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Expression of CD27, CD28 and IL-17A in peripheral blood from patients with colorectal carcinoma

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Abstract. – OBJECTIVE: To compare the different expressions of CD27, CD28, IL-17A, IFN-γ and TNF-α in the peripheral blood sampled from patients with colorectal carcinoma and healthy volunteers.

PATIENTS AND METHODS: Vδ2 T cells were isolated from the peripheral blood mononuclear cells (PBMCs) of patients with the colorectal carcinoma (CRC, n = 30) and healthy controls (HC, n = 21). The proportion of CD27, CD28, IL-17A, IFN-γ and TNF-α of Vδ2 T cells was detected by the flow cytometry.

RESULTS: We found that the proportion of IL-17A of Vδ2 T cells in PBMCs was higher in the CRC vs. the HC group ($p < 0.05$). A significant positive correlation was observed between the expression of IFN-γ and TNF-α of Vδ2 T cells. In the CRC patients, the proportions of IL-17A of CD27- Vδ2 T cells and CD28+ Vδ2 T cells were higher than those of CD27+ Vδ2 T cells and CD28- Vδ2 T cells, whereas the expression of IFN-γ and TNF-α of CD27-Vδ2 T cells was lower than that of CD27+ Vδ2 T cells.

CONCLUSIONS: Vδ2 T cells from PBMCs had higher expression of IL-17A in CRC patients than that in the HC group. The expression of IFN-γ and TNF-α of Vδ2 T cells from PBMCs was positively correlated. The cytokine profiles of peripheral Vδ2 T cells were likely determined by a CD27 and CD28 involving mechanism.

Key Words:
Colorectal carcinoma, γδ T cells, CD27, CD28, IL-17A.

Abbreviations

CRC = colorectal carcinoma; HC = healthy control; PBMC = peripheral blood mononuclear cell; FCM = Flow cytometry method; EST = early stage of tumor; LST = late stage of tumor; LGD = low grading of dysplasia; MGD = moderate grading of dysplasia; HGD = high grading of dysplasia.

Introduction

According to the different expressions of T cell receptor (TCR), T cells can be divided into αβ T cells, NKT cells and γδ T cells. The Vδ2 chain of TCR co-exists with Vγ9 chain, while most of γδ T cells in peripheral circulating blood are Vγ9Vδ2 T cells. γδT cells play an important role in body's innate immunity. They can produce a variety of pro-inflammatory cytokines and chemokines, infiltrate tumor tissues, destroy tumor cells, identify and kill a variety of tumor cells through various mechanisms[1-3]. Previous reports[4-6] have demonstrated a significant anti-tumor effect of γδT cells and phase I clinical trials have been carried out to evaluate the feasibility of γδT cells as an adoptive immunotherapy. There have also been *in vitro* and *in vivo* studies on the immune killing effect of γδT cells on colorectal cancer cells[7-9].

IL-17A is an important pro-inflammatory cytokine, mainly secreted by Th17 cells. IL-1β, IL-6, IL-23A, TGF-β and other factors can stimulate its secretion[10,11], and γδT cells are also an important source of IL-17A *in vivo*[12,13]. Currently, the specific role of IL-17 + γδT cells in tumor growth remains controversial[14-16]. Some studies showed that γδT cells can promote neutrophil aggregation in tumor lesions and inhibit tumor growth[15]. For example, during the antineoplastic chemotherapy, early invasion of IL-17 + γδT cells in the tumor bed along with high expression of IL-17A can inhibit tumor growth[16]. Other studies found that IL-17A expression of tumor-infiltrating γδT cells may promote tumor angiogenesis, thereby, promoting tumor growth[14].

IL-17A may also be involved in the development of human colorectal cancer[17-19]. Le Gouvello et al[17] reported an increased in IL-17A expression in human colorectal cancer tissues. It was

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found that gene expression was associated with multiple mismatch repair genes (MSS) in patients with colorectal cancer, and higher IL-17A expression was evidenced in patients with colorectal cancer than those with other types of cancers. Moreover, the gene defects such as K-Ras, PIK3CA and TP53 may lead to intestinal barrier dysfunction. Intestinal microbes in the tumor microenvironment may induce expression of IL-17A and other factors, thus, further promote the deterioration of straight adenocarcinoma[19].

In this study, we examined Vδ2 T cells accounted for the proportion of peripheral lymphocytes and its surface CD27, CD28 expression in human peripheral blood from healthy volunteers and patients with colorectal cancer. The content of IL-17A, IFN-γ and TNF-α expression level in Vδ2 T cells was also assessed. Results from our work may provide new understanding of γδ T cells subgrouping and inflammatory cytokines expression in Vδ2 T cells in colorectal cancer patients.

Patients and Methods

Patients

In this study, thirty patients who were diagnosed with colorectal carcinoma (CRC) and underwent treatment at the Affiliated Hospital of Xuzhou Medical College were enrolled between April 2013 and September 2013. This CRC group comprised 22 males and 8 females, aged 24-83 years old (median age, 58 years old). All of these patients had confirmed malignant adenoma by histology. They had no history of autoimmune disease or acute infection, and were not under treatment by chemotherapy, radiotherapy, immunotherapy or other therapies that may influence the immune system. Twenty-one healthy volunteers who had never suffered from malignancies or bacterial infections were recruited as healthy controls (HC). This control group composed of 14 males and 7 females, aged 27-68 years (median age, 52 years old). The general characteristics of the participants in this study were summarized in Table I. Our work was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical College. Written informed consent was obtained from each subject prior to the procedure of blood sampling.

