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Original article

IL-7Rα^{low} memory CD8^{+} T cells are significantly elevated in patients with systemic lupus erythematosus

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Abstract

Objective. Human effector memory (EM) CD8^{+} T cells include IL-7Rα^{high} and IL-7Rα^{low} cells with distinct cellular characteristics, including the expression of cytotoxic molecules. Both NK cells and the NK cell-associated molecule 2B4 that is expressed on CD8^{+} T cells promote cytotoxicity. Here we analysed the expression of 2B4 on IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells and its contribution to cytotoxicity. We also analysed the frequency of IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells in patients with SLE or lupus and in healthy individuals given the potential role of cytotoxic CD8^{+} T cells in the pathogenesis of lupus.

Methods. We used flow cytometry to measure the expression of 2B4 on IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells as well as the frequency of these cell populations in the peripheral blood of healthy individuals and patients with SLE. Also, 2B4-mediated cytotoxicity was quantitated in IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells using target cells with CD48 antigen.

Results. We found that IL-7Rα^{high} EM CD8^{+} T cells had higher levels of 2B4 expression compared with IL-7Rα^{low} EM CD8^{+} T cells. Triggering 2B4 enhanced the cytotoxic function of IL-7Rα^{low} EM CD8^{+} T cells against target cells. We also noticed that patients with SLE had an increased frequency of IL-7Rα^{low} EM CD8^{+} T cells that correlated with disease manifestation.

Conclusion. Our findings show that SLE patients have increased IL-7Rα^{low} EM CD8^{+} T cells, possibly contributing to tissue damage through 2B4-mediated cytotoxicity.

Key words: human CD8^{+} T cells, IL-7 receptor alpha (α) chain, NK receptor, 2B4, cytolytic function, systemic lupus erythematosus.

Introduction

Memory CD8^{+} T cells recognize specific antigens in target tumours and virally infected cells, and are therefore vital for host defense. Although persistent antigenic stimulation may aid the maintenance of memory T cells [1], the cytokines IL-7 and IL-15 also play an important role in the generation and maintenance of memory CD8^{+} T cells by promoting cell survival and proliferation, respectively, even in the absence of antigen [2, 3]. Memory CD8^{+} T cells can be divided into central and effector memory (EM) populations based on their capacity to migrate to secondary lymphoid tissues such as lymph nodes and spleen. We recently identified two unique subsets of human EM CD8^{+} T cells that express high and low levels of IL-7 receptor alpha chain (IL-7Rα^{high} and IL-7Rα^{low}, respectively) in peripheral blood [4]. IL-7Rα^{low} EM CD8^{+} T cells are largely antigen-exposed (CD27^{-}CD28^{-}) cells with increased expression of the cytotoxic molecule perforin compared with IL-7Rα^{high} EM CD8^{+} T cells. Hence the development of IL-7Rα^{low}

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cells is likely driven by repetitive antigenic immune stimulation [4]. This finding is supported by other studies that have found increased IL-7Rα^{low} memory CD8$^+$ T cells in individuals with chronic or latent viral infections such as CMV, EBV, HCV and HIV [5–8].

SLE or lupus is an autoimmune inflammatory disease of unknown aetiology. Both genetic and environmental factors are likely involved in the pathogenesis of SLE, leading to the development of autoantibodies and inflammation [9, 10]. CD4$^+$ T cells are critical drivers of the B cell-dependent autoantibody response in lupus [9, 11]. Conversely, the role of CD8$^+$ T cells in the development of lupus is less understood, despite the fact that these cells comprise a significant portion of peripheral lymphocytes and play a major role in immune responses via cytotoxicity and cytokine production. Interestingly, recent studies have documented expansion of cytotoxic CD8$^+$ T cells in patients with SLE, which was correlated with disease activity [12, 13]; the presence of CD8$^+$ T cells in the periglomerular area was also correlated with the presence of lupus nephritis [14]. These findings suggest a possible role of CD8$^+$ T cells in the pathogenesis of SLE.

The molecule 2B4 (CD244), which is associated with NK cells, belongs to the signalling lymphocyte activation molecule (SLAM/CD150) family [15, 16]. In addition to NK cells, 2B4 is expressed on other immune cells, including $\gamma\delta$ T cells and subsets of CD8$^+$ T cells. Upon binding its natural ligand, CD48, 2B4 may serve as a co-stimulatory molecule promoting NK and CD8$^+$ T cell function such as degranulation, cytokine production and proliferation [17–25]. However, recent studies have also reported an inhibitory role of 2B4, suggesting that this molecule may have a dual function [26, 27]. This study explored the biological significance of 2B4 expression on IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells in human peripheral blood. Specifically, we investigated whether IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells differentially expressed 2B4, and whether it served as an activator or inhibitor of cytotoxicity. We also determined whether the frequency of such cell subsets was altered in SLE, an inflammatory condition with chronic immune stimulations similar to those observed in latent and chronic viral infections.

