Int J Clin Exp Pathol 2015;8(4):3659-3669
www.ijcep.com /ISSN:1936-2625/IJCEP0006599

Original Article

Abnormal immunomodulatory ability on memory T cells in humans with severe aplastic anemia

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Received February 2, 2015; Accepted March 30, 2015; Epub April 1, 2015; Published April 15, 2015

Abstract: Severe aplastic anemia (SAA) is a bone marrow failure disease induced by hyperfunctional autoimmunic Th1 lymphocytes. Memory T cells (TM) are a component of the adaptive immune system. They ensure the host of more aggressive and faster immune response to efficiently eliminate the specific antigens after re-exposure and thus play a key role in T-cell functions. In this study we investigate the quantities and functions of memory T cells in SAA patients before and after immunosuppressive therapy (IST) to further clarify the mechanism of SAA apoptosis of bone marrow hematopoietic cells. Results showed that the percentage of CD4+ effector T cells in peripheral blood and bone marrow lymphocytes was decreased in SAA patients. The ratio of CD4+ memory T lymphocytes to CD8+ memory T subsets (CD4+/CD8+TM) in SAA patients was also lower. The percentage of CD8+ effector T cells in peripheral blood and CD8+ central memory T cells in the bone marrow lymphocytes was significantly higher in newly diagnosed patients. Furthermore, the median expressions of perforin and granzyme B on memory T cells were higher in SAA patients compared to those in normal controls. After IST, the quantities and functions of memory T cells return to normal level. Therefore, we concluded that the abnormal immunomodulatory ability on memory T cells may contribute to the imbalance of Th1/Th2 subsets and thus lead to over-function of T lymphocytes and hematopoiesis failure in SAA.

Keywords: Severe aplastic anemia, memory T cells, perforin, ganzyme B

Introduction

Severe aplastic anemia (SAA) is a kind of bone marrow failure mainly characterized by severe peripheral pancytopenia. Recently SAA has been recognized an immune-mediated destruction of hematopoietic cells caused by abnormally activated T lymphocytes (especially CD8+ cells), leading to hyperfunction of downstream Th1 cells and cytotoxic T lymphocytes [1, 2]. Anti-lymphocyte globulin/anti-thymocyte globulin-based intensive immunosuppressive treatment (IIST) has achieved increased clinical efficacy in recent years [3, 4].

Memory T cells play an important role in host defense against viruses, malignancies and allogeneic cells. According to the ability to express chemokine receptor CCR7, CD4+ and CD8+ memory T cells can be divided into central memory T cells (TCM, CD3+CD45RA-CCR7+), and effector memory T cells (TEM, CD3+ CD45RA-CCR7-). TEM circulate through nonlymphoid peripheral tissues with effector phenotypes and have distinct migratory and functional characteristics [5], and produce IFN-γ, perforin, ganzyme B, and IL-4 but little IL-2. TCM circulate through secondary lymphoid tissues with strong proliferative capacity and might contribute to the maintenance of TEM pool, produce IL-2 but little IFN-γ and no perforin. However, the origin of these memory subsets and whether they are interrelated are not completely understood. Recently study have found that CD45RA+ cells in autoimmune diseases are often reduced, particularly in the active or relapse stage of the disease, representing the transformation of quiescent cells to activated memory lymphocytes [6]. In systemic lupus erythematosus (SLE), multiple sclerosis (MS) and other autoimmune diseases, it has been shown that the quantitative abnormality of memory T cells correlates with the inflammatory process of the disease. Moreover, these patients have

Table 1. Characteristics of untreated SAA patients

Case	Age/sex	Granulocyte (×109/L)	Hemoglobin (g/L)	Platelet (×109/L)	Abnormal chromosome	Therapy
1	13/F	0.21	50	14	Absence	None
2	43/M	0.34	72	12	Absence	None
3	22/M	0.06	36	5	Absence	None
4	18/M	0.41	74	10	Absence	None
5	8/F	0.29	68	9	Absence	None
6	29/M	0.16	56	7	Absence	None
7	27/F	0.23	61	13	Absence	None
8	16/F	0.25	53	9	Absence	None
9	20/M	0.38	70	11	Absence	None
10	23/F	0.34	64	10	Absence	None
11	15/F	0.42	72	14	Absence	None

more memory T cells with impaired function. Therefore, immune memory plays a significant role in the pathogenesis of autoimmune disease. Nevertheless little is known about any potential role for them in SAA. The memory T cells that damage bone marrow hematopoiesis in SAA patients show heterogeneity. The abnormalities of quantities and functions of memory T cells and its subsets with the potent effector capacity may relate to the abnormal immune status in the pathogenesis of SAA. This study aimed to investigate the quantities and functions of memory T cells in SAA patients to further clarify SAA apoptosis of bone marrow hematopoietic cells.