Isolation of PBMCs and FACS staining

Human peripheral blood mononuclear cells (PBMCs) were isolated by lymphocyte separation medium. The following monoclonal antibodies were used: FITC Mouse Anti-Human Vδ2 TCR (clone B6), APC Mouse anti-Human CD27 (clone M-T271), PerCP-Cy5.5 Mouse Anti-Human CD28 (clone CD28.2), PE Mouse anti-Human IL-17A (clone SCPL1362), PE Mouse anti-Human IFN-γ (clone 4S.B3), and PE Mouse anti-Human TNF (clone Mab11). All Abs used in the present study were purchased from BD Biosciences (Franklin Lakes, NJ, USA). Four-color flow cytometries were performed using FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Flow Cytometric Detection of Intracellular Cytokines

Blood samples were obtained from patients with colorectal carcinoma and healthy donors. The staining procedure was performed according to the instructions provided by the manufacturer. PBMCs were plated at 1×10^9 cells/l, 200 μl/well in 96-well plates, and cultured in RPMI-1640 culture medium containing 10% fetal bovine serum in the presence of Brefeldin A (Leukocyte Activation Cocktail, with BD GolgiPlug, BD Biosciences) at 37°C, in 5% CO_2 for 5 h. After stimulation, the cells were harvested and stained with FITC Mouse Anti-Human Vδ2 TCR, APC Mouse anti-Human CD27 and PerCP-Cy 5.5 Mouse Anti-Human CD28. After incubation for 15 min in the dark at 37°C, FIX & PERM A (AN DER GRUB Company, Bio Research Gmbh, Wien, Austria) was added to the media. The cells were incubated in the dark for another 15 min, then washed twice with PBS, and centrifuged for 5 min at 2000 rpm. After FIX & PERM B (AN

Table I. Characteristics of participants recruited in this study.

Group	CRC	HC
N	30	21
Age (median, range)	58 (24-83)	52 (27-68)
Males	22	14
Females	8	8
Tumor stage		
I-II	3-13	N/A
III-IV	10-4	N/A
Dysplasia		
LGD	11	N/A
MGD	17	N/A
HGD	2	N/A

Note: CRC, colorectal carcinoma; HC, healthy controls; LGD, low grading of dysplasia; MGD, moderate grading of dysplasia; HGD, high grading of dysplasia.

DER GRUB Company) was added, the cells were then stained with PE Mouse anti-Human IL-17A, PE Mouse anti-Human IFN-γ and PE Mouse anti-Human TNF, separately. After fixation, the cells were incubated in the dark for 15 min, washed twice with PBS, and centrifuged for 5 min at 2000 rpm. The proportion of cytokine-producing Vδ2 T cells was determined by four-color cytometry. A total of 100000-150000 events were acquired for each sample and analyzed using FlowJo 7.6.1 (TreeStar, Ashland, OR, USA).

Statistical Analysis

All data were presented as means ± SEM. Differences between the groups were analyzed by using the Kruskal-Wallis test (non-parametric, two-tailed, and unpaired). The difference within each group was tested using a nonparametric Mann-Whitney U test. Spearman's rank correlation test was used to examine the correlation between two variables. A p-value < 0.05 was considered significant.

Results

Expression of Vδ2 T Cells and Cytokines in the CRC Tumor Group and the Control group

Our results showed that the proportion of Vδ2 T cells was similar in the CRC and the HC groups (Figure 1). However, the expression of IL-17A was higher in the CRC group than in the control group (5.99 ± 0.80% *vs.* 3.48 ± 0.57%, p = 0.012) (Figure 2). A significant increase in the expression of these markers was detected for male versus female patients (5.96 ± 0.85% *vs.*

