Int J Clin Exp Pathol 2017;10(3):3424-3430
www.ijcep.com /ISSN:1936-2625/IJCEP0045128

Original Article

Prognostic significance of preoperative CD8+ central memory T cells for operable and advanced gastric cancer

Guo-Qing Liu, Song-Wen Hua, Ke Pan

Department of General Surgery, The Second Xiangya Hospital of Central South University, Xiangya, Hunan, PR China

Received November 25, 2016; Accepted November 28, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: *Background:* CD8+ central memory T cells (CD8+ Tcm) have superior antitumor potential. The purpose of this study was to evaluate the percentage of CD8+ Tcm cells in peripheral blood of patients with advanced gastric cancer and analyze its prognostic significance in operable and advanced gastric cancer. *Methods:* A total of 274 patients with primary gastric cancer underwent curative operation were enrolled in this study. The percentage of CD8+ Tcm in peripheral blood of patients was analyzed using flow cytometry, comparing with 102 healthy donors. The correlation between CD8+ Tcm and survival outcome was analyzed by the Kaplan-Meier with Log-Rank test and Cox's regression methods, respectively. *Results:* Percentage of CD8+ Tcm from gastric cancer was lower than healthy control (6.2%±3.8% VS 13.7%±6.2%; P<0.01), which also had associated to the lymph node involvement. Patients with high CD8+ Tcm had a significantly higher median survival than those with low CD8+ Tcm (45.32 months VS 23.52 months, P=0.02). Percentage of CD8+ Tcm also was confirmed as an independent prognostic factor for gastric cancer in multivariate analysis. *Conclusions:* Percentage of CD8+ Tcm can be considered as an independent prognostic factor for operable and advanced gastric cancer, which also associated with the lymph nodes metastasis.

Keywords: Central memory T cells, survival, gastric cancer

Introduction

Gastric cancer accounted for fourth most prevalent cancer worldwide, which also considered as the second most common cancer and the third leading cause of cancer-related deaths in China [1, 2]. Despite improvements in comprehensive treatments, prognosis of gastric cancer is far from satisfying [3, 4]. Surgery is regarded as only curative treatment approach for localized and operable gastric cancer [5]. However, high rates of metastasis and relapse are major obstacles to improving long-term survival after a curative resection, 50%-90% patients with gastric cancer die of cancer due to local or distant recurrent [6-8]. There are several risk factors associated with outcome of gastric cancer, such as lymph node status, resection margins, tumor differentiation, and adjuvant treatment [9-11]. Despite important role of the immune system in controlling cancer development, there was no study focusing on relationship of immunity and the prognosis of gastric cancer.

T cells, an important component of immune system, responses for direct recognition and killing of tumor cells and immunologic surveillance [12]. Memory T cells are a subset of T cells participating in cell-mediated immunity responses, characterized by keeping survive for a long period until reencounter antigen and rapidly proliferating again eliminated, play effector functions and release various cytokines [13, 14]. CD8+ central memory T (CD8+ Tcm), as cytotoxic memory T cells, present as CD8+ CD45RO+CD62L+, which not only survive longer, but also can eradicate tumor cells in setting of the second recognition of tumor-associated antigen (TAA) [15]. Several studies have showed that memory T cells associated with the prognosis of malignancies including colorectal cancer, pancreatic and esophageal adenocarcinoma [16-19]. However, there was no

Table 1. Correlation between the percentage of CD8+ Tcm and clinicopathologic characteristics

Characteristic	CD8+ Tcm		*P*
	<6.2% (n=198)	≥6.2% (n=76)	
Age (years)			0.810
≥60	97	36	
<60	101	40	
Gender			0.162
Male	134	58	
Female	64	18	
Tumor site			0.798
Gastric cardia	52	23	
Gastric body	92	33	
Gastric antrum	54	20	
Tumor size (cm)			0.646
≥5	63	22	
<5	135	54	
Tumor invasion depth			0.992
T1+T2	65	25	
T3+T4	133	51	
Lymph node involvement			0.000
N0	103	22	
N1	95	54	
Pathological differentiation			0.917
Well/Moderate	116	44	
Poor	82	32	

CD8+ Tcm, C8+ central memory T cells.

study concerning the relationship of memory T cells with gastric cancer, especial for CD8+ central memory T cells.

