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Gastric cancer patients display a distinctive population of IFNg$^+$IL10$^+$ double positive CD8 T cells, which persists longer during prolonged activation

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ABSTRACT

IL10 is generally regarded as a broad-spectrum regulatory cytokine. However, the role of IL10 in CD8 T cells remains controversial. In this study, we investigated the characteristics of endogenous IL10 by CD8 T cells in gastric cancer (GC) patients. Using intracellular staining, we found that in both GC patients and healthy controls, the majority of IL10-expressing CD8 T cells also presented concurrent IFNg expression. Interestingly, the frequency of IFNg$^+$IL10$^+$ CD8 T cells was significantly higher in GC patients than in healthy controls, while the frequency of IFNg$^+$IL10$^-$ CD8 T cells was significantly lower in GC patients than in healthy controls. Compared to the IFNg$^-$IL10$^-$ CD8 T cells, both IFNg$^+$IL10$^-$ and IFNg$^+$IL10$^+$ CD8 T cells presented significantly higher expression of activation/inhibitory markers. Interestingly, the IFNg$^+$IL10$^+$ cells presented lower PD1 and TIM3 and higher KLRG1 than the IFNg$^+$IL10$^-$ CD8 T cells. Remarkably, the IFNg$^+$IL10$^+$ CD8 T cells, but not the IFNg$^+$IL10$^-$ CD8 T cells, were highly enriched in the CD45RO$^+$CXCR5$^+$ subset. Prolonged activation resulted in significant enrichment of IFNg$^+$IL10$^+$ CD8 T cells over time. Interestingly, compared to the CD45RO$^+$CXCR5$^-$ CD8 T cells, the CD45RO$^+$CXCR5$^+$ CD8 T cells presented stronger proliferation capacity at later stages of stimulation, and higher viability throughout the stimulation process. Overall, our investigation demonstrated that GC patients were enriched with a distinctive population of IFNg$^+$IL10$^+$ double positive CD8 T cells, which resembled T follicular cytotoxic cells and could persist longer during prolonged activation.

1. Introduction

Gastric cancer (GC) is one of the most common causes of cancer-related deaths worldwide [1], and its development is strongly linked to *Helicobacter pylori* (*H. pylori*) infection. However, only a tiny fraction of all *H. pylori*-infected individuals eventually develop GC [2]. The majority of infected cases remain asymptomatic through life. It is now understood that the chronic inflammatory response initiated by *H. pylori* critically contributes to GC pathogenesis [3]. Typically starting from the antral region that incites an inflammatory response, this *H. pylori*-mediated inflammation can gradually spread to the upper stomach over time and cause increasingly severe mucosal atrophy, intestinal metaplasia, and malignant transformation at the gastric lining [4,5].

Many branches of the immune system are involved in this process. *H. pylori* could directly elevate the expression of spermine oxidase in gastric epithelial cells and macrophages, and induce DNA damage through the production of reactive oxygen species [6,7]. *H. pylori* and high salt diet also synergistically increase the production of several proinflammatory cytokines, such as IL1, IL6, and TNF, which possess angiogenic and pro-metastatic roles in tumor [8,9]. Adaptive immune responses involving B cells and T cells likely also contributed to GC initiation. Mice infected with *Helicobacter* spp. showed symptoms of human GC patients, but not when B cells and T cells were absent [10,11]. Further evidence demonstrated that Th1-mediated IFNg responses contributed to the gastric atrophy in mice infected with *Helicobacter* spp. [12,13].

On the other hand, proinflammatory responses might contribute to

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GC suppression once the tumor is established. Meta-analysis of tumor-infiltrating lymphocytes (TILs) in GC patients indicated that the frequency of tumor-infiltrating $CD3^+$ T cells, especially the $CD8^+$ TILs, was positively associated with overall survival [14], while high expression of Foxp3, a transcription factor for regulatory T cells, in TILs was associated with reduced survival. High tumor-infiltrating $CD3^+$, $CD8^+$, and $CD45RO^+$ T cell frequency in addition was associated with lower lymph node metastasis and reduced recurrence following surgical resection [15,16].

IL10 is a pleiotropic cytokine with multiple protective roles during inflammation. IL10 is shown to suppress innate and adaptive immune responses, mediate tissue repair, and maintain epithelium integrity [17]. In addition, IL10 can directly dampen the signal transduction of CD8 T cells, thus increasing the threshold required for antigen recognition and activation [18]. Interestingly, polymorphisms that increase IL10 production are associated with elevated GC risk [19–21], which is contrary to the hypothesis that IL10 may prevent inflammation-induced disruption of the epithelium. Also, there is evidence that IL10 can promote the activation and expansion of tumor-resident CD8 T cells in murine tumor studies [22,23], which is in contrast with the common notion that IL10 inhibited CD8 T cell responses.