Materials and methods

Study subjects

A total of 45 patients with SAA including 26 men and 19 women with median age 30 (age range 8-56) were enrolled in the present study, including 11 newly diagnosed cases and 34 cases in remission after immunosuppressive therapy (IST). All patients were newly diagnosed in the Hematology Department of General Hospital Tianjin Medical University from November 2013 to November 2014, according to international AA Study Group Criteria (Marsh et al., 2009). The disease was considered SAA if pancytopenia with at least two of the following parameters were met: a neutrophil count less than 0.5×10^9/L, a platelet count less than 20×10^9/L, and a reticulocyte count less than 20×10^9/L with hypocellular bone marrow. VSAA was diagnosed in the cases SAA with the neutrophil count $<0.2\times10^9$/L [16, 17]. Patients were excluded if they had congenital AA or other autoimmune diseases. All patients were screened for paroxysmal nocturnal hemoglobinuria (PNH) by flow cytometry using anti-CD55 and anti-CD59 antibodies, and no PHN clones had been found. Patients' features are listed in **Table 1**. Patients in remission were those who improved after immunosuppressive therapy (antithymocyte globulin, cyclosporine, glucocorticoid) and hematopoietic stimulating factors (granulocyte colony-stimulating factor, recombinant human erythropoietin, recombinant human thrombopoietin, and/or IL-11 in combination). All of the patients in remission achieved bone marrow hematopoietic recovery and became transfusion independent, while some had normal peripheral blood cell counts but still required drug therapy (**Table 2**).

25 normal healthy individuals were selected as controls (12 men and 13 women, median age 29, age range 22-56, **Table 3**). The study was approved by the Ethics Committee of Tianjin Medical University General Hospital. Informed written consents were obtained from all patients and normal controls or their parents in accordance with the Declaration of Helsinki.

Monoclonal antibodies

Anti-CCR7-PE, anti-CD3-APC, anti-CD45RA-FITC, anti- CD4-PerCP, anti-CD8-PerCP and the mouse isotype controls were purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

Measurement of quantities of memory T cells and its subsets from peripheral blood and bone marrow

Blood and bone marrow samples were collected in heparin-anticoagulant tubes from the patients and normal individuals. Effector memory T cells (TEM, CD3+CD45RA-CCR7-) and central memory T cells (TCM, CD3+ CD45RA-CCR7+) were identified with a single platform four-color flow cytometric analysis. 50 μl whole blood or bone marrow was immunostained in TruCount tubes (BD), followed by lysis in 1.0 milliliter FACS RBC lysing solution (BD). Data acqui-

Table 2. Characteristics of remission SAA patients

Case	Age/sex	Granulocyte (×109/L)	Hemoglobin (g/L)	Platelet (×109/L)	Abnormal chromosome	Therapy
1	19/F	5.34	99	121	Absence	ATG+ CsA
2	56/M	6.89	101	98	Absence	ATG+ CsA
3	37/M	7.59	132	103	Absence	ATG+ CsA
4	32/F	6.21	108	94	Absence	ATG+ CsA
5	16/F	10.38	97	135	Absence	ATG+ CsA
6	10/F	7.45	93	126	Absence	ATG+ CsA
7	54/M	9.76	112	89	Absence	ATG+ CsA
8	34/F	8.39	128	94	Absence	ATG+ CsA
9	29/F	4.66	94	141	Absence	ATG+ CsA
10	41/F	8.47	96	113	Absence	ATG+ CsA
11	30/F	5.74	105	76	Absence	ATG+ CsA
12	46/M	9.21	109	99	Absence	ATG+ CsA
13	33/F	9.55	99	117	Absence	ATG+ CsA
14	26/M	6.36	110	132	Absence	ATG+ CsA
15	44/F	10.75	102	122	Absence	ATG+ CsA
16	48/M	8.04	111	98	Absence	ATG+ CsA
17	39/M	7.94	103	85	Absence	ATG+ CsA
18	41/F	7.09	98	91	Absence	ATG+ CsA
19	39/F	10.86	92	86	Absence	ATG+ CsA
20	40/M	8.93	121	145	Absence	ATG+ CsA
21	53/F	4.68	98	66	Absence	ATG+ CsA
22	28/F	8.37	114	92	Absence	ATG+ CsA
23	16/F	5.82	119	104	Absence	ATG+ CsA
24	33/F	9.67	106	108	Absence	ATG+ CsA
25	37/M	7.15	100	93	Absence	ATG+ CsA
26	46/M	5.46	112	122	Absence	ATG+ CsA
27	37/F	6.28	96	73	Absence	ATG+ CsA
28	43/M	8.54	98	79	Absence	ATG+ CsA
29	51/F	4.38	123	95	Absence	ATG+ CsA
30	28/F	6.17	93	78	Absence	ATG+ CsA
31	23/M	6.39	97	81	Absence	ATG+ CsA
32	20/M	7.66	104	86	Absence	ATG+ CsA
33	19/F	10.40	115	110	Absence	ATG+ CsA
34	24/M	3.63	107	105	Absence	ATG+ CsA

sition and analysis were carried out by using FACS-Caliburflow cytometer (BD Biosciences, US), with the Cell Quest software (Becton Dickinson, version 3.1) (**Figure 1**).