In this study, we try to study the percentage of CD8+ central memory T cells in peripheral blood of patients with gastric cancer and analyzed its prognostic significance in operable and advanced gastric cancer.

Materials and methods

Patients

A total of 274 patient undergone curative resection for histologically confirmed stage III gastric cancer at the Department of General Surgery in the Second Xiangya Hospital of Central South University between June 1, 2010 and June 1, 2015 were enrolled in this retrospective study. Inclusion criteria were histologically confirmed stage III gastric cancer, more than 18 years of age and life expectancy more than 6 months. Patients with preoperative acute and severe comorbidity and adjuvant treatments were excluded, such as systemic infection, autoimmune diseases or inflammation and preoperative chemotherapy. Clinical and histopathological features of all patients were collected from the clinical records by one surgeon and check by another surgeon, including gender, age, tumor site, tumor size, T stage, lymph node status, TNM and pathological differentiation. Histopathological and clinic staging were evaluated though postoperative histopathological examination and clinical assessment according to the UICC TNM classification, respectively.

5 ml EDTA-treated blood samples of all patients were obtained on the day before operation to detect the percentage of CD8+ Tcm within 8 h. The blood samples of 102 healthy volunteers were collected to study the percentage of CD8+ Tcm, sever as control.

This study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University. Written informed consent was obtained from individual patients.

Flow cytometry analysis

PBMCs were isolated from 5 ml blood sample though Ficoll-Hypaque (Haoyang Biological Manufacture, Tianjin, China) gradient centrifugation, then stained with antibodies labeled with CD8-FITC, CD62L-PE, CD4-PerCP and CD45RO-APC, incubated with antibodies for 30 min on 4°C in dark. Then, the cells were washed twice with PBS, analyzed using a FACSCalibur flow cytometer (BD, United States). The cells with phenotype of CD8+CD45RO+CD62L+ were considered as CD8+ Tcm. The percentage of CD8+ Tcm was analyzed in the plot of flow cytometer with Cell^{Quest} Software (BD, United States).

Follow-up

All Patients were followed up in regular intervals through outpatient visit including physical examination, laboratory examinations and imaging studies including blood routine test, biochemistry and tumor markers every 3 months for the first 2 years, every 6 months for the next 3 years, and once annually thereafter. Enhanced abdominal CT or MRI scans were

