Peripheral CD4CD8 Double Positive T Cells: A Potential Marker to Evaluate Kidney Damage Susceptibility During SLE

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Research

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Abstract

Background: Lupus nephritis (LN) has a high incidence in Systemic lupus erythematosus (SLE) patients, but there is a lack of sensitive predictive markers and mechanisms. The purpose of the study is to reveal the association between the CD4+CD8+ double positive T lymphocytes (DPT) and lupus nephritis (LN), and to investigate the immune mechanism of LN.

Methods: The study is composed of 395 samples from the General Hospital of Western Theater Command. Collected patients include SLE, lupus nephritis (LN), nephritic syndrome (NS) and nephritis patients. Peripheral blood lymphocyte subsets were performed by the Flow cytometry method. Biochemical measurements were performed in accordance with the recommendations proposed by national center for clinical laboratories.

Results: The proportions of DPT cells in LN group were significantly higher than in SLE group ($t=4.012$, $p<0.001$), NS group ($t=3.240$, $p=0.001$) and nephritis group ($t=2.57$, $p=0.011$). In LN group, the risk of kidney damage increased significantly in the DPT cell proportion dependent manner. In cases of high DPT cells proportion, the risk of LN was 5.136 times higher than when the proportion DPT cell was within the normal range. Moreover, hypertriglyceridemia and hyperuricemia were also independent risk factors.

Conclusion: The proportion of DPT cells was a potential marker to evaluate LN susceptibility. When assessing the risk of kidney damage during SLE with DPT cell proportion, we can effectively exclude the interference of NS and nephritis.

Introduction

Systemic lupus erythematosus (SLE), as an autoimmune inflammatory disease, is usually characterized by multiple organ damage. Various tissues and organs can be attacked by immune complex deposition and lymphocyte infiltration in SLE. Renal involvement is the most common in SLE patients. Lupus nephritis (LN) constitutes one of the main clinical challenges in patients and is a cause of significant morbidity and mortality[1]. The LN cumulative incidence is relatively high in Asians (55%) and African lineages (51%) with SLE. The outcome of patients with newly diagnosed LN was significantly better in recent years[2]. Especially early diagnosis and treatment of LN can greatly improve the prognosis of patients with SLE[3]. However, there is a lack of high sensitivity and coincidence markers for early diagnosis of LN.

Peripheral CD4 + CD8 + double-positive T lymphocytes (DPT) are regarded as extrathymic cells, and rarely be described because of the small proportion in humans. Recently years, people pay more attention to DPT cells due to the improvement of detection technology. Studies have shown that increased proportions of peripheral DPT cell were observed in target organs of various autoimmune diseases and acute viral infections. However, the role and repartition of extrathymic DPT cell remain largely uncharacterized[4]. The purpose of this study was to explore the predictive value of DPT cell in the occurrence of LN in SLE patients by retrospective analysis method.

Methods

Study design and participants

This study was conducted between 2017 and 2019, in the population covered by the General Hospital of Western Theater Command, constituted by 395 individuals with renal impairment or SLE. In all of participant, there were 79 patients with SLE, 100 patients with LN, 108 patients with nephrotic syndrome (NS) and 108 patients with nephritis (including glomerulonephritis and pyelonephritis). This study consisted on a detailed questionnaire and physical examination. A blood sample for further biochemical analysis was also collected. Participants were selected using a simple random sampling scheme. Diagnosis of SLE, LN, NS and nephritis is according to the WHO diagnostic criteria.

In this research, inclusion criteria of SLE are chronic, inflammatory, variable autoimmune disease of connective tissue that occurs chiefly in women and is typically characterized by fever, skin rash, fatigue, joint pain, and no kidney damage. The inclusion criteria of LN are glomerulonephritis associated with systemic lupus erythematosus that is typically characterized by proteinuria and hematuria and that often leads to renal failure. The inclusion criteria of nephrotic syndrome are an abnormal condition that is marked by deficiency of albumin in the blood and its excretion in the urine due to altered permeability of the glomerular basement membranes. The inclusion criteria of nephritis is acute or chronic inflammation of the kidney caused by infection, degenerative process, or vascular disease. All participants were given a brief description of the objectives of the study, after which they signed an informed consent form.