In this study, we investigated the endogenous production of IL10 by $CD8^+$ T cells in GC patients. Interestingly, we found that CD8 T cells that produced cytokines could be separated into two distinctive subsets, the $IFNg^+IL10^-$ single positive type and the $IFNg^+IL10^+$ double positive type, the latter being more enriched in GC patients. These two subsets were not only different in cytokine production, but also different in the expression of surface markers, exhaustion markers, and proliferation and survival capacity.

2. Materials and methods

2.1. Study participants

This study included 26 patients with primary gastric adenocarcinoma, including 14 stage II and 12 stage III patients as defined by the American Joint Committee on Cancer [24]. All the patients were *H. pylori* infected. A total of 26 age- and sex-matched healthy volunteers were recruited as controls. The demographic data of patients and controls are presented at Table 1. The exclusion criteria were age outside of the 18 to 65 years range, familial history of GC, previous or ongoing chemotherapy and radiation therapy, autoimmune conditions, and other malignancies. All patients and controls donated 50 to 100 mL of peripheral blood samples, from which peripheral blood mononuclear cells (PBMCs) were harvested by Ficoll density gradient centrifugation. The diagnosis, sample collection, and experimentation were performed at the No. 960 Hospital, following approval by the Ethics Committee of No. 960 Hospital. All participants provided written informed consent.

2.2. $CD8^+$ T cell stimulation

CD8 T cells were isolated from total PBMCs via negative magnetic selection using EasySep Human $CD8^+$ T Cell Enrichment Kit (Stemcell). Purity, assessed by staining of $CD3^+CD8^+$ cells, was consistently above 97%. The CD8 T cells were then incubated in RPMI 1640 supplemented with L-glutamine, penicillin-streptomycin, and 10% fetal bovine serum (all from Gibco). For stimulation, Human T activator (CD3/CD28) beads (Gibco) were added at 2 beads per cell, and IL2 (Gibco) was supplemented at 50U/mL. The medium and IL2 were replaced every 3 days. At time points specified in the experiments, centrifugation was performed at 300 *g* for 5 min to separate the cells and the supernatant.

Table 1
Demographic data of the study participants.

	Control	Patient (Stage II)	Patient (Stage III)
N	26	14	12
Age, mean ± SD (year)	54 ± 7.7	52 ± 5.4	55 ± 4.8
Female, N (%)	11 (42)	5 (36)	4 (33)
Smoking, N (%)	8 (31)	4 (29)	4 (33)
Alcohol, N (%) Never	14 (54)	6 (43)	4 (33)
Moderate	7 (27)	4 (29)	5 (42)
Heavy	5 (19)	4 (29)	3 (25)

2.3. Surface and intracellular staining

Violet Dead Cell Stain (Invitrogen) and different combinations of surface anti-human staining antibodies (BioLegend), including CD3, CD8, PD1, TIM3, KLRG1, CXCR5, and CD45RO, were added for 30 min on ice. The cells were then washed twice by centrifugation at 300*g* for 5 min, and permeabilized using CytoFix/CytoPerm (BD) for 15 min on ice. After washing, intracellular staining antibodies (BioLegend), including IFNg and IL10, were added for 30 min on ice in Perm Wash Buffer (BD). Samples were washed twice and acquired in BD LSR instrument. For each experiment, 5×10^4 lymphocytes at minimum were collected.

2.4. Cytokine quantification

IFNg and IL10 concentration was measured using respective Human ELISA Kits (Invitrogen) according to the manufacturer's instructions. Standard curves were established using pure cytokine samples included in the kits.

2.5. Thymidine incorporation assay

CD8 T cells after magnetic negative selection were incubated with anti-human CD45RO and CXCR5 for 30 min on ice. The $CD45RO^+CXCR5^+$ CD8 T cells and $CD45RO^+CXCR5^-$ CD8 T cells were sorted using FACSAria instrument (BD). Following stimulation, cells were plated at 1×10^4 cells per well in a 96-well plate, and were incubated with tritiated thymidine (0.1 μCi/mL; Amersham Biosciences) for 6 hours, and the radioactivity was counted in a liquid scintillation counter.

2.6. Statistical analysis

Data were given as scatterplots or mean ± SD. Distribution was examined using D'Agostino-Pearson test. Differences between groups were examined using tests specified in the figure legend. Two-tailed $p < 0.05$ was considered significant.