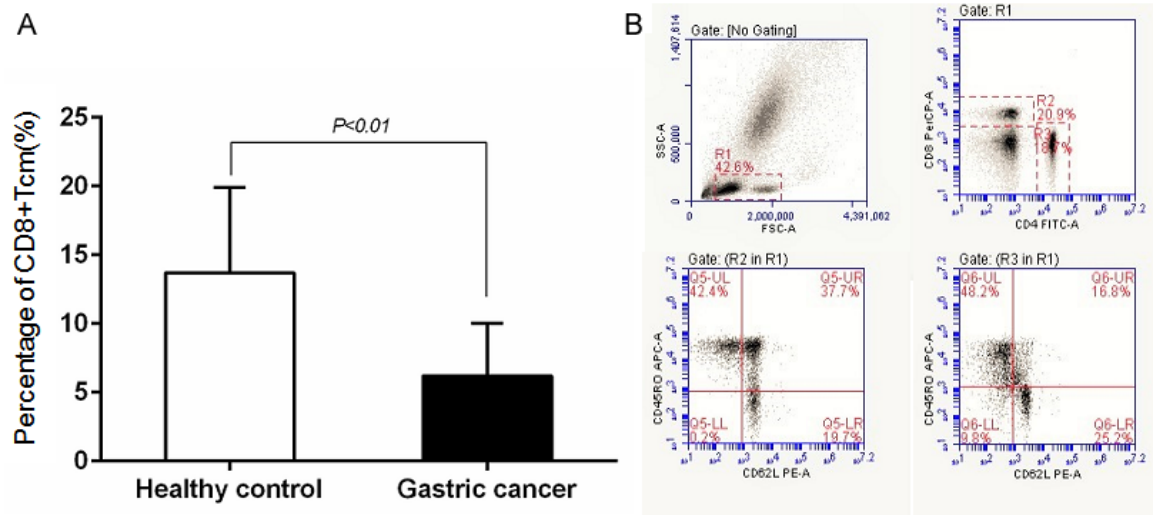

Figure 1. Evaluation of CD8+ Tcm in peripheral blood by flow cytometry. A. Showed the results of the analyzing difference of CD8+ Tcm between patients with gastric cancer (n=274) and healthy control (n=102) (mean percentage of CD8+ Tcm in gastric cancer, 6.2%±3.8%; in healthy subjects, 13.7%±6.2%, P<0.01). B. Showed the plots of flow cytometry data of Tm and its subsets in peripheral blood of gastric cancer.

obtained generally every 12 months. Clinical follow-up lasted from the date of surgery to either the time of death or July 2015.

Statistical analysis

Analyses were performed with SPSS 20.0 (IBM, USA). P<0.05 (two sided) was considered statistically significant. Overall survival was defined as the duration from date of surgery to death, which accurately identified by hospital records and follow-up results. The χ^2 test or Fisher's exact test was used to compare different of qualitative variables between two groups while quantitative values were analyzed by independent student's t test. The overall survival and survival curve was studied in Kaplan-Meier analyses by using the log-rank test. The Cox regression model was used to assess the hazard ratio and multivariate analysis.

Results

Patients

Baseline clinicopathologic characteristics of 274 patient undergone curative resection for histologically confirmed stage III gastric cancer are shown in **Table 1**. There were 192 males and 82 females, with 61±3.2 years average age. The most frequently site involved by tumor was the stomach body, accounted for 125 of 274 patients. There were 90 patients with T1 or T2 tumor, and 184 patients with T3 or T4 tumor according to the TNM staging. In pathology, 160 patients presented as well/moderately differentiation adenocarcinoma while 114 patients had poor differentiation tumor. Moreover, there was no statistical significant association between the percentage of CD8+ Tcm and clinicopathologic characteristics including age and gender of patients, tumor size, histological differentiation type and T stage, except lymph node status (**Table 1**).

CD8+ Tcm in peripheral blood of gastric cancer

In peripheral blood, the mean percentage of CD8+ Tcm from 274 patients with gastric cancer was 6.2%±3.8%, which was significantly statistically lower than that of healthy control (n=102; 13.7%±6.2%; P<0.01), see in **Figure 1**. The median percentage of CD8+ Tcm from patients with gastric cancer was 6.2% (range 0.8%-36.4%), which was considered as cutoff values. All 274 patients with gastric cancer were divided into high CD8+ Tcm (≥6.2%) group and low CD8+ Tcm (<6.2%) group.

Prognostic significance of CD8+ Tcm for gastric cancer

The median follow-up time was 21.4 months (6.1-79.57 months). At the end of this study, 184 (67.15%) of the 274 patients studied had

