor or patient and lines represent means. Histograms and dot plots are from one representative donor or patient. ** P<0.001; *** P<0.001

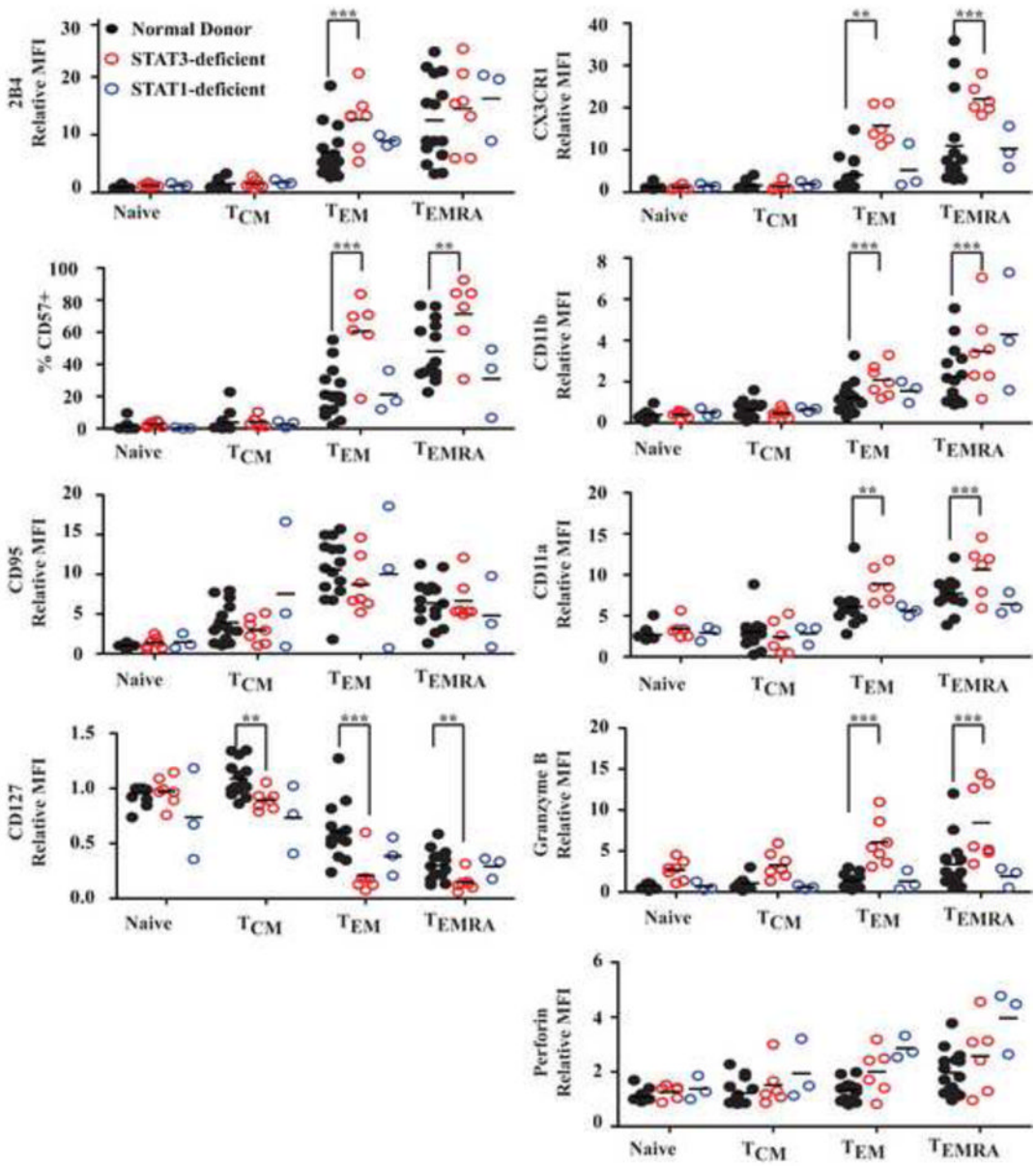

Figure 7. CD8$^+$ T cells from STAT3-deficient display a more activated phenotype

Subsets of naïve, T_{CM}, T_{EM} and T_{EMRA} CD8$^+$ T cells in PB of healthy donors (n=15), STAT3-deficient (n=7), STAT1-deficient (n=3) and IL21R-deficient (n=2) patients were assessed for expression of 2B4, CD57, CD95, CD127, CX3CR1, CD11a, CD11b, granzyme B or perforin. Graphs represent fold-change in MFI of the molecules relative to naïve cells, or the % of positive cells. **, P<0.001; ***, P<0.001