Materials and methods

Patients and healthy subjects

This work was approved by the institutional review board of Seoul National University Hospital and the institutional review committee of Yale University. Peripheral blood was drawn from healthy volunteers and patients with SLE after obtaining informed consent. Disease activity was evaluated using the SLEDAI score according to the criteria of the ACR, revised in 1982 [28]. Patients with SLE and healthy individuals were matched for gender (all females) and age [mean (S.D.) 35.8 (9.3) *vs* healthy 33.3 (7.0)]. Patients were taking the following medications: prednisone ($n = 12$), HCQ ($n = 16$), MTX ($n = 5$), AZA ($n = 4$) and MMF ($n = 5$).

Reagents and flow cytometry

Baf/3 cells stably expressing either green fluorescent protein (GFP, Baf/3-GFP) or human CD48 (Baf/3-CD48) (generously provided by Dr Carsten Watzl) were used as target cells in a cytotoxicity assay [15]. As previously described [4], Peripheral blood mononuclear cells (PBMCs) were purified and stained with goat anti-human IL-7Rα antibodies (Abs) (R&D Systems, Minneapolis, MN, USA), then stained with donkey anti-goat IgG Abs (Santa Cruz Biotech, Santa Cruz, CA, USA) and Abs to CD8 and CCR7 (BD Pharmingen, San Diego, CA, USA). Cells were sorted into IL-7Rα^{high} and IL-7Rα^{low} EM (CCR7$^-$CD45RA$^{+/-}$) CD8$^+$ T cells using a FACSAria (BD Immunocytometry Systems, San Jose, CA, USA). Some PBMCs were stained with Abs to IL-7Rα, CD8, CCR7, CD45RA, CD27, CD28, perforin, granzyme B, and 2B4 (BD Pharmingen) or isotype control, and analysed on an LSRII flow cytometer (BD Immunocytometry Systems). Collected data were analysed using FlowJo software (Tree Star, Ashland, OR, USA). In cytotoxicity and degranulation (CD107a) assays [29], sorted CD8$^+$ T cells were stimulated for 2 days with anti-CD3 (clone HIT3a, 5 μg/ml, BD Pharmingen) and CD28 Abs (clone CD28.2, 2 μg/ml, BD Pharmingen) in the presence of IL-15 (5 ng/ml, R&D Systems).

Cell-mediated cytotoxicity assay

Target cells were incubated with 100 μl $Na_2{}^{51}CrO_4$ (1 mCi/ml; PerkinElmer Life Science, Boston, MA, USA) for 1.5 h at 37°C. Then the ^{51}Cr-labelled cells were washed twice with RPMI media containing 2% FBS. In the chromium release assay, activated CD8$^+$ (IL-7Rα^{high} or IL-7Rα^{low}) T cells were incubated for 6 h with the indicated percentage of ^{51}Cr-labelled target cells in a 96-well round-bottom plate. The ^{51}Cr released was measured using a Packard Cobra gamma counter (GMI, Albertville, MN, USA). To block cytotoxicity, Abs to 2B4 (clone eBioPP35, 2.5 μg/ml; eBioscience, San Diego, CA, USA), CD48 (clone eBio156-4H9, 2.5 μg/ml; eBioscience) or the isotype control were added. Results were expressed as the percentage of specific lysis determined at each effector-to-target (E:T) ratio by the following formula: per cent cytotoxicity = [(mean cpm experimental release − mean cpm spontaneous release)/(mean cpm maximal release − mean cpm spontaneous release)] × 100.

Statistical analysis

All data are expressed as mean (S.D.) or mean (S.E.M.). Data were compared using the two-tailed Student's *t*-test or Mann–Whitney U test. $P < 0.05$ was considered significant. The Pearson's correlation analysis test was applied to SLEDAI and the frequency of IL-7Rα^{low} EM CD8$^+$ T cells. All statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, La Jolla, CA, USA) and SPSS 18.0 (SPSS, Chicago, IL, USA).

Results

Human IL-7Rα^{low} EM CD8^{+} T cells express high levels of 2B4 compared with IL-7Rα^{high} EM CD8^{+} T cells.