3. Results

3.1. Gastric cancer patients presented an enrichment of IFNg/IL10-double positive CD8 T cells

We recruited 26 GC patients and 26 healthy controls. Circulating CD8 T cells were isolated from total PBMCs using magnetic sorting. The isolated CD8 T cells were then treated with anti-CD3/CD28 and IL2 to stimulate cytokine production. Using intracellular staining, we identified IFNg-expressing and IL10-expressing CD8 T cells in these subjects (Fig. 1A). The frequency of IFNg-expressing IL10-nonexpressing ($IFNg^+IL10^-$) CD8 T cells was at 12.9 ± 0.5% (mean ± SEM) in healthy controls and significantly lower at 8.7 ± 0.4% in GC patients (Fig. 1B). The frequency of IFNg-expressing and IL10-expressing ($IFNg^+IL10^+$) CD8 T cells, on the other hand, was at 1.2 ± 0.1% in healthy controls and markedly higher at 3.8 ± 0.3% in GC patients (Fig. 1C). The frequency of total IFNg-expressing ($IFNg^+IL10^-$ plus $IFNg^+IL10^+$) CD8 T cells was at 14.2 ± 0.5% in healthy controls and slightly lower at 12.5 ± 0.4% in GC patients (Fig. 1D). Overall, these data demonstrated that GC patients presented an upregulation of the

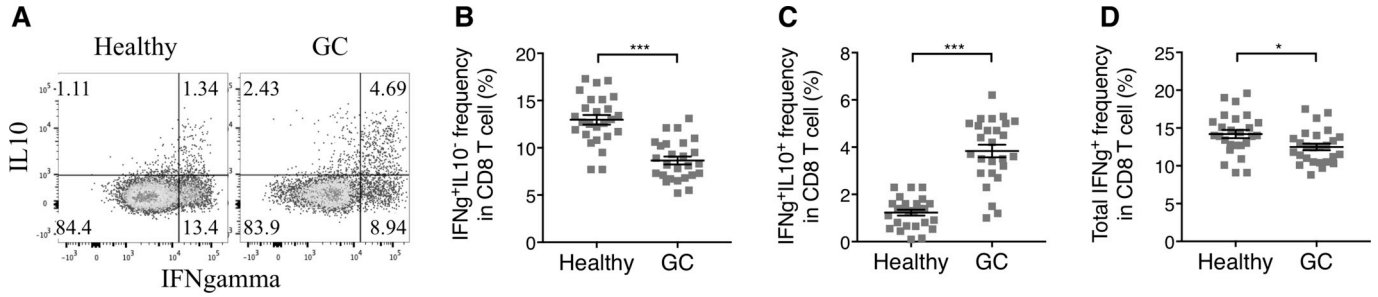


Fig. 1. IFNg-expressing and IL10-expressing CD8 T cells in GC patients compared to healthy controls.(A) Expression of IFNg and IL10 in circulating CD8 T cells. Fresh circulating CD8 T cells were stimulated using anti-CD3/CD28 beads and IL2 for 24 hours, and the exocytosis of the final 5 hours was inhibited by brefeldin A and monensin. Intracellular staining was then performed. One representative from the healthy group and the GC group was shown. (B) The frequencies of $IFNg^+IL10^-$ cells in CD8 T cells from 26 healthy controls and 26 GC patients. (C) The frequencies of $IFNg^+IL10^+$ cells in CD8 T cells from 26 healthy controls and 26 GC patients. (D) Total $IFNg^+$ cell ($IFNg^+IL10^-$ plus $IFNg^+IL10^+$) frequency in CD8 T cells from 26 healthy controls and 26 GC patients. (B to D) Student's *t*-test. *p < 0.05. ***p < 0.001.