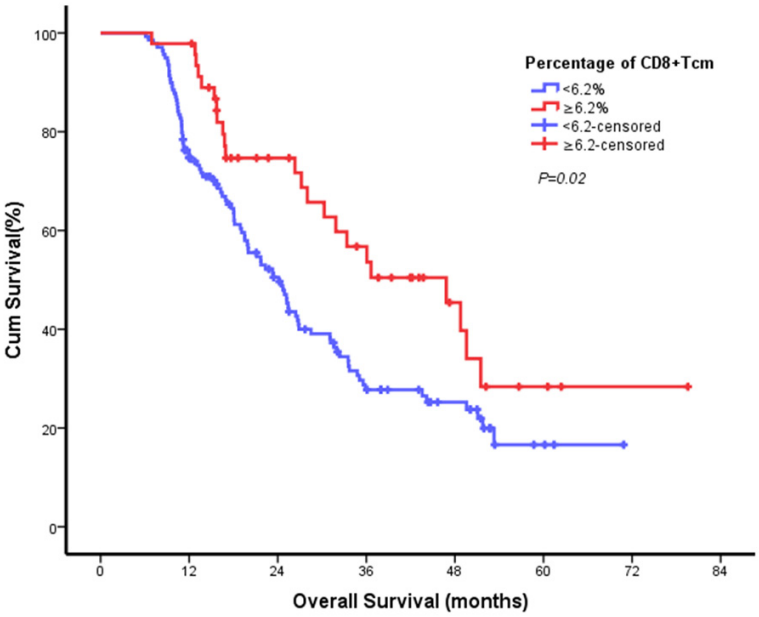

Figure 2. Prognostic significance of CD8+ Tcm for gastric cancer. Panel showed patients with high CD8+ Tcm had a significantly higher median survival than those with low CD8+ Tcm (45.32 months VS 23.52 months, P=0.02).

died. In purpose of evaluating the prognostic significance of preoperative CD8+ Tcm for operable gastric cancer, we found that patients in high CD8+ Tcm group in peripheral blood had a statistically significantly better postoperative prognosis than those with low CD8+ Tcm in overall median survival (45.32 months VS 23.52 months, P=0.02), (**Table 2**; **Figure 2**).

A multivariate analysis enrolled sex and gender of patients, tumor size, histological differentiation type, T stage, the percentage of CD8+ Tcm and lymph node status into the COX regression model to identify independent prognostic factors for operable gastric cancer. The result showed that the percentage of CD8+ Tcm (Hazard ratio, 0.468; 95% CI, 0.279-0.762; P<0.01) and lymph node status (Hazard ratio, 1.033; 95% CI, 0.651-1.314; P<0.05) and pathological differentiation (Hazard ratio, 1.257; 95% CI, 0.832-2.025; P<0.01) were the independent prognostic factors (**Table 2**).

Discussion

Antitumor immunity was considered as the first line of defense to control tumor growth [20]. However, patients with cancer always present as immunosuppression status, especially in advanced diseases [21]. It has been revealed that the peripheral or infiltrating T cells associated with clinical stage and prognosis of tumor [14, 15]. Memory T cell, as the major T lymphocyte subset in malignancy, which can be divided into effector memory (Tem) and central memory (Tcm) T cells distinguished partly by phenotype, homing, and function [12, 13, 22, 23]. CD8+ Tcm, with phenotype of CD8+ CD45RO+CD62L+, can survive long term after antigen elimination and present superior anti-tumor reactivity through promoting migration into lymph nodes and proliferate rapidly in the second response to tumor antigen [24]. Prior studies showed memory T cells had prognostic predictive power superior to standard staging systems [19, 20].

In our study, the percentages of CD8+ Tcm in peripheral blood from gastric cancer patients were statistically lower than those of healthy control, which was consistent with prior studies showed that percentage of memory T cells was lower in patients with gastric cancer [25]. It was suggested tumor associated antigen can induce naive T cells differentiate into memory T cells. However, in advanced cancer, high tumor loading, immunosuppression and immunologic tolerance in tumor microenvironments may decrease the percentage memory T cells, especially for CD8+ Tcm [26, 27]. Otherwise, we also found there was significant association between peripheral CD8+ Tcm and nodal involvement. It has been confirmed that CD8+ Tcm have high proliferative potential due to expression of CD62L, which is necessary for migration to lymph nodes [28]. Continuing releasing of tumor antigens from lymph node metastasis prompts the migration of Tcm to peripheral lymph zones [29].