We recently identified two unique subsets of EM CD8^{+} T cells (CCR7^{-}CD45RA$^{+/-}$) that express IL-7Rα^{high} and IL-7Rα^{low} in human peripheral blood [4] (Fig. 1A). Compared with IL-7Rα^{high} EM CD8^{+} T cells, IL-7Rα^{low} EM CD8^{+} T cells showed more expression of cytotoxic perforin, a molecule also expressed in terminally differentiated CD8^{+} T cells (Fig. 1B). In addition to perforin, the expression of granzyme B was higher in IL-7Rα^{low} EM CD8^{+} T cells than in IL-7Rα^{high} EM CD8^{+} T cells. Previous human and mouse studies have shown that terminally differentiated effector CD8^{+} T cells express NK-associated molecules such as CD57 and NKG2D [4, 30]. Thus we measured the expression of the NK cell-associated molecule 2B4, which promotes cytotoxicity, in IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells using flow cytometry (Fig. 1C, representative dot plot). The expression levels of 2B4 were significantly higher in IL-7Rα^{low} EM CD8^{+} T cells than in IL-7Rα^{high} EM CD8^{+} T cells [Fig. 1D, mean fluorescence intensity (MFI) (S.E.M.) 15.24 (5.61) *vs* 5.24 (0.91) and $P = 0.002$; mean % (S.E.M.) 17.73 (2.72) *vs* 47.74 (5.93) and $P < 0.001$]. Next, we determined whether TCR triggering with IL-15 would up-regulate the expression of 2B4 in purified IL-7Rα^{high} and IL-7Rα^{low} CD8^{+} T cells. IL-15 was used along with TCR triggering to restore impaired TCR-mediated proliferation of the IL-7Rα^{low} EM CD8^{+} T cells [31]. The combination of anti-CD3/CD28 Abs and IL-15 increased 2B4 expression in both IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells (Fig. 1E), but the levels of 2B4 in the latter remained above those in the former.

2B4-mediated cytotoxicity is elevated in IL-7Rα^{low} EM CD8^{+} T cells compared with IL-7α^{high} EM CD8^{+} T cells

We investigated whether the differential expression of 2B4 in IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells would have any functional implication by inducing cytotoxicity using target cells that expressed CD48 (Baf/3-CD48). The target cells were co-cultured with purified IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells that had first been stimulated with a combination of anti-CD3/CD28 Abs and IL-15. At low E:T ratios, the levels of cell lysis were higher in target cells co-cultured with IL-7Rα^{low} EM CD8^{+} T cells than in those co-cultured with IL-7Rα^{high} EM CD8^{+} T cells (Fig. 2A). However, similar levels of cell lysis were found when target cells were co-cultured with IL-7Rα^{high} or IL-7Rα^{low} EM CD8^{+} T cells at a high E:T ratio (Fig. 2A). IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells barely induced target cell lysis when they were co-cultured with Baf/3 cells expressing control GFP (Fig. 2B, IL-7Rα^{high} cell, data not shown). To further determine the specific role of the 2B4 and CD48 interaction in killing target cells, Baf/3-CD48 target cells were co-cultured with IL-7Rα^{low} EM CD8^{+} T cells in the presence of Abs to 2B4, CD48, both or an isotype control. Target cells treated with anti-2B4 or anti-CD48 Abs or both had less cell lysis than the same cells treated with the isotype control (Fig. 2C). Although the blocking effect of anti-2B4 Abs appeared to be weaker than that of anti-CD48 Abs, the combination of the two Abs synergistically increased the effect of blocking on target cell lysis. We next measured the expression of CD107a, a lysomal-associated membrane protein-1, by IL-7Rα^{low} EM CD8^{+} T cells because this molecule is mobilized to the cell membrane when the cytotoxic molecules perforin and granzyme B are released from cytotoxic cells [29]. IL-7Rα^{low} EM CD8^{+} T cells had increased CD107a expression when co-cultured with target cells expressing CD48. This was blocked by adding anti-CD48 Abs during the culture (Fig. 2D). Overall, these findings suggest that IL-7Rα^{low} EM CD8^{+} T cells with high levels of 2B4 expression have greater 2B4 and CD48-mediated cytotoxicity compared with IL-7Rα^{high} EM CD8^{+} T cells.