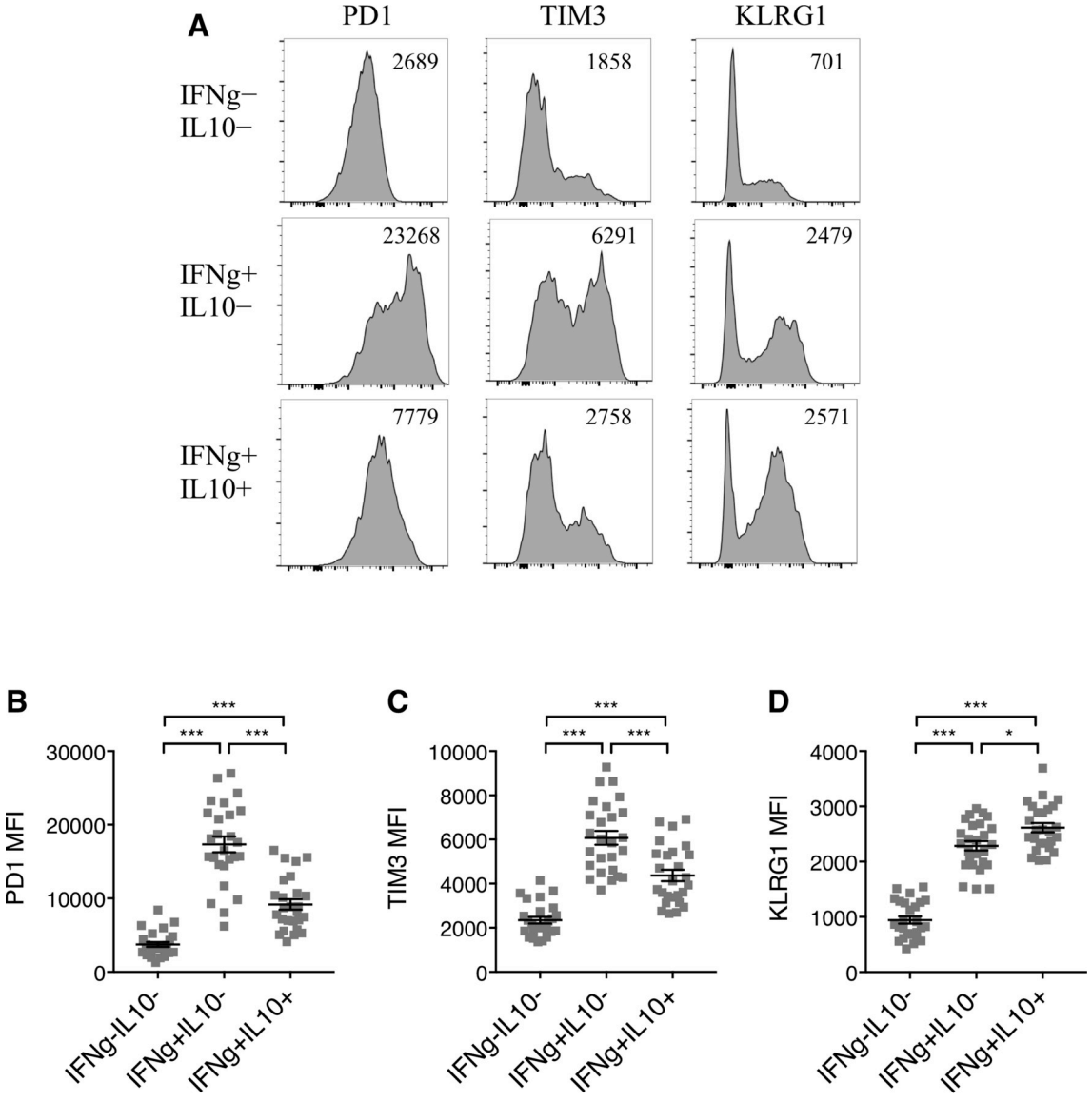


Fig. 2. Inhibitory marker expression by $IFNg^-IL10^-$, $IFNg^+IL10^-$, and $IFNg^+IL10^+$ CD8 T cells. (A) Expression of PD1, TIM3, and KLRG1 by pre-gated $IFNg^-IL10^-$, $IFNg^+IL10^-$, and $IFNg^+IL10^+$ CD8 T cells from one representative GC individual. Numbers represent the mean fluorescence intensity (MFI). (B) The MFI of PD1 in $IFNg^-IL10^-$, $IFNg^+IL10^-$, and $IFNg^+IL10^+$ CD8 T cells from 26 GC patients. (C) The MFI of TIM3 in $IFNg^-IL10^-$, $IFNg^+IL10^-$, and $IFNg^+IL10^+$ CD8 T cells from 26 GC patients. (D) The MFI of KLRG1 in $IFNg^-IL10^-$, $IFNg^+IL10^-$, and $IFNg^+IL10^+$ CD8 T cells from 26 GC patients. (B to D) ANOVA followed by Tukey's multiple comparisons. ***p < 0.001.

