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T-cell dysfunction in natural killer/T-cell lymphoma

Xiaoyan Feng[a,b,c*], Miaomiao Meng[a,b,c*], Hongwen Li[a,c], Yuyang Gao[a,c], Wenting Song[a,b,c], Ruiqing Di[a,d], Zhaoming Li[a,c], Xudong Zhang[a,c], and Mingzhi Zhang[a,c]

[a]Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China; [b]Academy of Medical Sciences of Zhengzhou University, Zhengzhou, Henan, China; [c]Lymphoma Diagnosis and Treatment Centre of Henan Province, Zhengzhou, Henan, China; [d]Nursing Department, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

ABSTRACT

Natural killer/T-cell lymphoma (NKTCL) is an incurable aggressive T-cell lymphoma closely correlated with Epstein–Barr virus (EBV) infection. Chronic and consistent viral infection induces T-cell exhaustion. Herein, we describe T-cell dysfunction in NKTCL patients for the first time. Peripheral blood mononuclear cells (PBMCs) from age-matched healthy donors (HDs) and NKTCL patients were collected, and lymphocyte distributions, multiple surface inhibitory receptors (IRs), effector cytokine production and cell proliferation were determined by flow cytometry. PBMCs from HDs were cocultured with NKTCL cell lines to verify the clinical findings. IR expression was further assessed in NKTCL tumor biopsies using multiplex immunohistochemistry (mIHC). NKTCL patients have higher frequencies than HDs of inhibitory T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs). T-cell distribution also varies between NKTCL patients and HDs. T cells from NKTCL patients demonstrated higher expression levels of multiple IRs than HDs. Meanwhile, T-cell proliferation and interferon-γ production was significantly reduced in NKTCL patients. More importantly, the number of EBV-specific cytotoxic cells was lower in NTKCL patients, and these cells demonstrated upregulation of multiple IRs and secreted fewer effector cytokines. Interestingly, NKTCL cells caused normal PBMCs to acquire T-cell exhaustion phenotypes and induced generation of Tregs and MDSCs. In line with ex vivo finding, mIHC results showed that CD8+ T cells from NKTCL tumor biopsies expressed much higher level of IRs compared with reactive lymphoid hyperplasia individuals. The immune microenvironment of NKTCL patients exhibited T-cell dysfunction and accumulation of inhibitory cell components, which may contribute to impaired antitumor immunity.

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Introduction

Natural killer/T-cell lymphoma (NKTCL) is a subtype of non-Hodgkin's lymphoma originating from NK cells and cytotoxic T cells and has a higher incidence in South Asia and Latin America[1]. Radiotherapy, the introduction of gemcitabine and asparaginase-based chemotherapy, has greatly improved the clinical outcomes of NKTCL patients. However, a high percentage of patients still do not respond to initial treatment and progress to refractory or relapsed disease, typically with a median survival time of several months[2,3]. Therefore, novel treatment strategies for NKTCL are urgently needed.

NKTCL is closely related to Epstein–Barr virus (EBV), a member of the human herpesvirus family, which encodes a series of products that mimic growth, transcription and anti-apoptotic factors, thus contributing to immune escape and tumor onset[4]. To date, most studies on NTKCL have focused on tumor cells, and the immuno-microenvironment in NKTCL remains largely unknown.

T cells are key mediators of antitumor immunity that specifically recognize and react to tumor-expressing antigens and have proven critical for cancer immunotherapy. However, in the scenario of chronic and consistent virus infection, the differentiation and development of T cells changes to an exhausted state characterized as follows. 1. Progressive loss of effector function and proliferation capacity: loss of interleukin 2 (IL-2) and tumor necrosis factor (TNF-α) production occurs early, and defects in IFN-γ production occur at more severe stages of exhaustion. 2. Sustained and high expression of multiple IRs, including PD1, CTLA4, TIM3, TIGIT, and LAG3. Numerous IRs have been identified that can negatively regulate the function, activation, or other properties of T cells. 3. Altered expression of transcription factors. 4. Skewed metabolism and epigenetic programs[5–8]. It is now clear that T-cell exhaustion occurs in not only chronic viral infections but also autoimmune disorders and cancers[9,10].

Given the relationships among NKTCL, EBV infection and T-cell exhaustion, we sought to explore T-cell immunity in NKTCL patients in comparison with that of healthy donors (HDs) and found that the immune microenvironment of NKTCL patients is characterized by T-cell dysfunction and the accumulation of inhibitory cell components.

CONTACT Mingzhi Zhang mingzhi_zhang1@163.com Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China

*Contribute equally to this study.

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Materials and methods

Sample collection

Peripheral blood mononuclear cells (PBMCs) from 30 newly diagnosed NKTCL patients and 28 age-matched healthy donors (HDs) were isolated by density gradient centrifugation using lymphocyte separation medium (Biosharp, China) and used fresh or resuspended in 90% fetal bovine serum (Clark Bioscience, USA) containing 10% DMSO (Sigma Aldrich, USA) and stored at −80°C until use. The diagnosis of NKTCL was made according to the 2016 World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues[11]. Clinical characteristics of NKTCL patients and HDs were listed in supplementary table 1.

PBMCs from another 12 non-NKTCL patients, including 4 peripheral T cell lymphoma, not otherwise specified (PTCL-NOS), 3 angioimmunoblastic T cell lymphoma (AITL), 2 ALK positive anaplastic large cell lymphoma (ALCL), 2 EBV-associated lymphoproliferative disorders (EBV-LPD), 1 enteropathy T cell lymphoma, were also collected to verify IR expression.

All patients and HDs provided informed consent prior to blood sampling. The present study was approved by the Clinical and Research Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval number: 2021-KY0971-001).

Cell lines

The NKTCL cell lines KHYG-1 (EBV negative) and NKYS (EBV positive) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml recombinant IL2 (Sigma–Aldrich, USA), 100 U/mL penicillin and 100 g/mL streptomycin (Gibco, USA). KAI3 (EBV positive) cells were kept in RPMI 1640 medium with 20% FBS and 100 U/ml rIL2. Cell lines were maintained at 37°C in a humidified incubator containing 5% CO2. All three cell lines were obtained from Dr Wing C. Chan (City of Hope Medical Center).