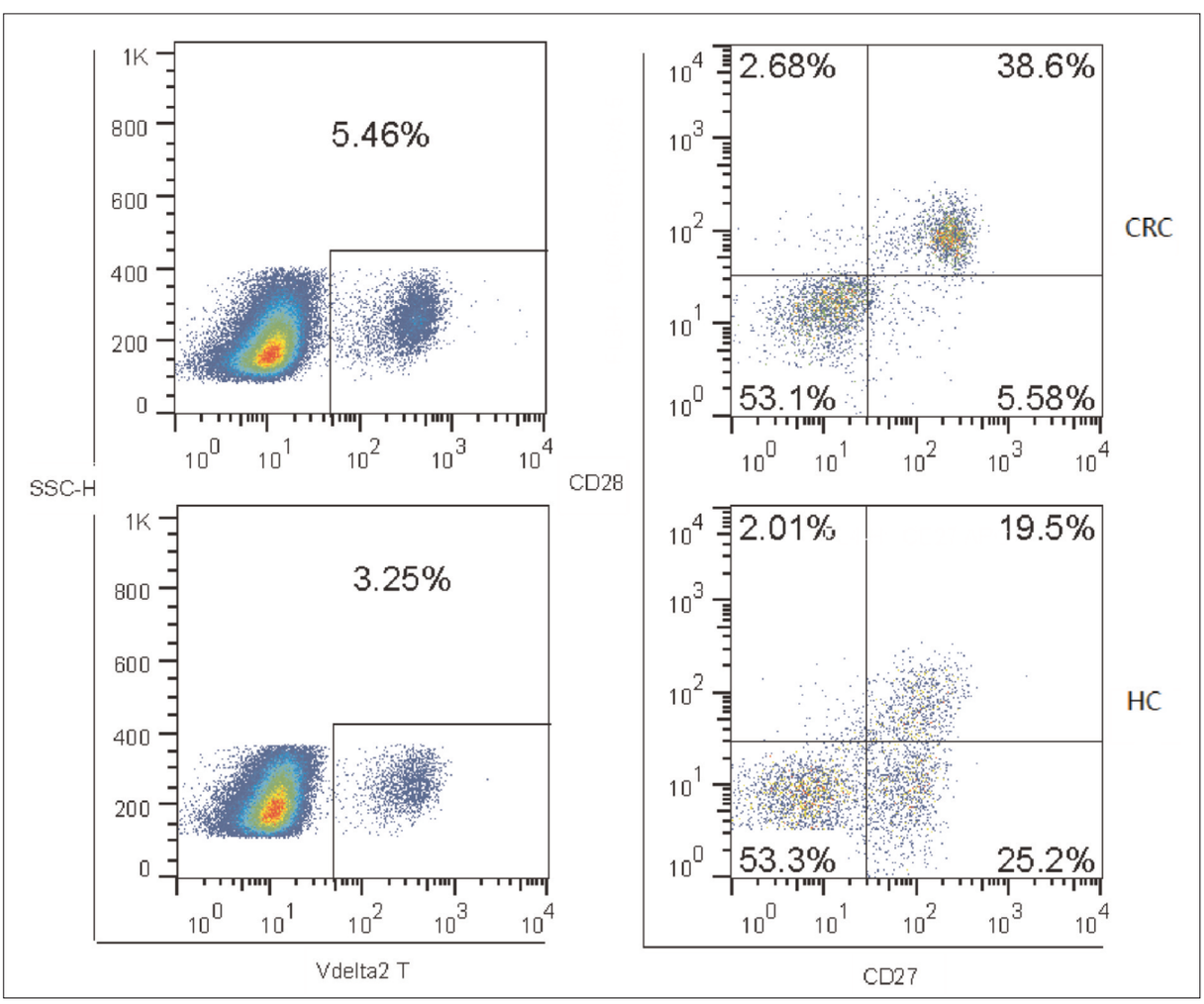

Figure 1. Cells flow chart of Vδ2 T cells and CD27, CD28 Vδ2 T cells in the CRC and the HC groups.

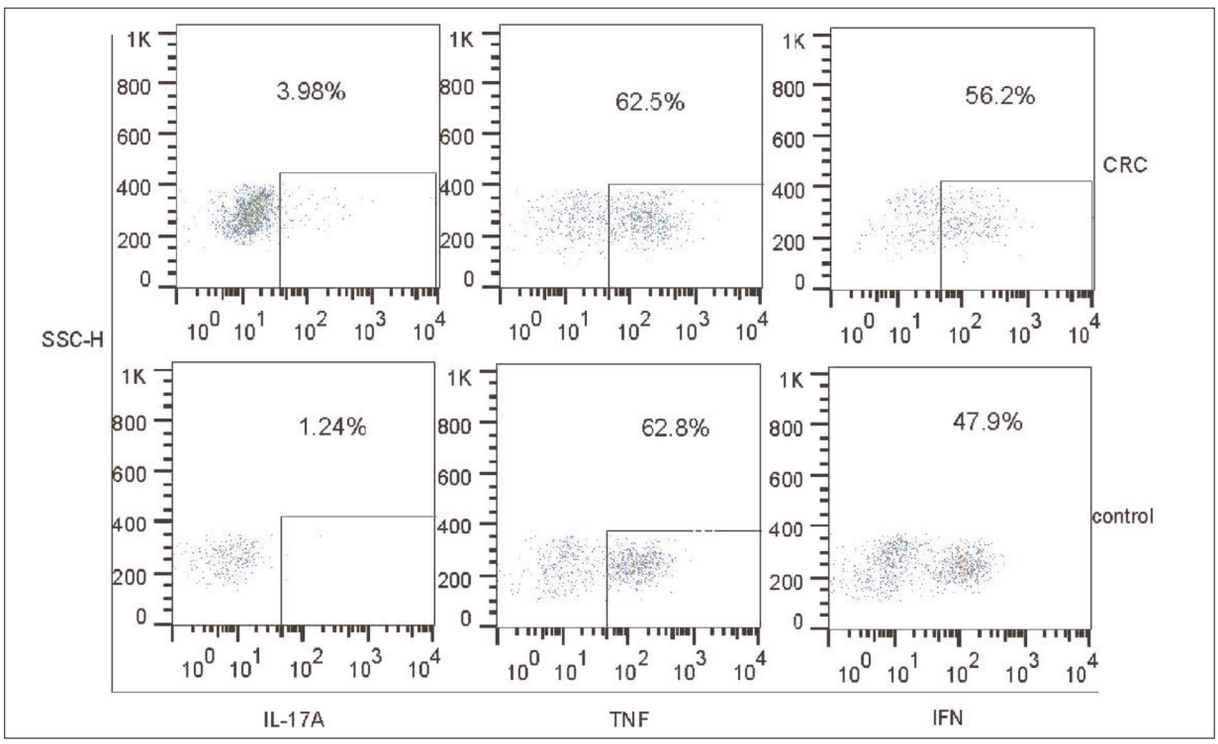

Figure 2. Comparison of Vδ2 T cells flow chart of IL-17A, TNF-α and IFN-γ expression in the CRC and the HC groups.