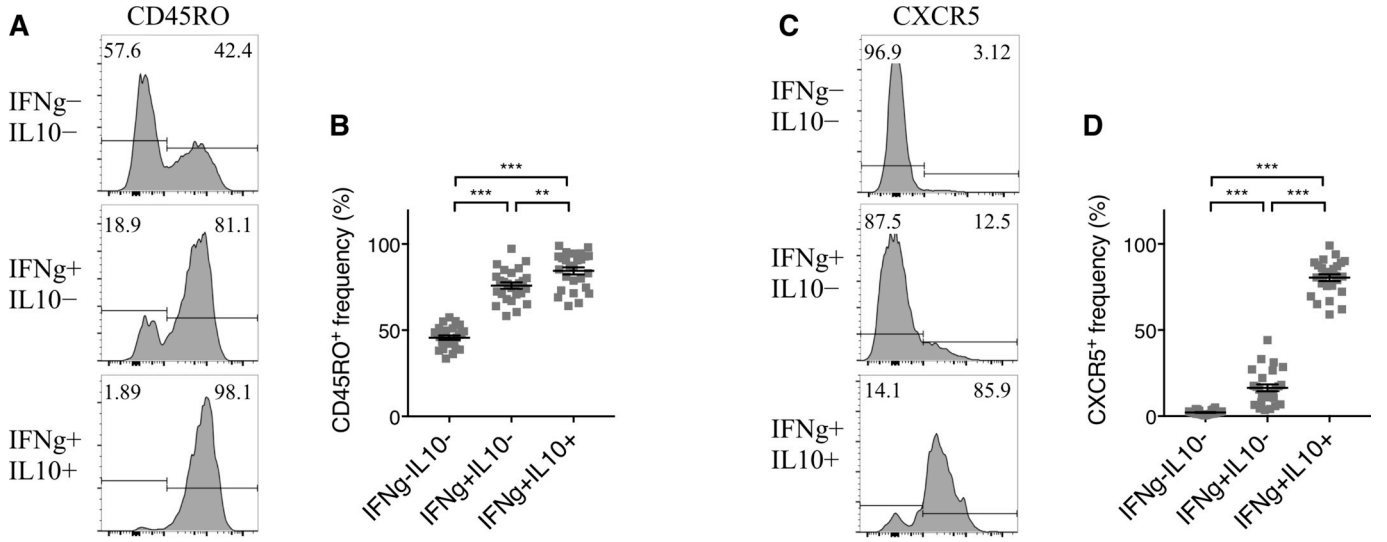


Fig. 3. CD45RO and CXCR5 expression by IFNg$^-$IL10$^-$, IFNg$^+$IL10$^-$, and IFNg$^+$IL10$^+$ CD8 T cells. (A) CD45RO expression by pre-gated IFNg$^-$IL10$^-$, IFNg$^+$IL10$^-$, and IFNg$^+$IL10$^+$ CD8 T cells from one representative GC individual. (B) The frequency of CD45RO$^+$ cells in IFNg$^-$IL10$^-$, IFNg$^+$IL10$^-$, and IFNg$^+$IL10$^+$ CD8 T cells from 26 GC patients. (C) CXCR5 expression by pre-gated IFNg$^-$IL10$^-$, IFNg$^+$IL10$^-$, and IFNg$^+$IL10$^+$ CD8 T cells from one representative GC individual. (D) The frequency of CXCR5$^+$ cells in IFNg$^-$IL10$^-$, IFNg$^+$IL10$^-$, and IFNg$^+$IL10$^+$ CD8 T cells from 26 GC patients. (B and D) ANOVA followed by Tukey's multiple comparisons. **p < 0.01. ***p < 0.001.

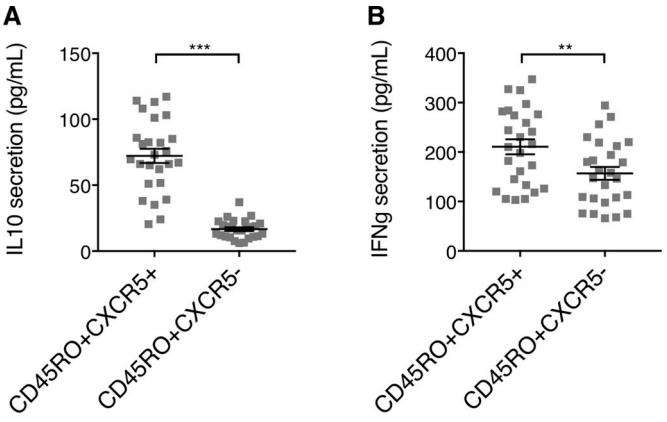


Fig. 4. IL10 and IFNg expression by sorted CD8$^+$CD45RO$^+$CXCR5$^+$ and CD8$^+$CD45RO$^+$CXCR5$^-$ T cells. (A) IL10 production by CD8$^+$CD45RO$^+$CXCR5$^+$ and CD8$^+$CD45RO$^+$CXCR5$^-$ T cells following 24-h stimulation using anti-CD3/CD28 beads and IL2. (B) IFNg production by CD8$^+$CD45RO$^+$CXCR5$^+$ and CD8$^+$CD45RO$^+$CXCR5$^-$ T cells following 24-h stimulation using anti-CD3/CD28 beads and IL2. Student's *t*-test. **p < 0.01. ***p < 0.001.

IFNg/IL10-double positive CD8 T cells, a downregulation of IFNg-single positive CD8 T cells, and a downregulation of IFNg-expressing CD8 T cells overall.

3.2. Characteristics of IFNg/IL10-double positive CD8 T cells

To characterize the IFNg/IL10-double positive CD8 T cell population in GC patients, we investigated the surface marker expression of these cells. First, the expression of inhibitory markers, including PD-1, Tim-3, and KLRG1, were examined by flow cytometry (Fig. 2A). Compared to the CD8 T cells that did not express IFNg or IL10 (IFNg$^-$IL10$^-$), the IFNg$^+$IL10$^-$ CD8 T cells presented significantly higher PD1 and TIM3 expression (Fig. 2B and C). Interestingly, the IFNg$^+$IL10$^+$ CD8 T cells presented significantly higher PD1 and TIM3 expression than IFNg$^-$IL10$^-$ cells and significantly lower PD1 and TIM3 expression than IFNg$^+$IL10$^-$ cells. The expression of KLRG1 was also higher in the IFNg$^+$IL10$^-$ CD8 T cells than in the IFNg$^-$IL10$^-$ CD8 T cells (Fig. 2D). The IFNg$^+$IL10$^+$ CD8 T cells presented slightly higher KLRG1 than the IFNg$^+$IL10$^-$ CD8 T cells.