Isolation and purification of CD8+TEM lymphocytes

Ten milliliters fresh human peripheral blood or bone marrow was obtained from SAA patients and normal controls. Peripheral blood mononuclear cells (PMMNCs) were isolated by density gradient centrifugation using Ficoll-Paque Plus solution (Amersham Bioscience, Uppsala, Sweden), followed by staining with fluorophore-conjugated monoclonal antibodies as described before (as mentioned above). CD8+ effector memory T cells were sorted and collected using a FacsAria flow cytometer, and the purity of these sorted cells was also measured. After detected by the multiparameter flow cytometry (BD Biosciences) and analyzed using the Cell Quest software program (Version 3.1, Becton Dickinson), the purity of CD8+ effector memory T cells was more than 90% (**Figure 2**).

RNA isolation and quantitative real-time PCR

PBMCs in human peripheral memory T cells were lysed in TRIzol reagent (Invitrogen, USA). Total RNA was dissolved in RNase-free water and quantified using a UV spectrophotometer (NanoDrop ND-1000, Thermo Scientific, USA). And 1 μg of RNA was reversed transcripted using TIANScript RT Kit (Invitrogen, USA). The sequences of primers specific for granzyme B, perforin and β-actin used have been listed below (**Table 4**), which were designed and synthesized by Sangon Biotech, Shanghai, China.

Real-time PCR was performed with 3 μL of each cDNA working solution in a final volume of 25 μL containing 12.5 μL 2x SYBR Green Real-time PCR Master Mix (TianGen Biotech) and 400 nM of each sense and antisense primer. The real-time PCR was carried out in a BIO-RAD iQ5 Real-Time System (BIO-RAD, USA), using the following thermal cycling profile for all genes of interest: 95°C for 5 min, followed by 40 cycles

Table 3. Characteristics of normal controls

Case	Age/sex	Granulocyte (×109/L)	Hemoglobin (g/L)	Platelet (×109/L)	Abnormal chromosome
1	23/M	8.13	124	151	Absence
2	24/M	7.39	145	286	Absence
3	24/M	6.98	160	213	Absence
4	24/F	9.56	139	137	Absence
5	25/M	8.26	127	265	Absence
6	24/F	9.14	122	201	Absence
7	24/F	7.30	158	186	Absence
8	26/M	7.74	132	194	Absence
9	42/F	8.91	137	241	Absence
10	33/F	5.88	146	190	Absence
11	34/F	6.23	141	266	Absence
12	29/M	9.04	125	148	Absence
13	31/M	5.78	159	169	Absence
14	28/F	5.68	154	253	Absence
15	29/M	6.03	128	210	Absence
16	29/F	8.52	134	155	Absence
17	37/M	9.11	147	172	Absence
18	56/M	4.23	121	134	Absence
19	35/F	5.16	126	209	Absence
20	49/F	5.87	144	214	Absence
21	30/F	5.47	137	233	Absence
22	22/M	7.22	143	127	Absence
23	36/M	6.39	119	145	Absence
24	41/F	4.41	151	138	Absence
25	43/F	5.23	128	195	Absence

of amplification (95°C for 15 s, indicated annealing temperature for 15 s, 72°C for 45 s). After normalization of the data according to the expression of b-actin mRNA, the levels of perforin and granzyme B were calculated by the $2^{-\Delta\Delta Ct}$ method ((Ct, target gene-Ct, β-actin)$_{sample}$-(Ct, target gene-Ct, β-actin)).

Statistical analysis

All statistical calculations were performed using SPSS Statistics 18.0. Statistical analysis was performed with the parametric unpaired t-test for normally distribution data, and the nonparametric test for skewed data. Statistical significance was accepted if $P<0.05$.

Results

Proportion of TEM subsets in untreated SAA patients

The percentage of CD4+ TEM cells in peripheral blood lymphocytes was (22.21±6.97)% in untreated SAA patients, and (28.18±8.18)% in normal controls. The percentage of CD4+ TEM cells in untreated SAA patients was lower than that in normal controls ($P<0.05$). The percentage of CD8+ TEM cells in peripheral blood lymphocytes was (18.24±7.12)% in untreated SAA patients, and (11.83±5.52)% in normal controls. The percentage of CD8+ TEM cells in untreated SAA patients was higher than normal controls ($P<0.05$). The ratio of CD4+ memory T lymphocytes to CD8+ memory T subsets in untreated SAA patients was also lower than remission group and normal controls ($P<0.05$). The percentage of CD4+ TEM cells in the bone marrow lymphocytes was (17.27±1.07)% in untreated SAA patients, and (27.30±4.29)% in normal controls. The percentage of CD4+ TEM cells in untreated SAA patients was lower than that in normal controls ($P<0.05$). There was no statistical difference between newly diagnosed SAA patients and normal controls in CD8+ TEM subsets in the bone marrow ($P>0.05$) (**Figure 3**).