Flow cytometry analysis

PBMCs or cocultured cells were collected and washed three times with phosphate-buffered saline (PBS) before surface staining at room temperature for 15 min. The antibodies used in the current study are listed in supplementary table 2. In total, $CD4^+$ T cells were gated as $CD3^+CD4^+$, $CD8^+$ T cells were gated as $CD3^+CD8^+$, NK cells were gated as $CD3^-CD56^+$, $CD4^+CD25^{high}CD127^-$ cells were gated as Tregs, and $HLA\text{-}DR^-CD11b^+CD33^+$ cells were considered MDSCs.

IR expression was detected by staining four IRs antibodies together with CD4 and CD8 antibody, then certain IR was gated out of $CD4^+$ or $CD8^+$ T cells. An anti-mouse Igκ negative control compensation particle set (BD Bioscience, USA) was used to set automatic compensation for multiple-color flow cytometry. Corresponding isotype control fluorescence was used for gating.

In certain circumstance, PD1 antibody (10 μg/ml, Selleck, USA) was used to treat PBMCs for 48 h, followed by the detection of IR expression.

All flow cytometry was performed on a BD FACS CantoII instrument (BD Bioscience, USA) and analyzed by FlowJo software version 10 (Treestar, USA).

T-cell proliferation

PBMCs were incubated with 5 μM CFSE (Invitrogen, USA) in the dark at 37°C for 15 min, quenched with ice-cold 20% FBS and washed three times with PBS; subsequently, 5×10^5 per well CFSE-labeled PBMCs were plated in a 96-well plate precoated with anti-CD3 (5 μg/ml, BD Bioscience, USA) and anti-CD28 (10 μg/ml, BD Bioscience, USA) antibodies. T cells stimulated by CD3/CD28 signaling were designated as W/T activation, while W/O represented a negative control with no stimulation. After 4 d of incubation, the cells were collected and stained with surface CD4 and CD8 antibodies, and proliferating cells were determined by the percentage of diluted CFSE signal within $CD4^+$ or $CD8^+$ T cells.

Cytokine production

PBMCs were stimulated with 500 ng/ml PMA (Sigma-Aldrich, USA) and 500 ng/ml ionomycin (Solarbio, China). One hour later, the protein transport inhibitors brefeldin A and monensin (BD Biosciences, USA) were added for an additional 5 h. Cells were then harvested and stained with surface CD8, CD3 and CD56 antibodies, followed by fixation and permeabilization using a commercial cytofix/cytoperm kit (BD Biosciences, USA) at 4°C for 30 min. Subsequently, intracellular IFN-γ and TNF-α were stained at 4°C for another 30 min before proceeding to flow cytometry. Cells with no stimulation were set as negative controls, designated as W/O activation.

Detection of EBV-specific CTLs

EBV-specific CTLs were recognized by staining HLA-A *02:01 restricted tetramers assembled with synthetic peptides from latent membrane protein 1 (LMP1) (YLQQNWWTL and YLLEMLWRL, MBL, Japan). PBMCs were incubated at room temperature for 15 min with PE-labeled tetramers and other surface markers as indicated. EBV-specific cytotoxic T cells (CTLs) were identified as $CD8^+tetramer^+$ cells. IR expression as well as IFN-γ production was detected as above-mentioned out of EBV-CTLs.

Coculture of PBMCs and NKTCL cell lines

PBMCs derived from HDs were cocultured with three NKTCL cell lines at a ratio of 5:1. Cells were harvested after the indicated timepoints and stained for surface markers to determine IR expression. For effector cytokine production, mixed cocultured cells were stimulated with PMA and ionomycin for 6 h, with additional protein transport inhibitors for the last 5 h.

Tregs and MDSCs induction assay

PBMCs (2×10^6) from HDs were cocultured with NKTCL cell lines at a ratio of 5:1. Cells were obtained at different time

points, and the percentages of $CD4^+CD25^{hi}CD127^{low}$ Tregs and $HLA\text{-}DR^-CD11b^+CD33^+$ MDSCs were determined.

To find out whether the full function of NKTCL cells is required by the induction of Tregs and MDSCs, NKTCL cells were pretreated with mitomycin C (50 μM, Selleck, USA) and then washed 3 times followed by co-culturation with PBMCs.

IR expression in tumor milieu

Biopsy from 5 NKTCL patients (1 biopsy from cervix and 4 from nasal) and 3 reactive hyperplastic lymphadenopathies were collected to proceed multiplex immunohistochemistry (mIHC) assay using commercial kit as indicated (Absin, China). In summary, CD8 antibody (1:200, Cell Signaling Technology, USA) together with PD1 (1:100, Cell Signaling Technology, USA), TIM3 (1:1000, Cell Signaling Technology, USA), LAG3 (1:200, Cell Signaling Technology, USA) were incubated and corresponding TSA fluorescence 570, 520, 700, 620, respectively, were used for detection. DAPI was stained for nuclear. Results were analyzed using CaseViewer software (3DHistech, Hungary) by numeration of five random high-power fields for each sample.

Statistical analysis

All the experiments performed in this study used separate PBMCs as independent repeats. The results are shown as the medians and ranges. The Mann–Whitney test was used to test for differences between two groups. Statistical analyses were performed with GraphPad Prism 5 software One-way ANOVA was used to compare 3 or more groups. Statistical analyses were carried out with Prism software (GraphPad Software, Inc.). Two-sided P values <0.05 were considered significant.

Results

Lymphocyte distribution and subset differences between NKTCL and HDs

We compared the percentages of $CD4^+$, $CD8^+$, NK cells, Tregs and MDSCs in PBMCs from both NKTCL patients and HDs (gating strategy is shown in (Figure 1a,b)) and found that inhibitory Tregs and MDSCs were remarkably accumulated in NKTCL patients (Figure 1c). CD8/Treg numbers, an index indicating antitumor immunity, were significantly decreased in NKTCL patients compared with HDs (Figure 1c).