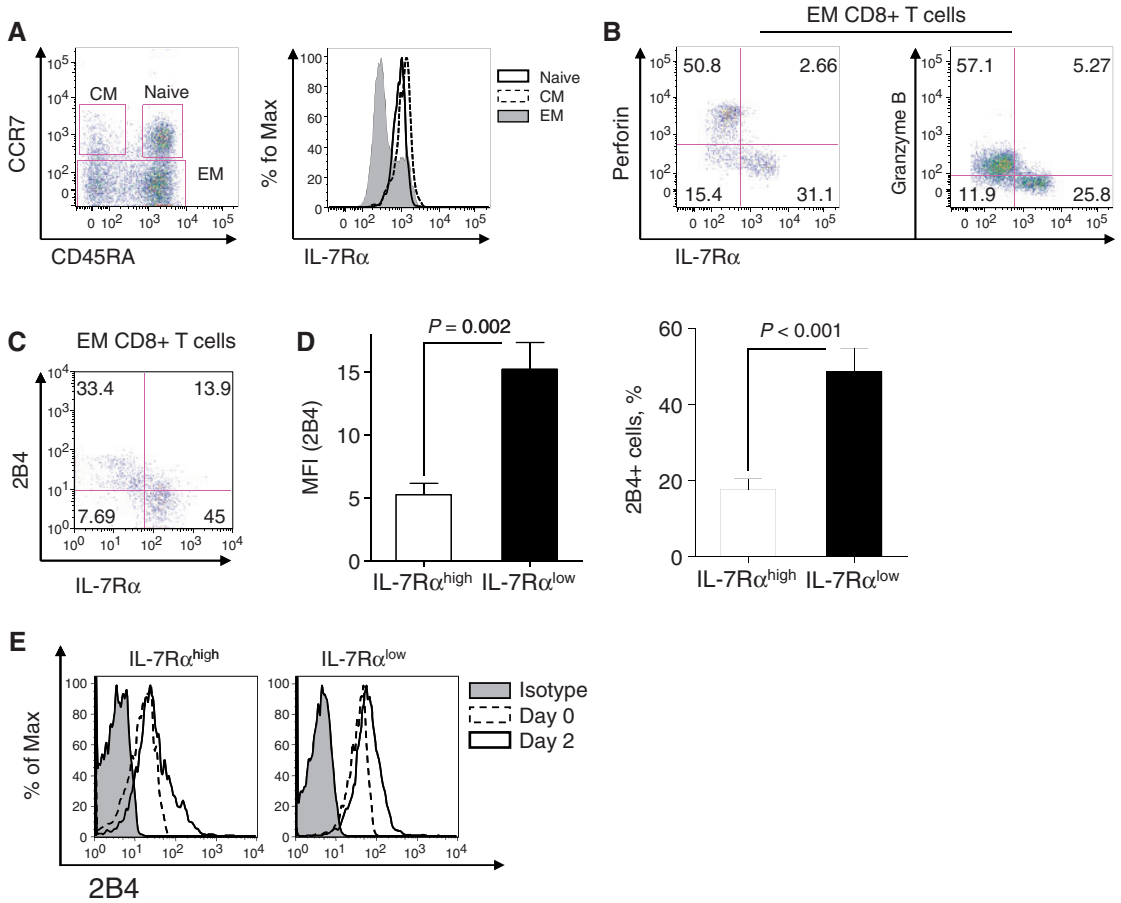
Patients with SLE have an increased frequency of IL-7Rα^{low} EM CD8^{+} T cells that are correlated with disease activity

Although the exact role of CD8^{+} T cells in lupus pathogenesis is yet to be determined, a recent study reported the expansion of perforin-expressing CD8^{+} T cells in the peripheral blood of patients with SLE [12]. Hence we measured the frequency of IL-7Rα^{low} EM CD8^{+} T cells with the expression of perforin, granzyme B and 2B4 in patients with SLE and healthy controls. Similar to healthy controls, IL-7Rα^{low} EM CD8^{+} T cells from lupus patients expressed higher levels of perforin, granzyme B and 2B4 than IL-7Rα^{high} EM CD8^{+} T cells (Fig. 3A and B). The incidence of IL-7Rα^{low} EM CD8^{+} T cells was much higher in patients with SLE than in age- and gender-matched healthy controls (Fig. 3C) [mean frequency (%) (S.E.M.) 32.33% (3.86) and 22.13% (2.41), respectively; $P = 0.027$]. Furthermore, the incidence of this cell subset in patients with SLE was directly proportional to the activity of the disease as measured by SLEDAI (Fig. 3D) ($r = 0.483$, $P = 0.026$).

Discussion

CD8^{+} T cells have been shown to express NK-cell associated molecules that affect cell function [20, 23–25, 32–34]. We recently reported the presence of two unique subsets of EM CD8^{+} T cells with high and low levels of IL-7Rα expression in human peripheral blood [4]. To better understand the biological significance of IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells, we investigated the expression of the NK-associated molecule 2B4 in these cell subsets as well as the impact of such molecular expression on cytotoxicity. In addition, we determined the frequency of IL-7Rα^{low} EM CD8^{+} T cells in patients with SLE and correlated it with disease severity. The results indicate that 2B4 is differentially expressed in IL-7Rα^{high} and IL-7Rα^{low} EM CD8^{+} T cells, contributing to cytotoxicity. In addition, patients with SLE had an increased frequency of IL-7Rα^{low} EM CD8^{+} T cells that correlated with disease activity compared with healthy controls. These findings provide new insight into understanding the

Fig. 1 2B4 is differentially expressed in IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells in human peripheral blood.