Proportion of TCM subsets in untreated SAA patients

The percentage of CD8+ TCM cells in the bone marrow lymphocytes was (0.56±0.35)% in untreated SAA patients, and (0.28±0.10)% in normal controls. The percentage of CD8+ TCM cells in untreated SAA patients was higher than that in normal controls ($P<0.05$). No significant differences in the proportion of TEM cell subsets between groups were detected in the bone marrow (**Figure 4**).

mRNA expression abnormalities of perforin and granzyme B in PWBCs from SAA patients

To determine the function of CD8+ memory T cells in SAA, the perforin and granzyme B mRNA expression on peripheral blood CD8+ TEM cells from untreated SAA patients, recovery patients and normal controls were evaluated. The levels of mRNA of perforin and granzyme B genes were dramatically increased CD8+ TEM cells from our patient samples compared with normal controls in the peripheral blood.

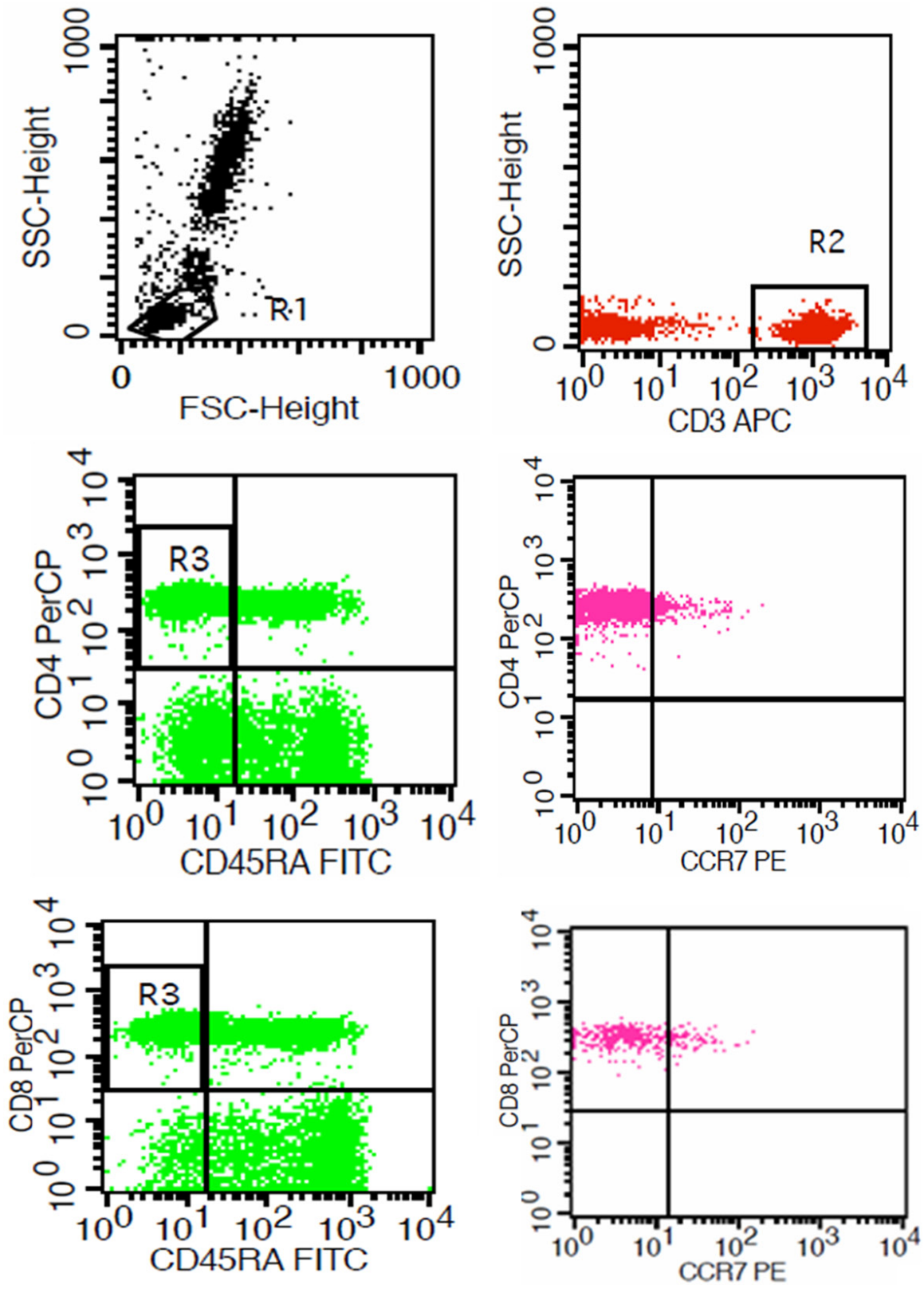

Figure 1. Flow cytometry (FCM) tests. Autoantibodies were detected on CCR7-PE, CD3-APC, CD45RA-FITC, CD4-PerCP (or CD8-PerCP).