The PD1, TIM3, and KLRG1 expression pattern by IFNg$^+$IL10$^+$ CD8 T cells strongly resembled the expression pattern by CXCR5$^+$CD8$^+$ T follicular cytotoxic (Tfc) cells, previously characterized in chronic LCMV infection [25]. Hence, the expression of CD45RO and CXCR5 were examined (Fig. 3A and C). On average, more than half of IFNg$^-$IL10$^-$ CD8 T cells were CD45RO-negative (Fig. 3B). The IFNg$^+$IL10$^-$ and the IFNg$^+$IL10$^+$ CD8 T cells, on the other hand, were predominantly CD45RO-positive, and the frequency of CD45RO$^+$ cells in IFNg$^+$IL10$^+$ CD8 T cells was significantly higher than that in IFNg$^+$IL10$^-$ CD8 T cells (Fig. 3B). The frequency of CXCR5-positive was lower than 5.0% in IFNg$^-$IL10$^-$ CD8 T cells from 26 GC patients (Fig. 3D). In IFNg$^+$IL10$^-$ CD8 T cells, however, 16.4% on average were CXCR5-positive. Remarkably, in IFNg$^+$IL10$^+$ CD8 T cells, 80.4% cells on average were CXCR5-positive.

Subsequently, we sorted total CD8 T cells into CD45RO$^+$CXCR5$^+$ CD8 T cells and CD45RO$^+$CXCR5$^-$ CD8 T cells. Each subset was then treated in vitro with anti-CD3/CD28 and IL-2 for 24 hours. The IFNg and IL10 production was measured using a multiplex assay. The CD45RO$^+$CXCR5$^+$ CD8 T cells and the CD45RO$^+$CXCR5$^-$ CD8 T cells displayed markedly different capacity in IL10 production, with the former expressing high levels of IL10, while the latter expressed only minimally detectable IL10 (Fig. 4A). Both the CD45RO$^+$CXCR5$^+$ CD8 T cells and the CD45RO$^+$CXCR5$^-$ CD8 T cells readily expressed IFNg, with the CD45RO$^+$CXCR5$^+$ CD8 T cells being more potent (Fig. 4B).

Together, these data demonstrated that the IFNg$^+$IL10$^+$ CD8 T cells displayed lower PD1 and TIM3 than the IFNg$^+$IL10$^-$ CD8 T cells, and could be enriched in the CD45RO$^+$CXCR5$^+$ CD8 T cell fraction.

IFNg$^+$IL10$^+$ CD8 T cells were enriched during prolonged stimulation.

To examine the dynamics of IFNg and IL10 expression following extended stimulation, the CD8 T cells were sorted from PBMCs and stimulated with anti-CD3/CD28 and IL-2 for a total of 12 days, and the frequencies of IFNg$^+$IL10$^-$ and IFNg$^+$IL10$^+$ CD8 T cells were examined on day 1, day 3, day 6, day 9, and day 12 of stimulation (Fig. 5A). The frequency of IFNg$^+$IL10$^-$ CD8 T cells increased from day 1 to day 6, then decreased from day 6 to day 12. The frequency of IFNg$^+$IL10$^-$ CD8 T cells, on the other hand, was increasing continuously until day 12. From day 1 to day 6, the frequency of IFNg$^+$IL10$^-$ cells was significantly