3.15 ± 0.73%, $p = 0.005$) (Table II). Strong positive correlations were observed between the expression of IFN-γ and TNF-α in both the CRC and the HC groups ($p < 0.001$ and $p = 0.003$, respectively). Significant correlation between the expression of IFN-γ and IL-17A was not evidenced in our study (Figure 3).

Expression of Vδ2 T Cells and Other Cytokines in Outer Periphery in the CRC Patients at Different Tumor Staging or Differentiation

Our results showed no difference in the Vδ2 T cells proportion in PBMC from patients with colorectal cancer at the early stage (I/II) and the advanced stage (III/IV). Figure 4 also shows similar expression of IL-17A, IFN-γ and TNF-α, and similar proportion of Vδ2 T cells and other factors in these patients with poor or moderate differentiation.

Comparison of the Expression of Various Cytokines in CD27+Vδ2 T Cells and CD27-Vδ2 T Cells Between the Tumor and the Control Groups

In the CRC group, the expression of IL-17A was significantly higher in CD27-Vδ2 T cells than that in CD27 + Vδ2 T cells (4.11 ± 0.59%

Table II. The proportion of peripheral Vδ2 T cells in the CRC and the HC groups and the proportion of cells expressed IL-17A, IFN-γ and TNF-α (mean ± SEM).

Group	Vδ2 T	IL-17A	IFN-γ	TNF-α
CRC (n = 30)	2.24 ± 0.55	5.99 ± 0.80*	50.81 ± 3.71	62.00 ± 3.91
Male (n = 22)	2.65 ± 0.72	5.96 ± 0.85**	50.57 ± 4.16	62.85 ± 2.46
Female (n = 8)	1.12 ± 0.25	6.06 ± 1.83	51.49 ± 8.56	59.66 ± 8.95
HC (n = 21)	2.40 ± 0.48	3.48 ± 0.57	46.25 ± 4.19	56.67 ± 5.10
Male (n = 14)	2.36 ± 0.52	3.15 ± 0.73	49.56 ± 5.02	60.91 ± 6.29
Female (n = 7)	2.48 ± 1.05	4.13 ± 0.92	39.76 ± 7.24	48.18 ± 8.46

*$p < 0.05$ compared to HC (n = 21); **$p < 0.01$ compared to males in HC (n = 14).

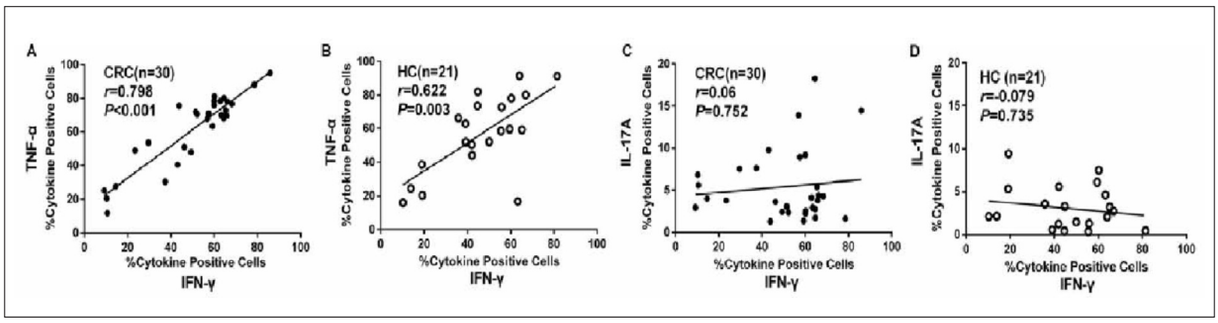

Figure 3. Correlation between the expression of IL-17A, IFN-γ and TNF-α in peripheral Vδ2 T cells in the CRC and the HC groups. Strong positive correlations were observed between the expression of IFN-γ and TNF-α in both the CRC group ***(A)*** and the HC group ***(B)***.

vs. 1.96 ± 0.35%, $p = 0.0001$), but such difference was not seen in the HC group (Figure 5B). IL-17A expression in CD27-Vδ2 T cells was significantly higher in the CRC group vs the HC group (4.11 ± 0.59% *vs.* 2.26 ± 0.44%, $p = 0.006$) (Figure 5B). By contrast, IFN-γ expression in CD27 + Vδ2 T cell were higher than that in CD27-Vδ2 T cells in both groups (33.01 ± 3.61% *vs.* 21.42 ± 2.63%, $p = 0.03$ and 32.83 ± 3.73% *vs.* 17.09 ± 2.52%, $p = 0.002$, for the CRC and the HC groups, respectively) (Figure 5C). And TNF-α expression in CD27 + Vδ2 T cell were also higher than that in CD27-Vδ2 T cells in both groups (35.75 ± 3.68% *vs.* 26.26 ± 3.36%, $p = 0.09$ and 37.68 ± 4.16% *vs.* 18.98 ± 3.20%, $p = 0.0012$, for the CRC and the HC groups, respectively) (Figure 5D).