Furthermore, we respectively analyzed the prognosis of 274 patients underwent curative

Table 2. Prognostic characteristics of patients underwent radical operation for gastric cancer

	Univariate			Multivariate		
	n	MS (months)	*P*	HR	95% CI	P
Age (years)			0.938			
≥60	133	25.40				
<60	141	26.67				
Gender			0.938			
Male	192	24.70				
Female	82	31.07				
Tumor site			0.854			
Gastric cardia	75	24.70				
Gastric body	125	26.80				
Gastric antrum	74	31.07				
Tumor size (cm)			0.182			
≥5	85	24.13				
<5	189	28.50				
Tumor invasion depth			0.486			
T1+T2	90	28.00				
T3+T4	184	24.90				
Lymph node involvement			0.027	1.033	0.651-1.314	0.032
N0	135	35.07				
N1	149	24.13				
Pathological differentiation			0.012	1.257	0.832-2.025	0.002
Well/Moderate	160	27.20				
Poor	114	25.23				
CD8+ Tcm%			0.020	0.468	0.279-0.762	0.001
≥6.2	76	45.32				
<6.2	198	23.52				

MS, median survival; CI, confidence interval; HR, hazard ratio; CD8+ Tcm, C8+ central memory T cells.

operation for advanced gastric cancer, and found percentage of CD8+ Tcm, lymph nodes status and pathological differentiation were independent prognostic factors of primary gastric cancer in both univariate and multivariate analysis. According to such results, central memory T cells, especially CD8+ Tcm, play a curial role in the antitumor immunity of patients with gastric cancer, which may be considered as novel immunity therapy target.

There were several limitations that possibly influence the results. One problem was the retrospective design of study. A large scale multicenter prospective study would allow further confirmation of the prognostic significance of CD8+ Tcm for gastric cancer. Furthermore, we cannot analyze absolute count of CD8+ Tcm, but the percentage of CD8+ Tcm though flow cytometry due to technology limitation and equipment lacking. We will cooperate with other study teams to furtherly confirmed the results in next program.

In conclusion, our study confirmed that patients with advanced gastric cancer have lower percentage of CD8+ Tcm in peripheral blood, which was correlated with lymph nodes metastasis. More important, we found that the percentage CD8+ Tcm was an independent prognostic factor for operable and advanced gastric cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ke Pan, Department of General Surgery, The Second Xiangya Hospital of Central South University, Renmin Middle Road No.139, Changsha 410011, Hunan, PR China. Tel: (86-731)-85295888; Fax: (86-731)-85295888; E-mail: 13278907790@sina.cn

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[3] Huang DD, Chen XX, Chen XY, Wang SL, Shen X, Chen XL, Yu Z, Zhuang CL. Sarcopenia predicts 1-year mortality in elderly patients undergoing curative gastrectomy for gastric cancer: a prospective study. J Cancer Res Clin Oncol 2016; 142: 2347-56.