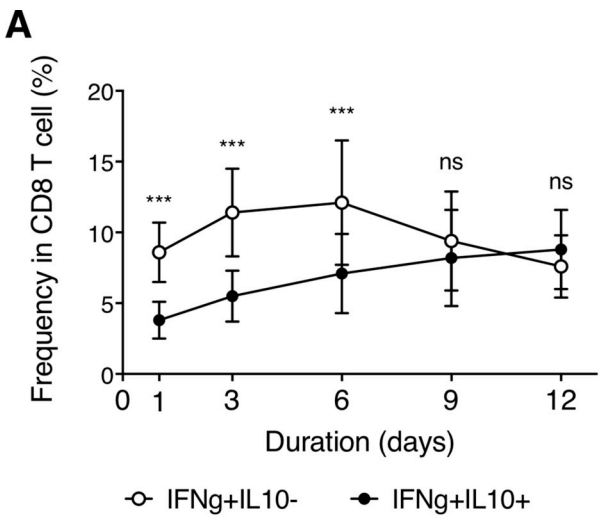
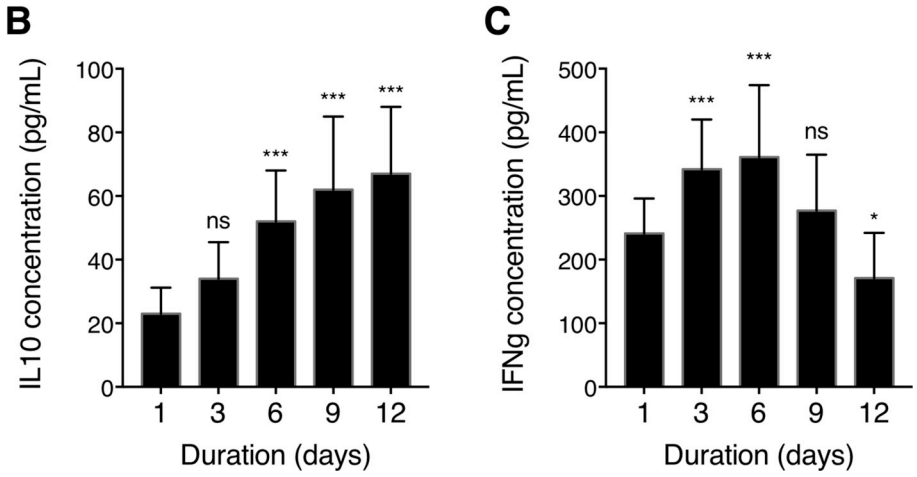


Fig. 5. IL10 and IFNg expression during extended stimulation. (A) CD8 T cells were stimulated with anti-CD3/CD28 beads and IL2 for 1 to 12 days. For each time point, exocytosis of the final 5 hours was inhibited by brefeldin A and monensin. The cells were then harvested and the IFNg and IL10 expression was examined using intracellular staining. Two-way ANOVA followed by Sidak's multiple comparisons. (B) The concentration of IL10 in the supernatant at each time point. (C) The concentration of IFNg in the supernatant at each time point. (B and C) One-way ANOVA followed by Dunnett's multiple comparisons. ns: not significant. *p < 0.05. ***p < 0.001.



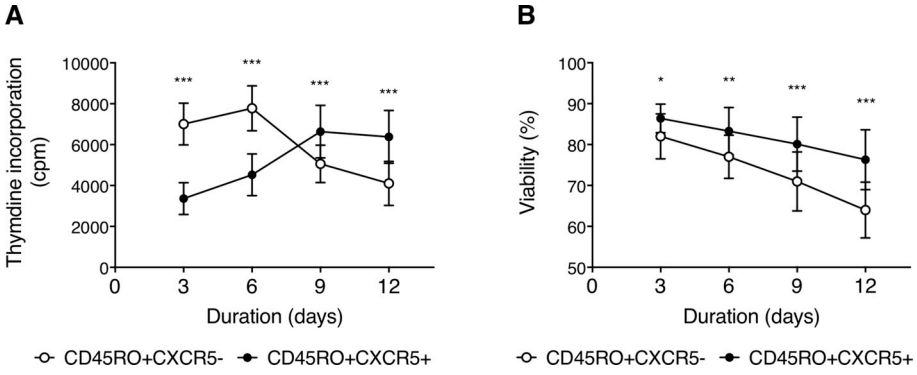


Fig. 6. Proliferation and viability of $CD45RO^+CXCR5^+$ and $CD45RO^+CXCR5^-$ CD8 T cells. $CD8^+CD45RO^+CXCR5^+$ and $CD8^+CD45RO^+CXCR5^-$ T cells were stimulated using anti-CD3/CD28 beads and IL2. Every 3 days, a portion of the cells were harvested to determine proliferation capacity and viability. (A) Radioactive thymidine incorporation of $CD8^+CD45RO^+CXCR5^+$ and $CD8^+CD45RO^+CXCR5^-$ T cells after 6-h pulsing with tritiated thymidine. (B) Viability of $CD8^+CD45RO^+CXCR5^+$ and $CD8^+CD45RO^+CXCR5^-$ T cells, measured using Violet Dead Cell Stain. (A and B) Two-way ANOVA followed by Sidak's multiple comparisons. *p < 0.05. **p < 0.01. ***p < 0.001.

higher than the frequency of $IFNg^+IL10^+$ cells. On day 9 or day 12, however, no significant differences were observed.

In the supernatant, we detected the cytokine concentration in the supernatant harvested at each time point. IL10 concentration presented an increasing trend, and compared to the concentration at day 1, the concentrations at day 6 to day 12 were significantly higher (Fig. 5B). IFNg, on the other hand, increased initially and decreased after day 6. Compared to the IFNg concentration on day 1, the IFNg concentrations were higher on day 3 and day 6, comparable on day 9, and lower on day 12 (Fig. 5C).

$CD45RO^+CXCR5^+$ CD8 T cells presented better proliferation and lower death than $CD45RO^+CXCR5^-$ CD8 T cells.