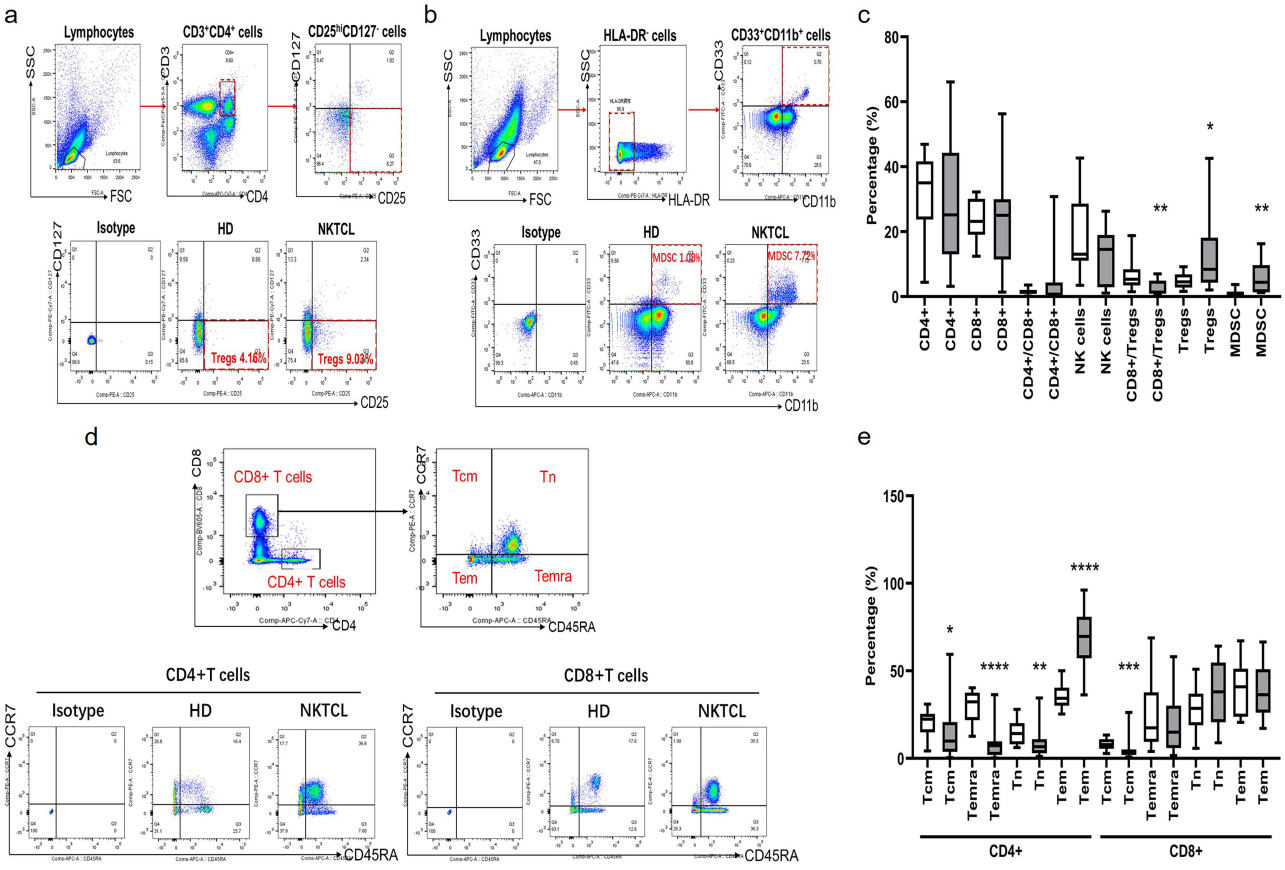

Figure 1. Lymphocytes distribution and subsets differed between NKTCL and HDs. Gating strategy and representative plots for $CD4^+CD25^{high}CD127^-$ Tregs and $HLA\text{-}DA^-CD11b^+CD33^+$ MDSCs in HD and NKTCL patients (a-b). Pooled data from 30 newly diagnosed NKTCL patients and 28 age-matched HDs (c). gating strategy and representative plots for different T cell subsets (d). Pooled data of T cell subsets of CD4 and CD8 in NKTCL patients and HDs (e). Gray bars indicate NKTCL patients, blank bars indicate HDs. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

We further divided $CD4^+$ and $CD8^+$ T cells into four subsets by the surface markers CD45RA and CCR, namely, naïve T cells (Tn, $CD45RA^+CCR7^+$), terminally differentiated T cells (Temra, $CD45RA^+CCR7^-$), central memory T cells (Tcm, $CD45RA^-CCR7^+$) and effector memory T cells (Tem, $CD45RA^-CCR7^-$), as gated in Figure 1d. Pooled data showed that NKTCL patients had a greater number of $CD4^+$ T cells expressing Tem; accordingly, the number of cells in the remaining lymphocyte subsets was decreased (Figure 1e). Regarding $CD8^+$ T cells, the number of Tcm-expressing cells was strikingly decreased in NKTCL patients. Our finding is similar to a previous report showing that exhausted T cells favor differentiation into effector/memory T cells instead of central memory T cells, impairing the long-term maintenance of antitumor immunity[9].

NKTCL patients highly express multiple inhibitory receptors

An important feature of T-cell exhaustion is the consistent upregulation of multiple IRs; therefore, we measured IRs, including CTLA4, PD1, TIM3, and TIGIT, on both $CD4^+$ and $CD8^+$ T cells. As shown in Figure 2a, NKTCL patients had much higher expression levels of all IRs. It was reported that T cells co-expressing IRs represent a more exhausted status[9,12], and our data revealed that both $PD1^+TIM3^+$ and $PD1^+TIGIT^+$ cells were enriched in NKTCL patients (Figure 2b). Next, IR expression was measured in various lymphocyte subsets, as shown in Figure 2c. In almost all the lymphocyte subsets, both PD1 and CTLA4 were strikingly upregulated in cells from NKTCL patients compared with those from HDs.