[4] Lee HH, Park JM, Song KY, Choi MG, Park CH. Survival impact of postoperative body mass index in gastric cancer patients undergoing gastrectomy. Eur J Cancer 2016; 52: 129-37.
[5] Liu XX, Pan HF, Jiang ZW, Zhang S, Wang ZM, Chen P, Zhao Y, Wang G, Zhao K, Li JS. "Fast-track" and "minimally invasive" surgery for gastric cancer. Chin Med J (Engl) 2016; 129: 2294-300.
[6] Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY, Stratton MR. A mutational signature in gastric cancer suggests therapeutic strategies. Nat Commun 2015; 6: 8683.
[7] Alberts SR, Cervantes A, van de Velde CJ. Gastric cancer: epidemiology, pathology and treatment. Ann Oncol 2003; 14 Suppl 2: ii31-6.
[8] Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 2015; 21: 449-56.
[9] Zhao LY, Chen XL, Wang YG, Xin Y, Zhang WH, Wang YS, Chen XZ, Yang K, Liu K, Xue L, Zhang B, Chen ZX, Chen JP, Zhou ZG, Hu JK. A new predictive model combined of tumor size, lymph nodes count and lymphovascular invasion for survival prognosis in patients with lymph node-negative gastric cancer. Oncotarget 2016; 7: 72300-72310.
[10] Zhuo C, Shao M, Chen C, Lin C, Jiang D, Chen G, Tian H, Wang L, Li J, Lin X. Chemotherapy effectiveness and prognosis of gastric cancer influenced by PTPN11 polymorphisms. Cell Physiol Biochem 2016; 39: 1537-52.
[11] Cong X, Li S, Xue Y. [Impact of preoperative lymphocyte to monocyte ratio on the prognosis of the elderly patients with stage II (-III (gastric cancer]. Zhonghua Wei Chang Wai Ke Za Zhi 2016; 19: 1144-8.
[12] Yang S, Gattinoni L, Liu F, Ji Y, Yu Z, Restifo NP, Rosenberg SA, Morgan RA. In vitro generated anti-tumor T lymphocytes exhibit distinct subsets mimicking in vivo antigen-experienced cells. Cancer Immunol Immunother 2011; 60: 739-49.
[13] Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999; 401: 708-12.
[14] Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 2004; 22: 745-63.
[15] Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, Palmer DC, Antony PA, Hwang ST, Rosenberg SA, Waldmann TA, Restifo NP. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc Natl Acad Sci U S A 2005; 102: 9571-6.
[16] Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005; 353: 2654-66.
[17] Lee HE, Chae SW, Lee YJ, Kim MA, Lee HS, Lee BL, Kim WH. Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br J Cancer 2008; 99: 1704-11.
[18] Enomoto K, Sho M, Wakatsuki K, Takayama T, Matsumoto S, Nakamura S, Akahori T, Tanaka T, Migita K, Ito M, Nakajima Y. Prognostic importance of tumour-infiltrating memory T cells in oesophageal squamous cell carcinoma. Clin Exp Immunol 2012; 168: 186-91.
[19] Liu HL, Guan CJ, Wu YJ, Hu MG, Zhao ZM, Liu R. Clinical significance of preoperative CD8+ central memory t cells for operable pancreatic adenocarcinoma. Dig Surg 2015; 32: 433-8.
[20] Chekmasova AA, Brentjens RJ. Adoptive T cell immunotherapy strategies for the treatment of patients with ovarian cancer. Discov Med 2010; 9: 62-70.
[21] DeVita VT Jr, Rosenberg SA. Two hundred years of cancer research. N Engl J Med 2012; 366: 2207-14.
[22] Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. J Clin Invest 2008; 118: 294-305.
[23] Gattinoni L, Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, Yu Z, Finkelstein SE, Theoret MR, Rosenberg SA, Restifo NP. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. J Clin Invest 2005; 115: 1616-26.
[24] Wang X, Berger C, Wong CW, Forman SJ, Riddell SR, Jensen MC. Engraftment of human central memory-derived effector CD8+ T cells in immunodeficient mice. Blood 2011; 117: 1888-98.
[25] Zhang R, Li F, Li H, Yu J, Ren X. The clinical significance of memory T cells and its subsets in gastric cancer. Clin Transl Oncol 2014; 16:257-65.

[26] Shimizu K, Kuriyama H, Kjaergaard J, Lee W, Tanaka H, Shu S. Comparative analysis of antigen loading strategies of dendritic cells for tumor immunotherapy. J Immunother 2004; 27: 265-72.
[27] Zhou Y, Bosch ML, Salgaller ML. Current methods for loading dendritic cells with tumor antigen for the induction of antitumor immunity. J Immunother 2002; 25: 289-303.
[28] Groom JR. Moving to the suburbs: T-cell positioning within lymph nodes during activation and memory. Immunol Cell Biol 2015; 93: 330-6.
[29] Kodera M, Grailer JJ, Karalewitz AP, Subramanian H, Steeber DA. T lymphocyte migration to lymph nodes is maintained during homeostatic proliferation. Microsc Microanal 2008; 14: 211-24.