To assess the proliferation capacity of $IFNg^+IL10^-$ and $IFNg^+IL10^+$ CD8 T cells, we took advantage of the observation that $IFNg^+IL10^+$ CD8 T cells were enriched in $CD45RO^+CXCR5^+$ cells. The total CD8 T cells were sorted into $CD45RO^+CXCR5^+$ and $CD45RO^+CXCR5^-$ subsets. Each subset was then stimulated with anti-CD3/CD28 and IL2 in vitro, and the proliferation capacity was examined using thymidine incorporation assay (Fig. 6A). The proliferation capacity of $CD45RO^+CXCR5^-$ CD8 T cells was significantly higher than that of

$CD45RO^+CXCR5^+$ CD8 T cells on day 3 and day 6, but quickly declined on day 9 and day 12, during which the proliferation capacity of $CD45RO^+CXCR5^+$ CD8 T cells continued to increase and exceeded that of $CD45RO^+CXCR5^-$ CD8 T cells.

The viability of $CD45RO^+CXCR5^+$ and $CD45RO^+CXCR5^-$ CD8 T cells was then examined using flow cytometry. Throughout the course of stimulation, the $CD45RO^+CXCR5^+$ CD8 T cells presented higher viability than the $CD45RO^+CXCR5^-$ CD8 T cells (Fig. 6B).

4. Discussion

IL10 is generally regarded as a broad-spectrum regulatory cytokine that dampens inflammatory responses, including those mediated by CD8 T cells [17,18]. In GC patients, IL10 expression level is positively associated with the density of microvessels and negatively associated with CD8 T cell infiltration in the tumor [26]. However, the appearance of IL10 is often accompanied by inhibitory immune cells, such as regulatory B cells, regulatory dendritic cells, M2-polarized macrophages, and tumor-associated macrophages [27–29]. Hence, some of the IL10-mediated suppressive effects, previously described in other studies, might have required the coexistence of other inhibitory molecules and cell types. Indeed, IL10 in IL10 receptor (IL10R)-expressing CD8 T cells has been shown to strongly induce the phosphorylation of STAT3 and STAT1 and promote the in situ proliferation of tumor-resident CD8 T cells [22,23]. In addition, in a coronavirus-infected murine encephalitis model, CD8 T cells that produced IL10 displayed higher cytokine and chemokine expression and stronger cytolytic activity [30].

In this study, we investigated CD8 T cells that expressed IL10 endogenously. In both healthy controls and GC patients, CD8 T cells that expressed IL10 could be observed following anti-CD3/CD28 and IL2 stimulation. Interestingly, IL10-single positive cells were rare, and the majority of IL10-expressing cells also presented concurrent IFNg expression. GC patients had more IFNg/IL10-double positive CD8 T cells than healthy controls, while healthy controls had more IFNg-single positive CD8 T cells than GC patients, which seemed to suggest that the IFNg/IL10-double positive CD8 T cell population arose due to disease. We later found that prolonged stimulation alone could result in higher levels of IFNg/IL10-double positive cells and lower levels of IFNg-single positive cells. Hence, it is possible that CD8 T cells in GC patients are continuously being challenged with tumor antigens and/or *H. pylori* antigens, resulting in prolonged activation and higher frequencies of IFNg/IL10-double positive cells. In the future, the antigen specificity of IFNg/IL10-double positive CD8 T cells should be investigated.

A major limitation of this study is that we have not conclusively demonstrated the function of IFNg/IL10-double positive CD8 T cells in gastric cancer patients. We found that certain features of the IFNg/IL10-double positive CD8 T cell subset, including lower PD1, lower TIM3, and higher KLRG1, resembled Tfc cells [25]. Indeed, we found that the IFNg/IL10-double positive cells were highly enriched in $CD45RO^+CXCR5^+$ CD8 T cells, while IFNg-single positive cells were enriched in $CD45RO^+CXCR5^-$ CD8 T cells. This feature would help us demonstrate the role of IFNg/IL10-double positive CD8 T cells in animal models using deletions and adoptive transfers. In past research, Tfc cells were shown to present stronger proliferation in response to PD1 blockade in exhausted CD8 T cells [31], suggesting that they might be candidates of antitumor immunotherapy. However, regulatory functions also have been demonstrated in IL10-expressing CD8 T cells. For example, IL4 and IL12-induced IL10-expressing CD8 T cells was shown to inhibit graft-versus-host disease *in vivo*, and suppressed naive and effector T cell activation and IgG/IgE production [32]. Interestingly, CD8 T cell-mediated IL10 was shown to resolve chemotherapy-induced neuropathic pain [33]. Hence, IL10 expression might serve multiple roles in CD8 T cells, and the function of IFNg/IL10-double positive CD8 T cells should be further investigated using animal models.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexcr.2019.06.032.

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