To find out whether IRs upregulation is a universe phenomenon in T cell lymphoma or is exclusive in NKTCL, we collected PBMCs from 12 non-NKTCL patients, as indicted in the method part, and found a similar upregulation of certain IRs, although it is not so significant as in NKTCL patients (supplementary figure S1).

Subsequently, PD1 antibody was used to treat PBMCs and the result showed that only PD1 expression was remarkably decreased after PD1 antibody exposure (supplementary figure S2), while the other IRs remained the same. This suggests PD1 antibody monotherapy might not be a satisfactory choice for NKTCL patients.

NKTCL patients displayed impaired T-cell proliferation and effector cytokine production

T cells were stimulated with anti-CD3/CD28 antibody to evaluate their proliferation capacity, depicted in Figure 3a. We showed that $CD4^+$ and $CD8^+$ T cells derived from NKTCL patients displayed decreased proliferation (Figure 3b).

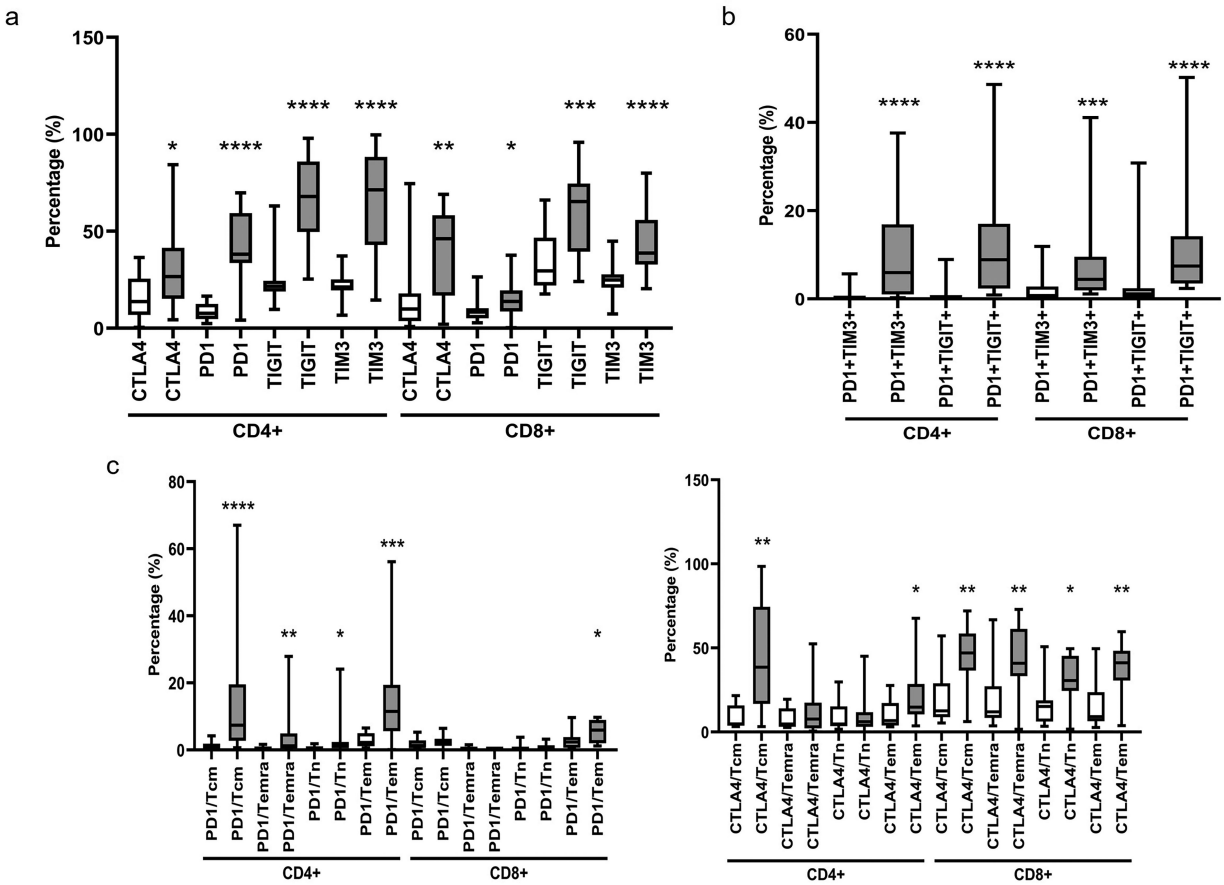

Figure 2. NKTCL patients highly expressed multiple inhibitory receptors. Pooled data of IR expression on both T cells of NKTCL patients and HDs (a). Percentage of T cells co-express IRs in CD4+ and CD8+ cells (b). Expression of PD1 and CTLA4 in different T cell subsets (c). Gray bars indicate NKTCL patients, blank bars indicate HDs. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