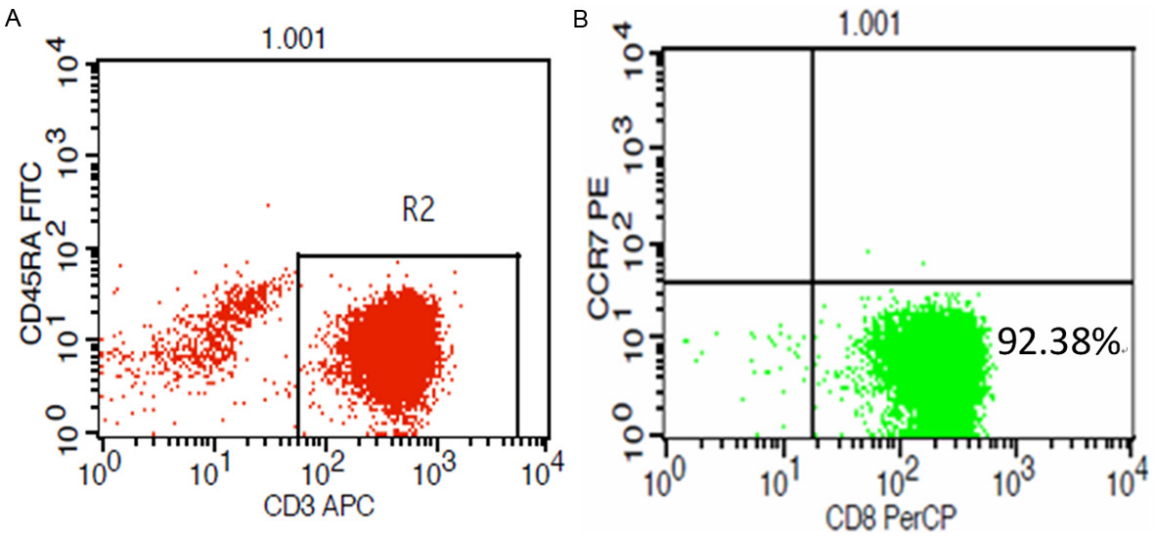

Figure 2. A and B. The purity of isolated CD8+ effector memory lymphocytes. After being isolated using a Facs Aria flow cytometer, we measured using multiparameter flow cytometry. The purity of CD8+ effector memory lymphocytes (CD3+CD8+CD45RA-CCR7-) was more than 90%.

Table 4. Primers Used for quantitative real-time PCR detection

Target gene	Primer sequences	Product
Perforin	F:5'-GAGGAGAAGAAGAAGAAGCACAA-3'	200 bp
	R: 5'-AGGGGTTCCAGGGTGTAGTC-3'	
Ganzyme B	F: 5'-CCAGCAGTTTATCCCTGTGAA-3'	235 bp
	R:5'-CACCTCTTGTAGTGTGTGTGAGTG-3'	
β-actin	F: 5'-TTGCCGACAGGATGCAGAA-3'	100 bp
	R: 5'-GCCGATCCACACGGAGTACT-3'	

We measured the levels of mRNA of these genes in AA patient samples and identified significantly higher expression of perforin in the SAA untreated group (5.46, range: 4.31-6.25) relative to the remission group (3.71, range: 1.44-5.83) and the control group (0.82, range: 0.39-1.22; $P<0.05$). Meanwhile, the relative Granzyme B mRNA levels evidently increased in the peripheral blood CD8+ TEM cells from untreated SAA patients (4.54, range: 2.71-5.56) compared to remission group (3.59, range: 1.86-5.48) and normal controls (0.97, range: 0.21-1.50; $P<0.05$; **Figure 5**).

Discussion

Severe aplastic anemia is a potentially life-threatening disease whose high mortality is usually due to complications such as infection, hemorrhage and severe anemia. It is known that our immune system is usually in a state of autoimmune tolerance in order to maintain the relatively stable internal environment of body. But stable dysfunction of the immune system can produce its reactive antibodies and/or its reactive T/B lymphocytes to attack its own cells or molecules, leading to autoimmune disease. In the past decades, studies have shown that SAA was an autoimmune disease with hematopoietic stem/progenitor cells impaired by hyperfunctional autoimmunic T lymphocytes (especially CD8+ cells) which activated abnormally by Th1 cells. The increased expression of TNF-α, IFN-γ, and IL-2 from SAA patients indicates that hematopoietic stem/progenitor cells are destroyed through Th1 cell response [7]. Liu et al. reported about increased expression of Fas antigen on bone marrow CD34+ cells of patients with SAA. Fas antigen, a receptor molecule that has contributed great progress to human programmed cell death and apoptosis, is enhanced by INF-γ and TNF-α. The recognition of the Fas expression antigen by the FasL expression CTL might give rise to excessive apoptosis of bone marrow hematopoietic cells in patients with SAA. Furthermore, CD34+ cells are likely the main targets of SAA immune injury [8]. Patients with SAA have a significant increase in CD8+ suppressor T lymphocytes [9, 10]. Moreover, the expression of perforin, granular enzyme and TNF-β were elevated obviously in these effector T cells as well as many other hemato-