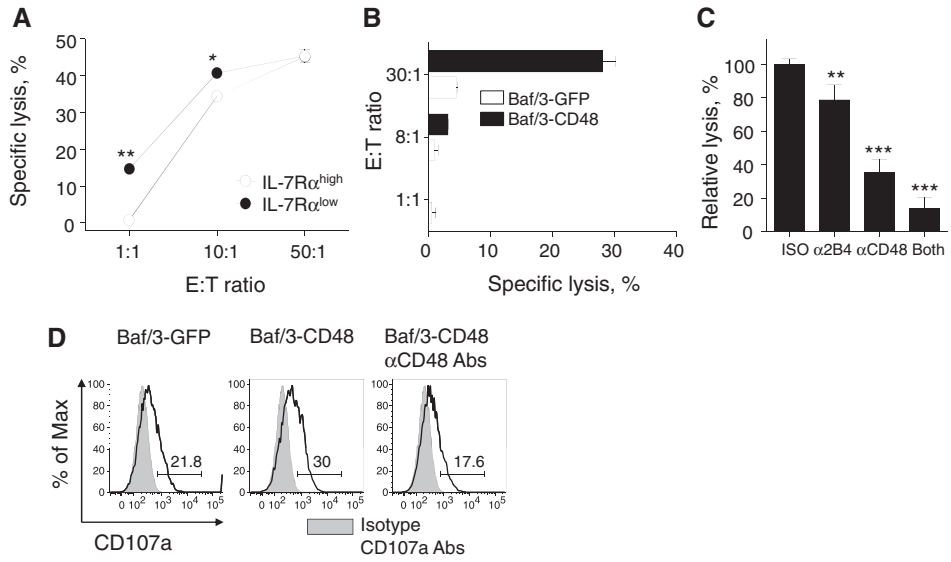


PBMCs from healthy individuals were stained with Abs to CD8, CCR7, CD45RA, IL-7Rα and 2B4 (**A**, **C** and **D**), perforin, and granzyme B (**B**). The stained cells were analysed on a flow cytometer. (**A**) Representative dot plot and histogram showing naïve, CM and EM CD8$^+$ T cell subsets as well as IL-7Rα expression by the individual CD8$^+$ T cell subsets. (**B**) Representative dot plots showing the expression of perforin, granzyme B and IL-7Rα by EM CD8$^+$ T cells. These data were gathered from three individuals (**A** and **B**). (**C**) Representative dot plot showing the expression of 2B4 and IL-7Rα by EM CD8$^+$ T cells. (**D**) MFI of 2B4 expression by IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells ($n = 7$, left panel). The frequency of 2B4 positive cells by IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells in healthy individuals ($n = 7$) as measured by flow cytometry (right panel). (**E**) Increased expression of 2B4 by IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells in response to a combination of anti-CD3/CD28 Abs and IL-15. CD8$^+$ T cells were sorted into IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells using a FACSAria. Sorted cells were cultured for 2 days with Abs to CD3 and CD28 (each at 5 μg/ml) in the presence of IL-15 (5 ng/ml), followed by staining with anti-2B4 or isotype Abs. Results are representative of two independent experiments. *P* values were obtained using the Mann–Whitney U test. Numbers in dot plots indicate the percentage of cells in each quadrant.

function and biological significance of IL-7Rα^{low} EM CD8$^+$ T cells in health and disease.

The molecule 2B4 is expressed in NK cells and subsets of CD8$^+$ T cells such as memory and activated cells [35]. We found that IL-7Rα^{low} EM CD8$^+$ T cells had increased expression of 2B4 compared with IL-7Rα^{high} EM CD8$^+$ T cells. IL-7Rα^{low} EM CD8$^+$ T cells are known to have terminally differentiated phenotypes with decreased CD27 and CD28 expression and limited TCR repertoires [4]. These findings suggest that the expression of 2B4 could be affected by the differentiation status of memory CD8$^+$ T cells. In fact, we noted up-regulation of 2B4 in both IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells upon TCR and IL-15 stimulation. In general, triggering 2B4 augments cytotoxicity and IFN-γ production in NK cells and CD8$^+$ T cells [20–25], although high levels of 2B4 expression could function as an inhibitory receptor [26, 27]. We noticed that 2B4, which is expressed at different levels by IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells, up-regulated cytotoxicity rather than inhibiting it. Upon antigenic triggering of 2B4 with CD48, IL-7Rα^{low} EM CD8$^+$ T cells showed increased cytotoxicity than IL-7Rα^{high} EM CD8$^+$

FIG. 2 Enhanced cytotoxicity of activated IL-7Rα^{low} EM CD8$^+$ T cells.