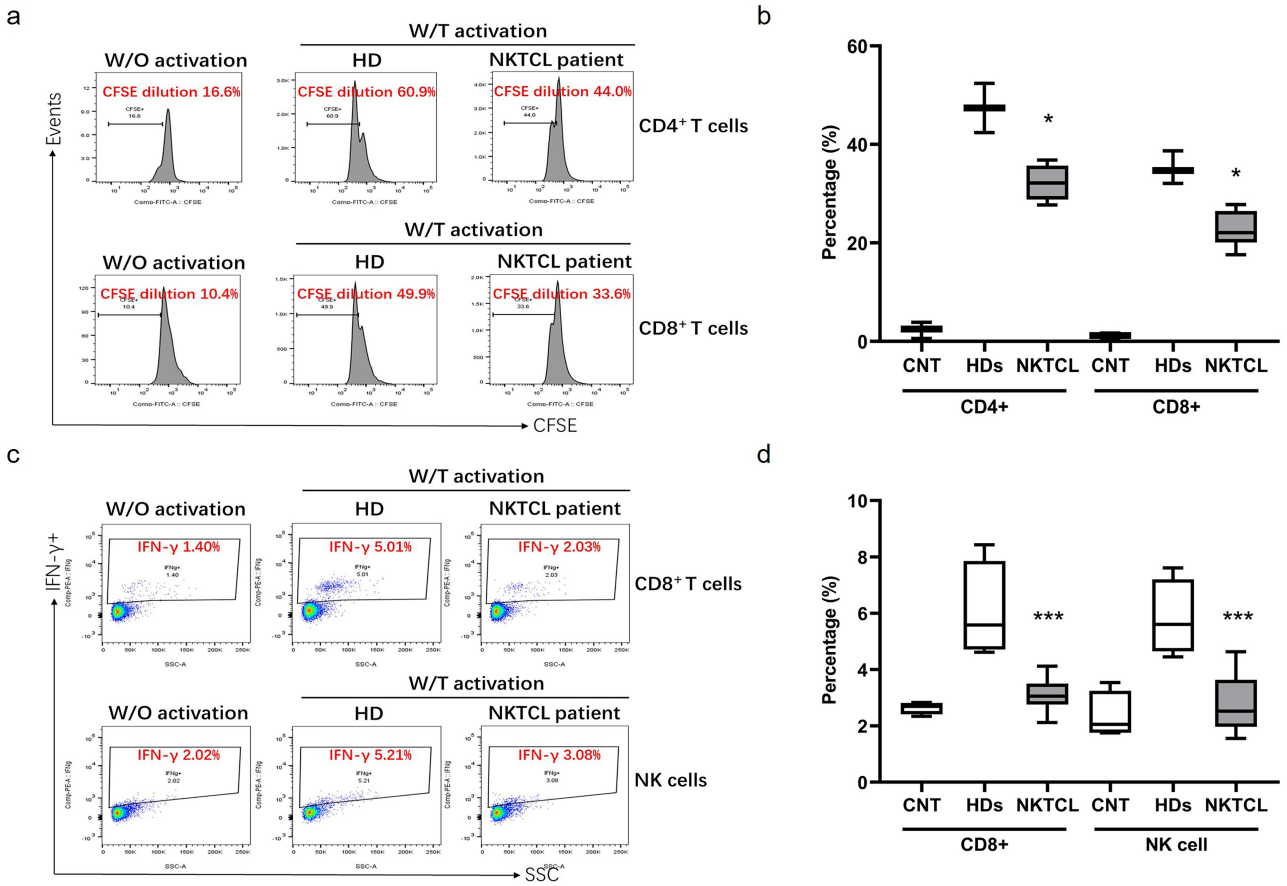

Figure 3. NKTCL patients displayed decreased T cell proliferation and effector cytokine production. Representative plots and pooled data of proliferation (a-b), IFN-γ production (c-d) of T cells in NKTCL patients and HDs. W/O: without, W/T: with, cnt: control. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

As indicated in Figure 3c, T cells or NK cells seldom secrete effector IFN-γ without activation. In contrast, IFN-γ production was strikingly increased after stimulation by TCR signaling activation. We compared intercellular IFN-γ percentages in T cells and NK cells and found that NKTCL patients exhibited defective effector cytokine production (Figure 3d).

Abnormality of EBV-specific cytotoxic T cells in NKTCL

EBV-specific CTLs exert crucial effects in limiting EBV infection and tumorigenesis. We utilized two EBV latent membrane protein-1 (LMP-1)-derived peptide-conjugated MHC-I restricted tetramers recognizing EBV-specific CTLs as described in the methods section. First, we found that NKTCL patients had fewer EBV-specific CTLs than HDs (Figure 4a). Moreover, EBV-specific CTLs from NKTCL patients upregulated multiple IRs, as shown in Figure 4b. In addition, EBV-specific CTLs derived from NKTCL patients produced less IFN-γ than those derived from HDs (Figure 4c, d). Our data suggest T cells and EBV-specific T cells display exhausted features in NKTCL patients.

NKTCL cells reprogram normal T cells to exhausted T cells

To verify the abovementioned clinical findings, we cocultured PBMCs derived from HDs with three NKTCL cell lines for different timepoints. All three NKTCL lines showed significantly increased expression of IRs, including PD1, TIM3, TIGIT, and LAG3 (Figure 5a,b). Moreover, the percentage of T cells co-expressing at least two IRs was greatly increased compared with PBMCs cultured alone (Figure 5c,d).

Regarding cytokine secretion, PBMCs from HDs were cultured with or without NKTCL cells for 7 d; TCR signaling activation was subsequently conducted to detect effector cytokine production. As shown in Figure 5e,f, T cells produced less IFN-γ and TNF-α after coculturing with NKTCL cells. Overall, healthy T cells acquired an exhaustion phenotype after coculture with NKTCL cell lines.

As Tregs and MDSCs are elevated in NKTCL patients compared with HDs, we cocultured normal PBMCs with NKTCL cell lines and found that tumor cells remarkably promoted an increase in both Tregs and MDSCs, as indicated in supplementary figure 3. To find out whether the fully function of NKTCL cells is required by the induction of Tregs and MDSCs, NKTCL cells were pretreated with mitomycin C for 30 min to inhibit their proliferation. As indicated in supplementary figure S3, these pretreated NKTCL cells still sustain the ability to induce generation of Tregs and MDSCs.

Highly expression of IRs in tumor milieu

Finally, we utilized biopsies from five NKTCL patients and three reactive hyperplastic lymphadenopathies to verify