Expression of Various Cytokines in CD27+Vδ2 T cells and CD27-Vδ2 T Cells Between the Tumor and the Control Groups

There was no significant difference in the T cells proportion of CD28+Vδ2 T cells and CD28-Vδ2 T cells between the tumor group and the control group (Figure 5E). Similarly, the expression of TNF-α and IFN-γ was not significantly different between the two groups or within each group ($p > 0.05$) (Figure 5G, 5H). In both groups, IL-17A expression in CD28+Vδ2 T cells was significantly higher than that in the CD28-Vδ2 T cells tumor group (3.99 ± 0.65% *vs.* 1.96 ± 0.56%, $p = 0.0002$) and in the control group (3.14 ± 0.69% *vs.* 1.16 ± 0.31%, $p = 0.001$). However, there was no significant difference be-

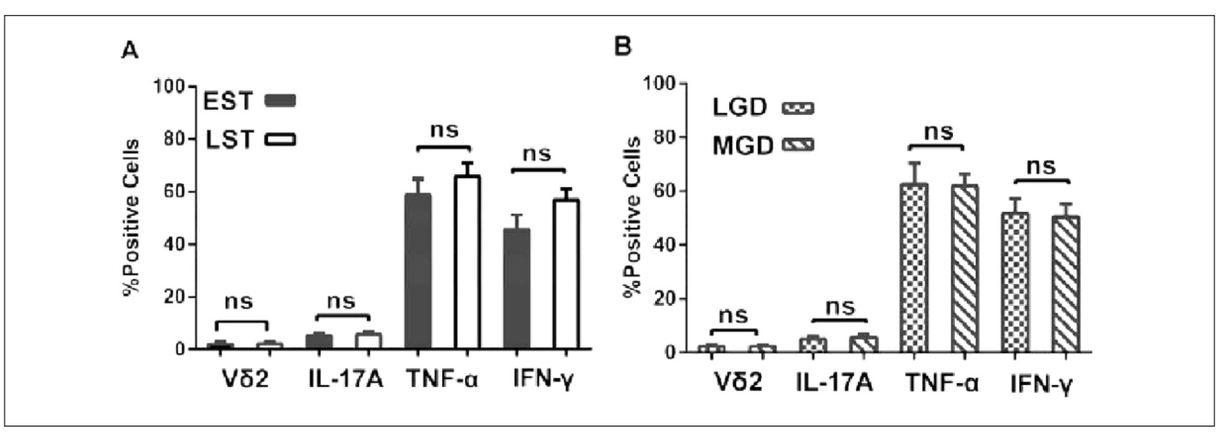

Figure 4. Expression of peripheral Vδ2 T cells and cytokines in patients with CRC at different tumor staging or differentiation. Tumor staging ***(A)*** and tumor differentiation ***(B)*** had no significant effects on the proportion of Vδ2 cells expressing IL-17A, IFN-γ and TNF-α (both $p > 0.05$). Data shown are mean + SEM. EST (early stage of tumor): n = 16; late stage of tumor (LST): n = 14; low grading of dysplasia (LGD): n = 11; moderate grading of dysplasia (MGD): n = 17. ns, not significantly different.

tween the two groups (Figure 5F). CD27 and CD28 for IL-17A expression in Vδ2 T cells exhibited a different pattern in that no significant association was found with the risk of tumor. IL-17A expression in CD27-Vδ2 T cells and CD28+Vδ2 T cells were higher than that in CD27+Vδ2 T cells and CD28-Vδ2 T cells when compared with CD27. The effects of CD28 on the expression of IFN-γ and TNF-α was not recognized in our study (Figure 5).

The Subsets of Cytokine Expression in the Vδ2 T Cells Within the Tumor Group and the Control Group or Between Two Groups

In the tumor group, the order of IL-17A expression, from high to low, was CD27-CD28+, CD27-CD28-, CD27+CD28- (Figure 6A). IL-17A expression in CD27-CD28+ was higher than that in CD27+CD28- and CD27-CD28- in the control group (Figure 6B). The expressions of IFN-γ and TNF-α in both groups were similar. Both CD27+CD28+ and CD27+CD28- expression were higher than CD27-CD28- (Figure 6C-6F). There was no significant difference in the proportion of Vδ2 T cell subsets between the tumor group and the control group. In both groups, CD27-CD28-Vδ2 T cell subset proportion was the highest (43.62 ± 4.18% and 38.40 ± 4.16%), while CD27- CD28 + Vδ2 T cell subsets proportion was the lowest (8.37 ± 0.92% and 8.03 ± 1.19%) (Figure 7A). Expression of IFN-γ and TNF-α in each subgroup was not significantly different between the two groups (Figure 7C, 7D), but the expression of IL-17A subgroups in the tumor group was higher than that in the control group (Figure 7B).