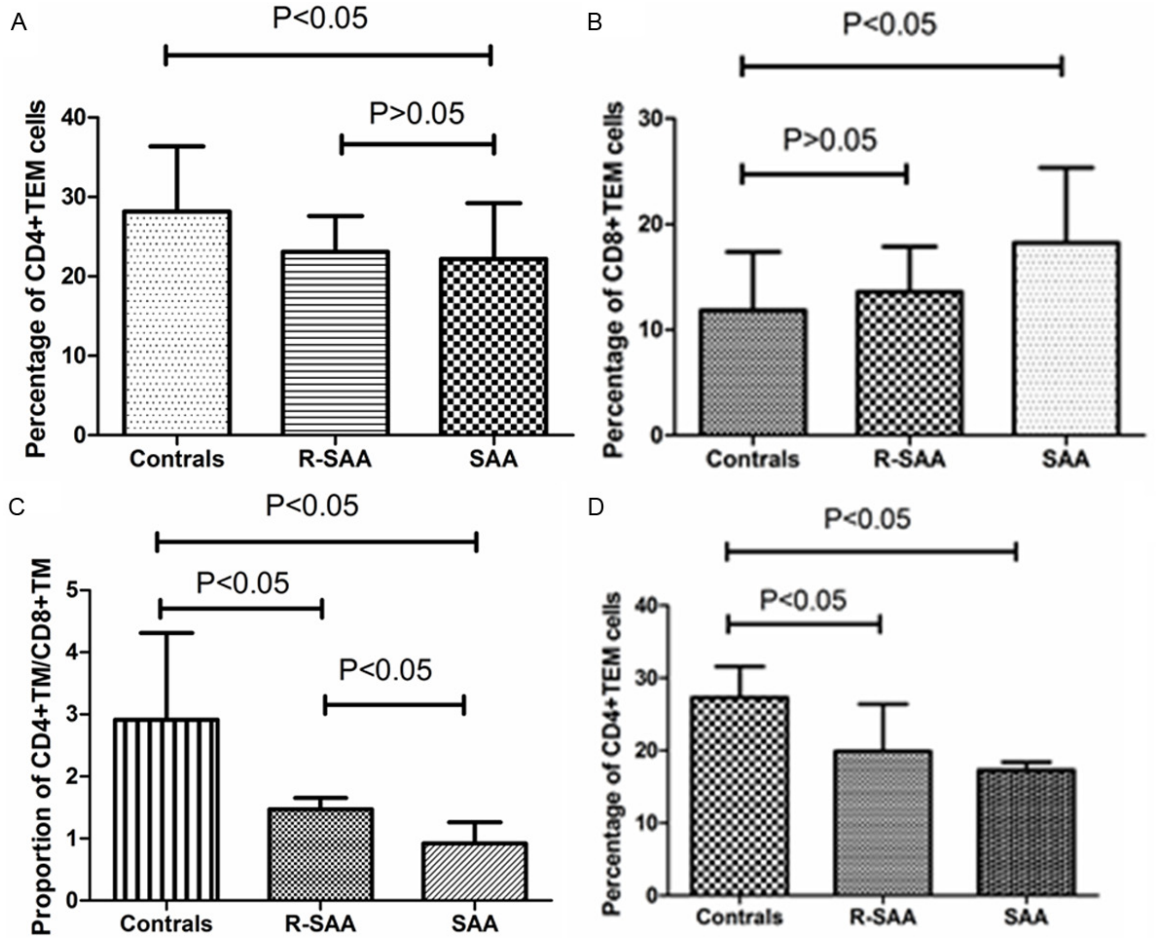

Figure 3. The changes of TEM cells and its subsets after intensive immune suppressive therapy in severe aplastic anemia patients. A. The percentage of CD4+ TEM cells in SAA, R-SAA and healthy controls in peripheral blood; B. The percentage of CD8+ TEM cells in SAA, R-SAA and healthy controls in peripheral blood; C. The ratio of CD4+ memory T lymphocytes to CD8+ memory T subsets in SAA, R-SAA and healthy controls in peripheral blood; D. The percentage of CD4+ TEM cells in SAA, R-SAA and healthy controls in the bone marrow.

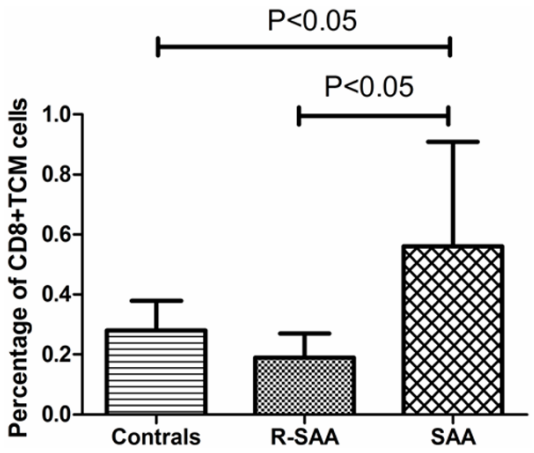

Figure 4. Percentage of CD8+TCM cells in SAA, R-SAA and healthy controls in the bone marrow.

poietic negative regulatory factors in SAA patients, suggesting that hematopoietic stem/progenitor cells might destroyed through lymphokine induced apoptosis [11-13]. Previous studies have demonstrated that activated DC1 cells increased in the bone marrow of SAA patients, promoting Th0 cells to polarize to Th1 cells and even increases in the quantity and function of CD8+ T cells, thus cause the hyperfunction of T lymphocytes and hematopoiesis failure in SAA [14, 15].