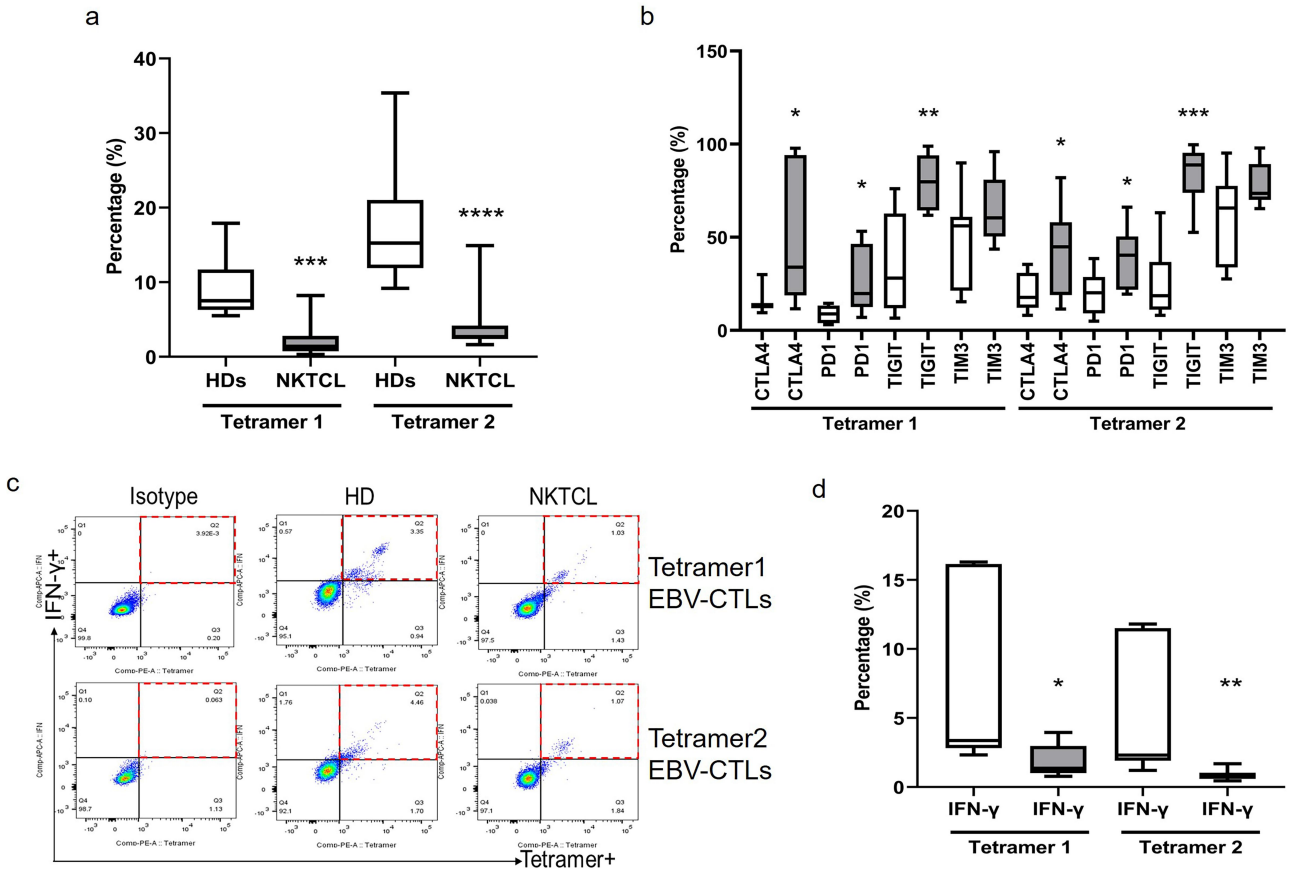

Figure 4. Abnormality of EBV-specific cytotoxic T cells in NKTCL. Percentage of EBV-specific CTLs recognized by two LMP1 epitope in NKTCL patients and HDs (a). IR expression (b) and IFN-γ production (c-d) of EBV-specific CTLs in NKTCL patients and HDs. Gray bars indicate NKTCL patients, blank bars indicate HDs. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

above ex vivo findings. All three IRs measured were much highly expressed in NKTCL tumor microenvironment, as shown in Figure 6a–e.

Discussion

NKTCL is a highly aggressive mature NK/T-cell neoplasm, and patients who experience relapse usually have a dismal outcome with an OS of just a few months. Therefore, novel therapies are needed for patients in the salvage setting. Recently, cancer immunotherapy has been widely applied in clinical practice; however, it is well established that the immunosuppressive tumor microenvironment (TME) poses a pivotal hurdle for successful tumor immunotherapy[13,14]. Many studies have shown that a large number of T cells in the TME are converted into functionally hyporesponsive states and consequently fail to eliminate cancer cells and promote tumor progression[7,12,15,16]. In this study, we investigated the nature of the T-cell defects in NKTCL patients and compared these T cells to those from age-matched HDs. The results showed that NKTCL patients are characterized by T-cell dysfunction, especially the accumulation of exhausted cells.

T-cell exhaustion, a state of acquired T-cell dysfunction initially described in the context of chronic lymphocytic choriomeningitis viral infection, occurs in many other chronic viral infections, autoimmune diseases and various cancers. Recently, T-cell exhaustion has been reported in hematologic malignancies. $CD8^+$ T cells from chronic lymphocytic leukemia (CLL) patients show a phenotype of exhaustion, evidenced by upregulation of IRs, impaired proliferative capacity and reduced ability to lyse target cells. However, these cells retain the capacity to produce cytokines. It is speculated that the "pseudoexhausted" state of CLL T cells may be caused by chronic stimulation by the characteristic CLLL B-cell receptor[17]. In multiple myeloma, enhanced T-cell exhaustion is more common at the tumor site than in the peripheral blood[18]. Isolated $PD1^+TIM3^+$ tumor-infiltrating lymphocytes (TILs) in diffuse large B-cell lymphoma (DLBCL) patients exhibit a signature of T-cell exhaustion, which can be restored by the blockade of PD1 or TIM3. In addition, these $PD1^+TIM3^+$ exhausted $CD8^+$ TILs localize inside $CD20^+$ malignant B-cell clusters[19]. Liu et al. demonstrated that exhaustion of T cells in the leukemic site contributed to B-ALL relapse after allogenic hematopoietic stem cell transplantation[20]. In keeping with these findings, our study also revealed T-cell exhaustion was significantly increased in NKTCL patients compared with HDs, and the percentage of T cells co-expressing IRs was increased, further indicating an exhausted status

To further explore whether the intricate dysfunction of T cells or tumor cells induced the reprogramming of T cells, we performed cocultures of NKTCL cell lines and PBMCs derived from HDs for various time points. NKTCL cell lines could promote the reprogramming of healthy T cells to