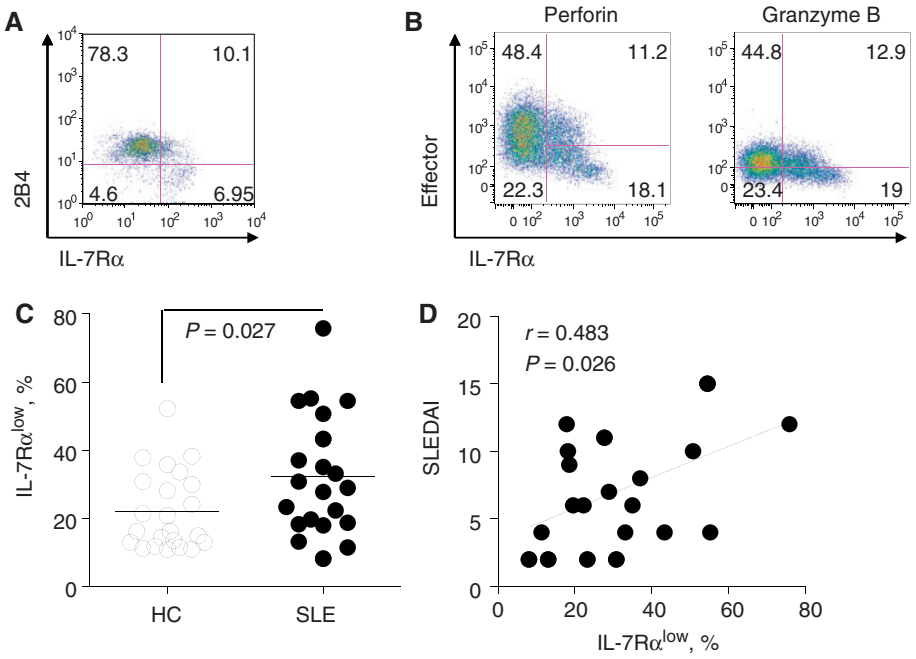
PBMCs were sorted into IL-7Rα^{high} and IL-7Rα^{low} EM (CCR7$^-$CD45RA$^{+/-}$) CD8$^+$ T cells using a FACSAria. To generate effector cells, sorted cells were cultured for 2 days with Abs to CD3 (5 μg/ml) and CD28 (2 μg/ml) in the presence of IL-15 (5 ng/ml). IL-7Rα^{high} (**A** and **B**) and IL-7Rα^{low} (**B**) EM CD8$^+$ T cells were used as effector cells (**E**) in a 6-h chromium release assay against target cells (T) expressing human CD48 (Baf/3-CD48) (**A** and **B**) or GFP (Baf/3-GFP, control protein) (**B**). The percentage of specific lysis was calculated after subtracting the medium-only background. Circles and bars indicate the mean of triplicate counts. The data were compiled from five independent experiments using PBMCs from five healthy individuals. (**C**) The results of a chromium release assay where IL-7Rα^{low} EM CD8$^+$ T cells (effector cells, E) were incubated with Baf/3-CD48 cells (target cells, T) at an E:T ratio of 40:1 in the presence of control Abs or Abs to 2B4, CD48, or both. Bars and error bars indicate the mean and S.D. of triplicate counts. Results are representative data from two (**C**) to four (**A** and **B**) independent experiments using PBMCs of five individuals. (**D**) Flow cytometric analysis of CD107a expression by IL-7Rα^{low} EM CD8$^+$ T cells that were stimulated for 2 days with a combination of anti-CD3 (5 μg/ml)/CD28 (2 μg/ml) Abs and IL-15 (5 ng/ml) followed by co-culturing with target cells (Baf/3-CD48 or Baf/3-GFP) at an E:T ratio of 40:1 in the presence or absence of anti-CD48 Abs. Numbers in histograms indicate the percentage of cells that stained positive for antibody. *P* values were obtained using a two-tailed Student's *t*-test ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). The data represent two independent experiments.

T cells. This finding could be secondary in part to the differential expression of 2B4 and cytotoxic molecules (e.g. perforin and granzyme B) by the two cell subsets.

CD8$^+$ T cells, possessing potent cytotoxicity, play a pathogenic role in organ-specific autoimmune diseases such as type I diabetes mellitus [36, 37]. However, the role of CD8$^+$ T cells in the pathogenesis or regulation of SLE is less understood. CD8$^+$ T cell depletion prevents or mitigates lupus in experimental animals [38–40], suggesting that CD8$^+$ T cells contribute to the pathogenesis of the disease. This notion is supported by human studies that have shown the expansion of memory CD8$^+$ T cells in the peripheral blood of patients with SLE [12, 13]. In particular, the expansion of perforin- and/or granzyme B-positive CD8$^+$ T cells has been found in patients with SLE, correlating with disease activity [12]. This observation is consistent with our results showing the expansion of IL-7Rα^{low} EM CD8$^+$ T cells with the expression of perforin and granzyme B as well as the correlation of such cell expansion with the degree of disease. The expansion of cytotoxic CD8$^+$ T cells causes tissue damage which could possibly lead to increased production of soluble nucleosomes, target antigenic structures in lupus, from target cells [12]. Interestingly, IL-7Rα^{low} EM CD8$^+$ T cells had increased expression of 2B4, which could promote their cytotoxicity. This raises the possibility of enhanced tissue damage in lupus by 2B4 expressed by IL-7Rα^{low} EM CD8$^+$ T cells through an interaction with CD48 expressed by other cells including lymphocytes, macrophages, dendritic cells and epithelial cells [15, 16].