The present study was aimed to elucidate the effect of memory T cells and its subsets from both in peripheral and bone marrow on SAA patients and obtain more evidence for the bone marrow failure in SAA. Here we have studied memory T cells in peripheral blood from patients with SAA and normal controls. Our results have demonstrated that there was a significant decrease in the proportions of CD4+/CD8+ TEM cells in the peripheral blood from

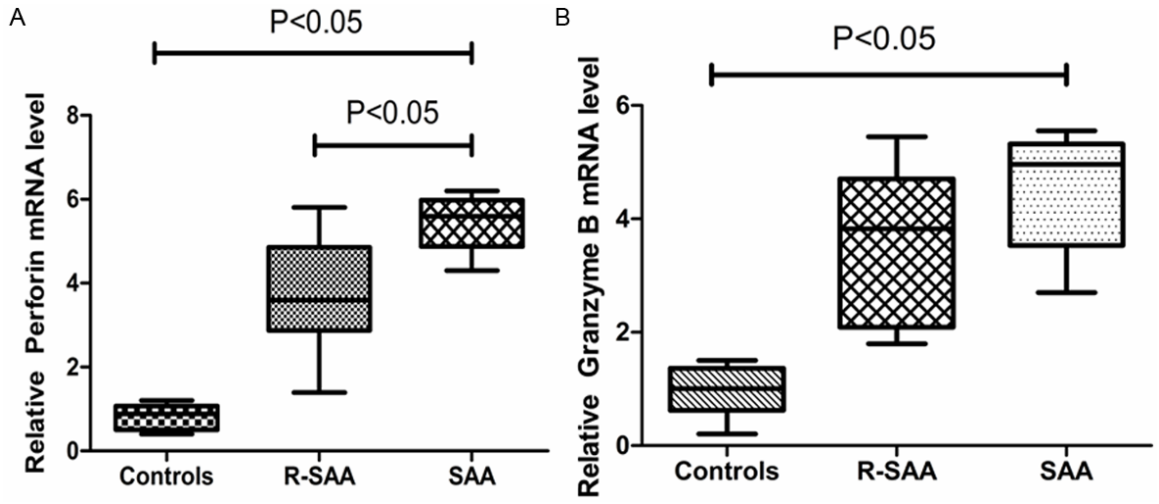

Figure 5. Perforin and granzyme B mRNA expression in different groups.

individuals with severe aplastic anemia, and the proportion of TM subsets (CD4+ TM/CD8+ TM) restored to normal levels in these patients after IIST. Furthermore, the ratio of CD8+ TCM cells in the bone marrow of SAA patients increases obviously. Based on these results, we suggested that abnormal immunomodulatory ability on memory T cells is responsible for T-cell proliferation, imbalance of Th1/Th2 subsets and even in the processes of bone marrow failure of SAA.

Memory T cells are the components of the adaptive immune system. They circulate between peripheral tissues, lymphoid organs and blood to ensure the host of more aggressive and faster immune response to efficiently eliminate the specific antigens after re-exposure [16]. According to the ability to express chemokine receptor CCR7, CD4+ and CD8+ memory T cells can be divided into central memory T cells (TCM, CD3+CD45RA-CCR7+), and effector memory T cells. Different surface molecular markers on memory T cells affect its tissue localization. TEM is mainly distributed in the blood, lymph nodes and peripheral non-lymphoid tissues and express high levels of integrin β1 and β2, cutaneous lymphocyte antigen (CLA) and CCR1, CCR3, CCR5 chemokine receptors. Those TEM cells who homing to the skin express CCR4 and CCR10 molecules. Other TEM cells who homing to the intestinal tract express integrin α4β7. Activated TEM cells migrate to peripheral sites of inflammation, and rapidly secrete both Th1 and Th2 cytokines [17]. TCM express CD62L, CCR7, CCR4, CCR6, CXCR3, CCR1 and CCR2 molecules, most of them exist in secondary lymphoid tissues and organs such as spleen, blood and lymph nodes. Additionally, memory T cells are critically dependent on instructive signals from specific cytokines, such as IL-7, to remain in a functionally competent state [18, 19]. IL-15 is the central driver of CD8+ memory T cell [20]. Such memory cells can rapidly secrete IFN-γ and IL-2, with increased sensitivity to TCR signaling and a reduced need for co-stimulation [21]. Following a second exposure to the same antigen, the CD8+ memory T cells develop into secondary effectors and eventually differentiate into secondary memory T cells. While the CD4+ T memory cells have not been extensively studied and complicated by the existence of multiple Th subsets [22].