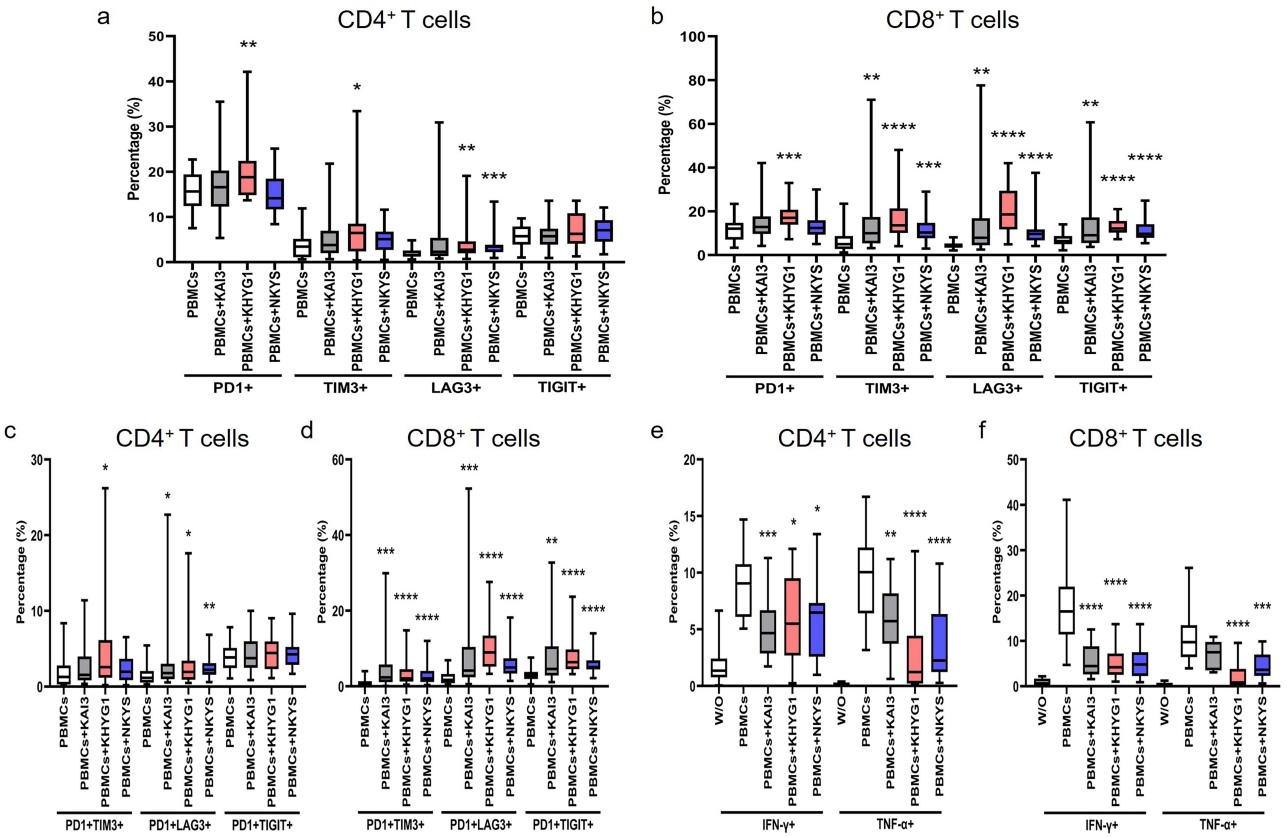

Figure 5. NKTCL cells educate normal T cells to exhausted T cells. Percentage of IRs (a-b), co-expressed IRs (c-d) and cytokine production (e-f) on $CD4^+$ and $CD8^+$ T cells derived from HDs after co-culturation with NKTCL cell lines. W/O: without activation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

exhausted T cells. Growing evidence indicates that cancer cells can directly induce T-cell exhaustion during crosstalk[21,22]; however, the detailed mechanisms remain to be fully illustrated.

In addition to alterations in the T-cell subset, we also found that immunosuppressive Tregs and MDSCs accumulated in NKTCL patients. Consistently, tumor cells induced the generation of these two cell fractions. It is well known that both Tregs and MDSCs suppress T-cell activation and therefore participate in promoting immune evasion and carcinogenesis[23,24]. Recent studies have suggested that both cell types can induce T-cell exhaustion via multiple mechanisms, including immunosuppressive cytokine secretion, the PD-1/PD-L1 signaling pathway, production of nitric oxide and reactive oxygen species, and expression of arginase 1 and IDO[25–27]. Targeting inhibitory Tregs and MDSCs represents a novel strategy to invigorate antitumor immunity.

In healthy individuals, lifelong asymptomatic infection by EBV is critically controlled by EBV-specific CTLs. Staining with EBV peptide-loaded HLA tetramers suggested that T cells specific for EBV antigens are maintained in the blood of healthy carriers at relatively high frequencies throughout life[28]. In certain circumstances, EBV-positive lymphoma can be controlled or even cured by the adoptive transfer of in vitro activated and expanded EBV-specific CTLs[29–31], suggesting that reconstitution of EBV-specific immunity may be a promising strategy for EBV-related malignancies. Our current study elaborated that the frequency of $CD8^+$ T cells recognizing two HLA-A2 restricted epitopes in LMP1 was much lower in NKTCL patients than in healthy individuals. Moreover, EBV-specific CTLs expressed high levels of IRs and demonstrated reduced IFN-γ secretion. Similarly, Li et al. demonstrated that EBV-specific CTLs in nasopharyngeal carcinoma were not only reduced in frequency but also lacked cytotoxic activity and failed to produce IFN-γ upon specific stimulation[32]. Collectively, these dysfunctional EBV-specific CTLs likely contribute to immune escape and carcinogenesis.