Discussion

In the tumor and the tumor microenvironment, γδT cells secrete a variety of cytokines including IL-17A, IFN-γ, and TNF-α. The anti-tumor immune response of γδT cells has a close relationship with these cytokines expression[1,20-21]. Kunzmann et al[22] reported an increase in IFN-γ expression level in the γδT cells from cancer patients by zoledronic acid and IL-2 immunotherapy. In our study, changes in expression IFN-γ and TNF-α cells in peripheral Vδ2 T cell were not observed in patients with colorectal cancer, and there was no

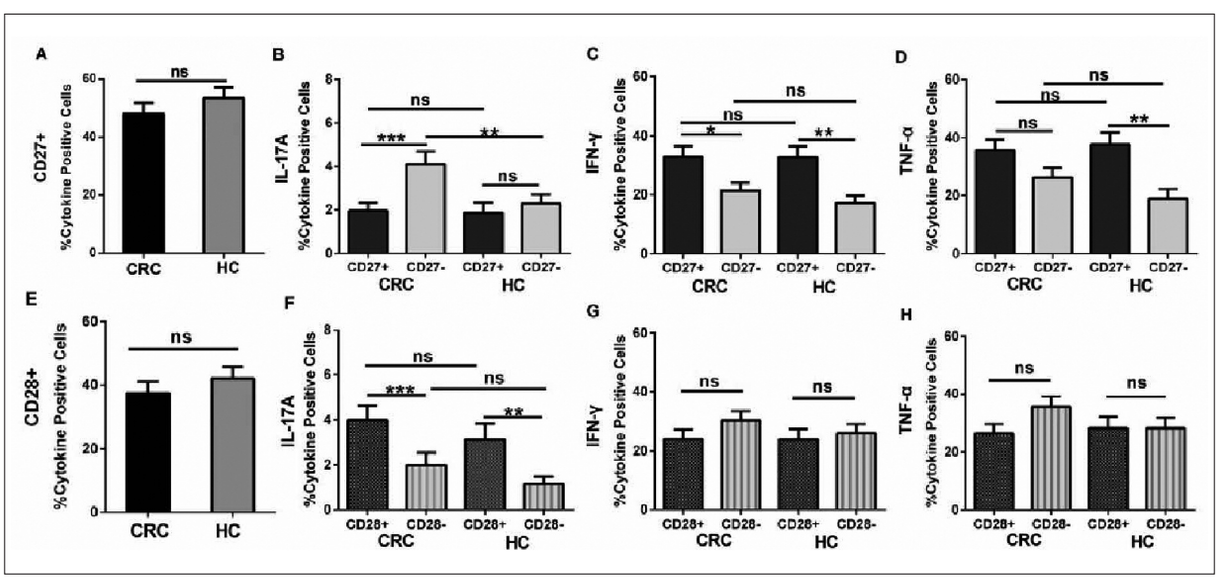

Figure 5. The expression of various cytokines in CD27 ± Vδ2 T cells and CD28 ± Vδ2 T cells in the CRC and the HC groups. In the CRC (n = 30) and HC (n = 21) groups, the proportion of CD27 + Vδ2 T cells with CD28 + Vδ2 T cells had no significant difference ***(A*** and ***E)***. ***B,*** Shows that in the CRC group, IL-17A expression in CD27-Vδ2 T cells was relatively higher than that in CD27 + Vδ2 T cells ($p = 0.0001$), and also than CD27-Vδ2 T cell in the HC group ($p = 0.006$). IFN-γ expression in CD27 + Vδ2 T cells within the CRC and the HC groups was higher than CD27-Vδ2 T cells ($p = 0.03$ and $p = 0.002$, respectively) ***(C)***. Similar trend in expression of TNF-α was also found in the HC group ($p = 0.0012$) ***(D)***. IL-17A expression in CD28 + Vδ2 T cells within the CRC and the HC groups was higher than CD28-Vδ2 T cells (Panel F). Within two groups, IFN-γ and TNF-α expression had no significant difference between CD28 + Vδ2 T cells and CD28-Vδ2 T cells ***(G-H).*** All illustrated data show means + SEM. ns = not significantly different. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