In addition to play a role in virus, bacteria and other pathogens, TM cells also related to the occurrence of many autoimmune diseases. In type 1 diabetes (T1D), memory T cells protect from the progress of the disease through secretion of immunoregulatory cytokines [23]. In systemic lupus erythematosus (SLE) [24], it has been shown that the quantitative abnormality of memory T cells correlates with the inflammatory process of the disease. Moreover, patients with multiple sclerosis (MS) have more memory T cells (especially CD8+ memory T cells) with impaired function, and this may be related to maintain continuous process after the occurrence of immune response in multiple sclerosis patients [25]. These studies above strongly suggest that the memory T cells are involved in

the autoimmune disease. Memory T cells show enhanced effector function which is more likely to be activated and have a rapid production of effector molecules. This process is controlled by transcription factors, such as Eomesodermin (*EOMES*) and T-box transcription factor 21 [26, 27]. T-bet is a kind of T-box transcription factor, which is the key regulator of Th1 development and function. There is evidence that T-bet and the cognate transcription factors *EOMES* closely related to the differentiation of effect and memory T cells [28]. The expression of T-bet in TM will be reduced while the expression of *EOMES* will be elevated. Moreover, those cells with high expression of T-bet will differentiate into effector cells, while the cells with low expression of T-bet will differentiate into memory cells [29]. Past studies have shown that *EOMES* directly regulates expression of perforin, granzyme B, and TNF-α, and promotes effector function in CD8+ T cells. Granzymes, a cell death-inducing serine proteases released from cytotoxic T lymphocytes and natural killer cells, considered to be the responsible molecule for target cell cytosol. Perforin, a Ca (2+)-dependent pore-forming protein, is released in the presence of Ca2+ and aggregate to form pores on the target cell membranes. These pores can cause disruption of the balance of osmotic pressure inside and outside of the cell and finally lead to the death of the target cell [30]. A critical cofactor for the granule exocytosis pathway is granzyme B, which enters the target cell through the pores formed by perforin where they cleave specific substrates that initiate DNA fragmentation and apoptosis [31, 32]. Studies have found that CD8+ TEM can rapidly exert effector functions when re-exposure to antigen, including the release of perforin and granzyme B [33]. Patients with SLE were characterized by higher proportions of perforin and/or granzyme B-positive lymphocytes, and the increase in circulating perforin or granzyme B-positive CD8+ T cells thoroughly reflected the activity of the disease [34]. The results obtained in the present study allowed us to better delineate a still-unknown role for CD8+ TEM lymphocytes in SLE pathogenesis. No studies showing the specific mechanism for memory T cells in SAA patients have yet been reported.

Our previous study has demonstrated that the quantity of CD8+ TEM cells in peripheral blood was significantly increased in SAA patients compared with normal controls, then next we use the method of qPCR to verify whether there is a hyperfunctional state of TEM in peripheral blood. The results indicate that the median expressions of perforin and granzyme B on CD8+ TEM cells were higher in SAA patients compared to those in normal controls. Based on these results, we consider that hyperfunction of memory T cells possibly caused by genetic mutation or antigenic stimulus could enhance the cytotoxicity of CD8+ T cells in SAA. The activated TM cells proliferate extremely quickly upon stimulating repeatedly by unknown antigen, causing the hyperfunction of antigen-specific lymphocytes attack against hematopoietic tissue, and thus ultimately led to the apoptosis of hematopoietic cells. It is noteworthy that the body's memory T cells return to a dormant state after the majority of the specific antigen is removed, and Th1 cells decreased gradually with hematopoietic function recovery. Additionally, because of the increase expression of CCR7 and CD62L, a mass of TCM cells can infiltrate to the bone marrow of SAA patients to attack hematopoietic stem/progenitor cells, which might also contribute to the immunopathogenesis of SAA. The activation of CD4+ memory T cells is associated with the deterioration of SAA, and the activation of CD8+ memory T cells may reflect the systemic immune dysfunction in SAA patients. Especially the abnormal changes of TCM cells subgroup, which may be related to sustain continuous process of the SAA after the occurrence of immune response.

In conclusion, the abnormal quantity and function of memory T cells in humans with severe aplastic anemia may be one of the reasons that cause the over-function of T lymphocytes and thus lead to hematopoiesis failure in SAA. Our research might allow the development of new therapeutic strategies based on memory T cells for the treatment of SAA. Nevertheless, the biological characteristics of memory T cells and its subsets need further study to explore the real target.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81170472, 81370607, and 81400085), Tianjin Municipal Natural Science Foundation (12JCZDJC21500), Health Industry Research

and Special Projects (201202017), Tianjin Science and Technology support key project plan (20140109), Health Industry Research and Special Projects (201202017), Tianjin Cancer Research of Major Projects (12ZCDZSY17900 and 12ZCDZSY18000), and the Tianjin Health Industry Key Projects (11KG135).

Disclosure of conflict of interest

None.

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