It is well recognized that T-cell exhaustion is mainly mediated by suppressive factors in the TME, including malignant tumor cells, immunosuppressive cells (i.e., Tregs, MDSCs, tumor-associated macrophages, cancer-associated fibroblasts, tumor-associated neutrophils, mast cells), inhibitory cytokines (i.e., TGF-β, IL-10, adenosine, ROS), receptor ligands (i.e., PD-1/PD-L1), transcription factors (i.e., NFAT, Nr4a, TOX, Blimp1), and metabolic regulation[6,7,9,33]. Considering the relationship between chronic viral infection and T-cell exhaustion, we speculate that EBV plays a critical role in the T-cell exhaustion observed in NKTCL patients. Previous studies have reported that EBV-encoded latent genes, especially the well-characterized LMP1 oncogene, can induce immune escape through several mechanisms. It has been reported that LMP1 induces the expression of IL10, a critical inhibitory cytokine in T-cell exhaustion[34]. Additionally, LMP1 and IFN-γ upregulate PD-L1 on tumor cells and inhibit

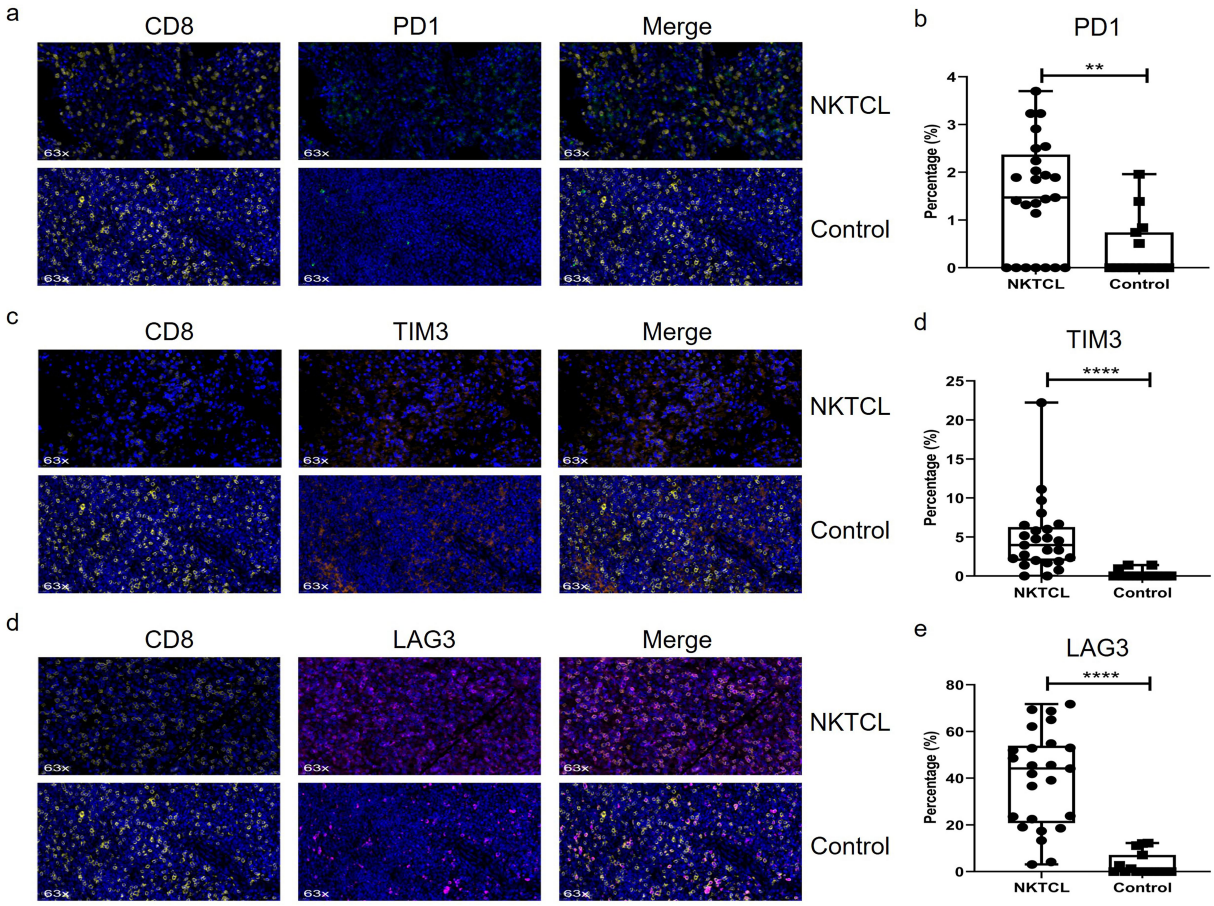

Figure 6. Upregulated IRs in tumor microenvironment of NKTCL. Representative images (a,c,d) and pooled data (b,d,e) of PD1, TIM3 and LAG3 expression in CD8+ T cells in both NKTCL and reactive hyperplastic lymphadenopathies (indicated as Control). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

T cells via an interaction with PD-1[35]. Moreover, the cis- and trans-presentation of $CD8^+$ T-cell epitopes is triggered by LMP1 through self-aggregation, mainly through the first transmembrane domain of LMP1[36]. However, the exact mechanism of EBV in T-cell exhaustion in NKTCL patients remains to be illustrated.

Restoring exhausted T cells by immune checkpoint inhibitors (ICIs) represents an inspiring strategy for cancer management that has yielded promising results and is a significant breakthrough in cancer immunotherapy. Accumulating evidence has also shown that ICIs generate promising results in different EBV-related diseases, including NKTCL[37,38]. An increasing number of clinical studies are dedicated to exploring the combinational and even upfront use of ICIs for treating NKTCL[39]. Recent studies revealed that exhausted T cells display phenotypic and functional heterogeneity. Terminally exhausted T cells, which co-upregulate multiple IRs, have been indicated to exhibit more extensive exhausted phenotypes and are unable to respond to anti-PD-1 checkpoint blockade therapy[40–42]. As mentioned above, our study found that NKTCL patients have a higher percentage of T cells co-expressing IRs than healthy controls, which may hamper the efficacy of ICIs. Further study is needed to verify this finding.

In conclusion, for the first time, we described the dysfunction of T cells in NKTCL, highlighted by T-cell exhaustion and the accumulation of inhibitory cells. A better understanding of diverse factors regulating defective T cells will pave the way for developing more effective immunotherapeutic and prophylactic strategies for treating chronic infectious diseases.

Acknowledgments

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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