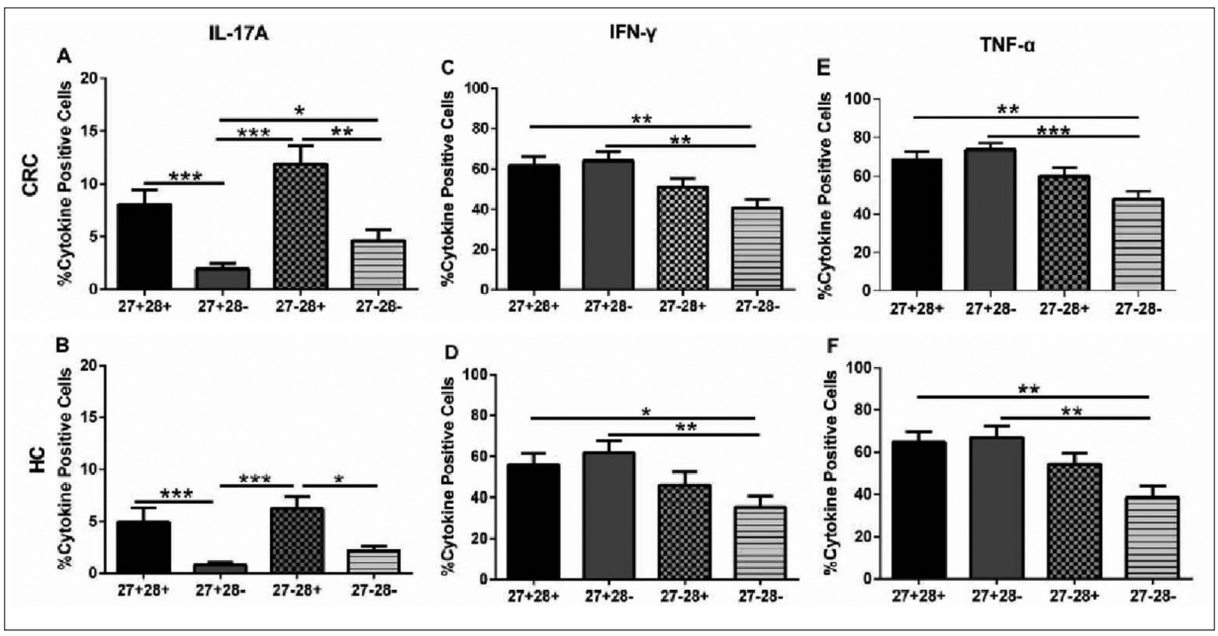

Figure 6. The expression of IL-17A ***(A-B)***, IFN-γ ***(C-D)***, and TNF-α ***(E-F)*** cytokines in CD27CD28Vδ2 T cells sub-group in the CRC group ***(A, C,*** and ***E)*** and the HC group ***(B, D,*** and ***F)***. A, IL-17A expression in Vδ2 T cell subsets of the CRC group (n = 30) is, from high to low, CD27-CD28 +, CD27-CD28-, CD27 + CD28-. B, IL-17A expression in CD27-CD28+ in the HC group (n = 21) was higher than that in CD27 + CD28- and CD27-CD28-. IFN-γ expression was similar comparing the CRC ***(C)*** to the HC group ***(D)*** subsets, whereas CD27+CD28+ and CD27+CD28- were significantly higher than CD27-CD28- in both groups-. For TNF-α expression in the CRC (E) and the HC group (F) subsets, CD27+CD28+ and CD27+CD28- were significantly higher than CD27-CD28-. Data shown are mean + SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

significant association between the expression of these cytokines and tumor staging or differentiation. However, we observed a strong correlation between IFN-γ and TNF-α expression in the peripheral Vδ2 T cells, consistent with the literature[23]. We speculated that the expression level of IFN-γ and TNF-α may, to some extent, signify the functional status of Vδ2 T cells.

Our study showed that IL-17A expression in peripheral Vδ2 T cells from colorectal cancer patients was significantly higher than that in healthy donors. The high Vδ2 T cells expression was not correlated with tumor staging and differentiation, and no correlation was found between the expression of IFN-γ and TNF-α. In contrast, previous studies[24] showed that expression of IL-17A and its stimulating factors such as IL1β, IL6, and TGF-β gradually increased with the tumor staging and differentiation, which may be an indicator of poor prognosis in colorectal cancer patients. It might be possible that the increase in IL-17A expression in periphery Vδ2 T cells from

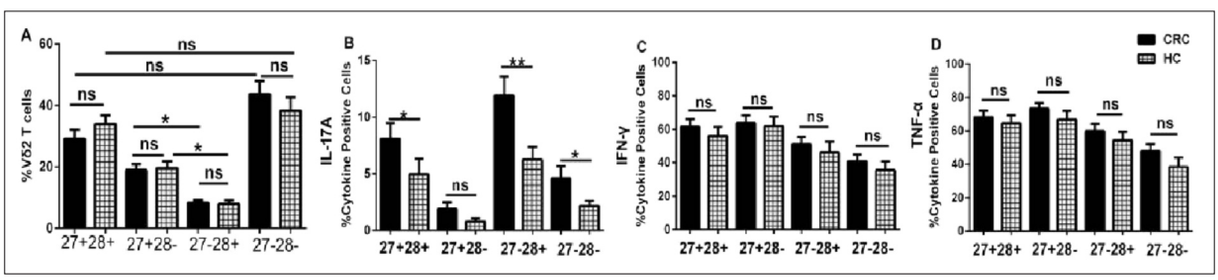

Figure 7. The expression of cytokines in CD27CD28Vδ2 T cells sub-group within the CRC and the HC groups. The ratio of Vδ2 T cell subsets was the lowest in CD27-CD28 + within the CRC (n = 30) and the HC group (n = 21), whereas each subpopulation ratio had no significant difference between the two groups ***(A)***. In addition to CD27 + CD28- group, IL-17A expression in other CRC subsets was higher than that in the HC group ***(B)***. Expression of IFN-γ ***(C)*** and TNF-α ***(D)*** of Vδ2 T cells was similar in the CRC group compared to the HC group. *$p < 0.05$, **$p < 0.01$.

patients with colorectal cancer was affected by other cells such as Th17 that secrete IL-17A. We believe that more complex mechanisms may be involved in regard to the role of IL-17+γδT cells in promoting or suppressing colorectal cancer. Systematic research is needed to provide further understanding of γδ T cells sub-grouping and various inflammatory cytokines expression in Vδ2 T cells in patients with colorectal cancer.