In conclusion, we found that the NK cell-associated molecule 2B4 is differentially expressed in IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells in human peripheral blood, and that it activates cytotoxicity. We also noticed that patients with SLE had an increased frequency of IL-7Rα^{low} EM CD8$^+$ T cells that correlated with disease activity. Our findings suggest the possible involvement of 2B4 in SLE-associated tissue damage by the expansion of IL-7Rα^{low} EM CD8$^+$ T cells.

Fig. 3 Increased frequency of IL-7Rα^{low} EM CD8$^+$ T cells in patients with SLE.



(**A** and **B**) Flow cytometric analysis of 2B4, perforin, granzyme B and IL-7Rα expression by EM (CD45RA$^-$CCR7$^{+/-}$) CD8$^+$ T cells in a patient with SLE. Numbers in dot plots indicate the percentage of cells in each quadrant. (**C**) The frequency of IL-7Rα^{low} EM CD8$^+$ T cells in patients with SLE ($n = 21$) and healthy individuals (HC, $n = 23$) as measured by flow cytometry. (**D**) Correlation of the frequency of IL-7Rα^{low} EM CD8$^+$ T cells with SLE disease activity index (SLEDAI). The data were compiled from three individuals (**A** and **B**). The *P* value was obtained using the two-tailed Student's *t*-test (**C**) or the Pearson's correlation (**D**).

Rheumatology key messages

- The frequency of IL-7Rα^{low} CD8$^+$ T cells is correlated with the disease activity of SLE.
- 2B4 is differentially expressed on IL-7Rα^{high} and IL-7Rα^{low} EM CD8$^+$ T cells.
- 2B4-mediated cytotoxicity is increased in IL-7Rα^{low} EM CD8$^+$ T cells.

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References

1 Gray D, Matzinger P. T cell memory is short-lived in the absence of antigen. J Exp Med 1991;174:969–74.

2 Tan JT, Ernst B, Kieper WC *et al*. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. J Exp Med 2002;195:1523–32.

3 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;28:325–39.

4 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;107:2855–62.

5 Boutboul F, Puthier D, Appay V *et al*. Modulation of interleukin-7 receptor expression characterizes differentiation of CD8 T cells specific for HIV, EBV and CMV. AIDS 2005;19:1981–6.

6 Paiardini M, Cervasi B, Albrecht H *et al*. Loss of CD127 expression defines an expansion of effector CD8+ T cells in HIV-infected individuals. J Immunol 2005;174: 2900–9.

7 Radziewicz H, Ibegbu CC, Hon H *et al*. Impaired hepatitis C virus (HCV)-specific effector CD8+ T cells undergo massive apoptosis in the peripheral blood during acute HCV infection and in the liver during the chronic phase of infection. J Virol 2008;82:9808–22.

8 Zhang SY, Zhang Z, Fu JL *et al*. Progressive CD127 down-regulation correlates with increased apoptosis of CD8 T cells during chronic HIV-1 infection. Eur J Immunol 2009;39:1425–34.

9 Herrmann M, Voll RE, Kalden JR. Etiopathogenesis of systemic lupus erythematosus. Immunol Today 2000;21: 424–6.

10 Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 2001;1:147–53.

11 Vratsanos GS, Jung S, Park YM, Craft J. CD4(+) T cells from lupus-prone mice are hyperresponsive to T cell receptor engagement with low and high affinity peptide antigens: a model to explain spontaneous T cell activation in lupus. J Exp Med 2001;193:329–37.

12 Blanco P, Pitard V, Viallard JF *et al*. Increase in activated CD8+ T lymphocytes expressing perforin and granzyme B correlates with disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 2005;52:201–11.

13 Fritsch RD, Shen X, Illei GG *et al*. Abnormal differentiation of memory T cells in systemic lupus erythematosus. Arthritis Rheum 2006;54:2184–97.

14 Couzi L, Merville P, Deminiere C *et al*. Predominance of CD8+ T lymphocytes among periglomerular infiltrating cells and link to the prognosis of class III and class IV lupus nephritis. Arthritis Rheum 2007;56:2362–70.

15 Endt J, Eissmann P, Hoffmann SC *et al*. Modulation of 2B4 (CD244) activity and regulated SAP expression in human NK cells. Eur J Immunol 2007;37:193–8.