Early researches[25,26] showed that IFN-γ + γδT cells and IL-17 + γδT cells had different physiological effects. For instance, Ribot et al[25] found that the co-stimulatory factor CD27 could act as a cell surface marker to distinguish IFN-γ + γδT cells from IL-17 + γδT in mouse, and that CD27 + Vδ2 T cells separated from pancreas, lymph and other tissues (such as lung and intestinal) could be stimulated to secrete IFN-γ. Additionally, IL-17A secretion was generally restricted to CD27-γδT cell subsets. Similar results were found in our study. IFN-γ expression in human peripheral CD27+Vδ2T cells was significantly higher than CD27-Vδ2 T cells, whereas IL-17A expression of CD27 + Vδ2 T cells was lower than CD27-Vδ2 T cells. Subgroups of γδT cells that bear various functions depend upon the differentiation process in the thymus, which involves a variety of molecular mechanisms[25,27-29]. Signaling pathways and transcription factors are key factors in functional differentiation. It is now generally accepted that γδ thymocytes express the transcription factors T-bet and RORγt that regulate the expression of *Ifng* and *Il17*, respectively, while maintaining a sustained synergistic effect[25,29,30]. This collaborative, functional characteristics allowed for dividing the γδT cells into CD27 + γδT cell subsets and CD27-γδT cell subsets in the thymus. This feature was maintained from the thymus to the periphery tissue during differentiation and maturation of γδT cells[25-27].

Ribot et al[31] and Laird et al[32] have examined the mechanisms of CD28 co-stimulatory on αβT cells for activation, but the effects of CD28 cells on γδT were still controversial in the literature. Ribot et al[33] found that γδT cell amplification can significantly be improved by CD28 receptor agonist. When mice with loss of expression of CD28 were infected with plasmodium *P. berghei*, IFN-γ + γδT cells and IL-17 + γδT cells were different from the selective activation effect of CD27 on IFN-γ+γδT cells, and unable to activate the amplification[34]. Our results showed that there was no significant difference in the expression of IFN-γ and TNF-α between the peripheral CD28+Vδ2 T cells and CD28-Vδ2 T cells, but IL-17A expression of CD28+Vδ2 T cells was significantly higher than CD28-Vδ2 T cells. We speculated that CD27 co-stimulatory may selectively promote the expression of IFN-γ and TNF-α in γδT cells and may inhibit the release of IL-17A. Moreover, the IL-17A expression in γδT cells may be affected mainly by CD28 co-stimulation.

According to the different expression of CD27 and CD28, Appay et al[35] classified the memory CD8 + T cells into three subgroups: CD27 + CD28 +, CD27 + CD28- and CD27-CD28-. These three subgroups can be used to describe the differentiation pathway of CD8+T cells that the expression of CD27 and CD28 continues downward and cytotoxic factors expression remains up-regulated. We found that the proportion of CD27-CD28-Vδ2 T cell subsets was higher in patients with colorectal cancer vs healthy volunteers, but IFN-γ and TNF-α expression in CD27-CD28-Vδ2 T cell subsets was significantly lower than the other subgroups. IL-17A expression was the highest in CD27-CD28 + Vδ2 T cells, while the lowest was in CD27 + CD28-Vδ2 T cells. These findings supported our data on CD27 and CD28 expression and on IL-17A expression in γδT cells. IL-17A expression in the Vδ2 T cell each subset was all higher in healthy volunteers versus cancer patients, indicating that the increase in IL-17A total in Vδ2 T cells of patients with colorectal cancer was not caused by abnormal functional expression of one specific subgroup.

Caccamo et al[36] divided Vγ9Vδ2T cells into four subgroups, namely the initial T cell (Tnative, CD45RA+CD27 +), central memory T cells (T_{CM}, CD45RA-CD27 +), effector memory T cells (T_{EM}, CD45RA-CD27-) and terminal differentiation T cells ($T_{EM\ RA}$, CD45RA+CD27-). After stimulation with antigen or cytokine, the expansion capacity of the group became weaker and the cytotoxicity increased gradually. Since IL-17A in $T_{EM\ RA}$ had the highest expression, it was thought that this subgroup classification may represent a differentiation of γδT cells. However, as mentioned above, different γδT cell subsets have different differentiation origins. CD27 expression was influenced not only by the periphery environmental stimuli. During early development in the thymus, γδT cells formed various CD27 expression and functional characteristic subgroups[25-27]. Our results suggested that, from a functional perspective, the use of CD27 and

CD28 on γδT cells for differentiation of subpopulations typing remained debatable. Therefore, full consideration should be given to the effect of CD27 and CD28 cells on γδT various cytokine expression.

Conclusions

Our study showed that expression of IL-17A was increased in Vδ2 T cells in colorectal cancer patients. We found a significant positive correlation between the expression of IFN-γ and TNF-α. Co-stimulatory receptors CD27 and CD28 played an important role in the γδT cells functional expression, either for the positive regulation (anti-infective or anti-tumor) or negative adjustment (autoimmune diseases) of γδT cells in potential clinical applications. These findings also indicated that increasing the anti-tumor cytokines such as IFN-γ and TNF-α secreted by γδT cells while reducing or removing the negative factors like IL-17A may be a beneficial strategy in anti-tumor therapies.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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