16 Brown MH, Boles K, van der Merwe PA *et al*. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. J Exp Med 1998;188: 2083–90.

17 Chuang SS, Kim MH, Johnson LA *et al*. 2B4 stimulation of YT cells induces natural killer cell cytolytic function and invasiveness. Immunology 2000;100:378–83.

18 Messmer B, Eissmann P, Stark S, Watzl C. CD48 stimulation by 2B4 (CD244)-expressing targets activates human NK cells. J Immunol 2006;176:4646–50.

19 Lee KM, Bhawan S, Majima T *et al*. Cutting edge: the NK cell receptor 2B4 augments antigen-specific T cell cytotoxicity through CD48 ligation on neighboring T cells. J Immunol 2003;170:4881–5.

20 Valiante NM, Trinchieri G. Identification of a novel signal transduction surface molecule on human cytotoxic lymphocytes. J Exp Med 1993;178:1397–406.

21 Sivori S, Parolini S, Falco M *et al*. 2B4 functions as a co-receptor in human NK cell activation. Eur J Immunol 2000;30:787–93.

22 Johnson LA, Goldfarb RH, Mathew PA. Regulation of IFN-gamma production following 2B4 activation in human NK cells. In Vivo 2000;14:625–9.

23 Speiser DE, Colonna M, Ayyoub M *et al*. The activatory receptor 2B4 is expressed in vivo by human CD8+ effector alpha beta T cells. J Immunol 2001;167:6165–70.

24 Dupre L, Andolfi G, Tangye SG *et al*. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. Blood 2005;105:4383–9.

25 Enose-Akahata Y, Matsuura E, Oh U, Jacobson S. High expression of CD244 and SAP regulated CD8 T cell responses of patients with HTLV-I associated neurologic disease. PLoS Pathog 2009;5:e1000682.

26 Lee KM, McNerney ME, Stepp SE *et al*. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. J Exp Med 2004; 199:1245–54.

27 Vacca P, Pietra G, Falco M *et al*. Analysis of natural killer cells isolated from human decidua: evidence that 2B4 (CD244) functions as an inhibitory receptor and blocks NK-cell function. Blood 2006;108:4078–85.

28 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992;35:630–40.

29 Alter G, Malenfant JM, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. J Immunol Methods 2004;294:15–22.

30 Franceschetti M, Pievani A, Borleri G *et al*. Cytokine-induced killer cells are terminally differentiated activated CD8 cytotoxic T-EMRA lymphocytes. Exp Hematol 2009;37:616–28.e2.

31 Kim HR, Hwang KA, Kang I. Dual roles of IL-15 in maintaining IL-7RalphalowCCR7- memory CD8+ T cells in humans via recovering the phosphatidylinositol 3-kinase/ AKT pathway. J Immunol 2007;179:6734–40.

32 Maasho K, Opoku-Anane J, Marusina AI, Coligan JE, Borrego F. NKG2D is a costimulatory receptor for human naive CD8+ T cells. J Immunol 2005;174: 4480–4.

33 Warren HS, Rana PM, Rieger DT *et al*. CD8 T cells expressing killer Ig-like receptors and NKG2A are present in cord blood and express a more naive phenotype than their counterparts in adult blood. J Leukoc Biol 2006;79: 1252–9.

34 Henson SM, Franzese O, Macaulay R *et al*. KLRG1 signaling induces defective Akt (ser473) phosphorylation and proliferative dysfunction of highly differentiated CD8+ T cells. Blood 2009;113:6619–28.

35 Peritt D, Sesok-Pizzini DA, Schretzenmair R *et al*. C1.7 antigen expression on CD8+ T cells is activation dependent: increased proportion of C1.7+CD8+ T cells in HIV-1-infected patients with progressing disease. J Immunol 1999;162:7563–8.

36 Pinkse GG, Tysma OH, Bergen CA *et al*. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. Proc Natl Acad Sci USA 2005;102:18425–30.

37 Blanco P, Viallard JF, Pellegrin JL, Moreau JF. Cytotoxic T lymphocytes and autoimmunity. Curr Opin Rheumatol 2005;17:731–4.

38 Mozes E, Kohn LD, Hakim F, Singer DS. Resistance of MHC class I-deficient mice to experimental systemic lupus erythematosus. Science 1993;261:91–3.

39 Chen SY, Takeoka Y, Pike-Nobile L *et al*. Autoantibody production and cytokine profiles of MHC class I (beta2-microglobulin) gene deleted New Zealand black (NZB) mice. Clin Immunol Immunopathol 1997;84: 318–27.

40 Reynolds J, Norgan VA, Bhambra U *et al*. Anti-CD8 monoclonal antibody therapy is effective in the prevention and treatment of experimental autoimmune glomerulonephritis. J Am Soc Nephrol 2002;13:359–69.