Measurements and blood sample collection

Flow cytometry was performed to detect peripheral blood lymphocyte subsets on the BD FACS CANTO flow cytometer (BD Corporation, USA). The test reagent (BD Multitest) was purchased from BD. Biochemical measurements, including total cholesterol (CHO), triglyceride (TG), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), Lipoprotein a (Lpa), Apolipoprotein a (ApoA), Apolipoprotein b (ApoB), cystatin C (Cys-C), blood urea nitrogen(BUN), Creatinine serum(CREA), Creatinine clearance rate (CCR), Uric acid (UA), Microalbuminuria (mALBU), urine creatinine (UCR), fibrinogen (FIB), D-dimer and High-sensitivity C-reactive protein (hs-CRP), were performed in accordance with the recommendations proposed by national center for clinical laboratories.

Flow cytometry

Flow cytometry was performed using a BD FACS CANTO ™ (BD Biosciences, USA). The reagent cocktail (10μl) containing CD3 fluorescein isothiocyanate (FITC), CD8 phycoerythrin (PE), CD45 peridinin chlorophy protein (PerCP) and CD4 allophycocyanin (APC) were added to 50 μl EDTA anticoagulated whole blood, and the samples were mixed and incubated for 30 min at room temperature in the dark. Erythrocytes were lysed by adding 500μl of ammonium chloride haemolysis agent for 15 min. The cells were washed, incubated with paraformaldehyde 2% in PBS, harvested on a FACS Canto [5]. A four-color direct immunofluorescence antibody is used for identifying and determining the percentages and

absolute counts of mature human T lymphocytes (CD3+), suppressor/cytotoxic (CD3+CD8+) T-lymphocyte subsets, and helper/inducer (CD3+CD4+) T-lymphocyte subsets in erythrocyte-lysed whole blood (Fig. 1).

Statistical analysis

The mean values of total clinical test results were collected in three months (once every month). Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were reported accounts and percentages. Analyses of t-tests and chi-square tests were used to test for differences between groups for continuous and categorical variables, respectively. Multivariate logistic regression analysis was used to identify independent predictors of nephropathy[6]. Analyses were performed using SPSS version 19.0 statistical software. A value of $P < 0.05$ (two-sided) was considered statistically significant. The P value is accurate to three decimal places when we calculated using SPSS.

Presentation Of Results

Overall, 395 individuals were enrolled in this study, including 154 cases of male, 241 cases of female. In all the cases, there were 79 patients with SLE, 100 patients with LN, 108 patients with nephrotic syndrome (NS) and 108patients with nephritis (including glomerulonephritis and pyelonephritis).

3.1 Test results and comparative analysis between SLE group and LN group

Because of the high probability of lupus nephritis in patients with SLE, we compared the clinical test results of the SLE group and the lupus nephritis group (Table 1). The proportions of sex and age were no significant difference between in the SLE group and the lupus nephritis group. As compared with SLE group, the levels of CHO, LDL-C, Lpa, ApoB and FIB were significantly higher in the lupus nephritis group in blood fatty and coagulative function detection. In liver and renal function biochemical examination, the levels of A/G, BUN, CREA, Cys-C, UA, mALBU and mALBU/UCR were significantly higher in lupus nephritis group than in the SLE group. In lymphocyte subsets examination, the proportion of CD3 + CD4 + CD8+ (DPT cell) was also significantly higher in lupus nephritis group than in the SLE group. There were no significant associations between 2 groups in the levels of TG, HDL-C, ApoA, D-Dimer, hs-CRP, CD3 + CD4 + CD8-, CD3 + CD4-CD8+, CD3 + CD4-CD8-and T4/T8.

Table 1
Clinical test information of the SLE group and the Lupus nephritis group.

Variable	SLE (n = 79)	Lupus nephritis (n = 100)	t/χ2 value	P value
Male (%)	14.10	13.00	0.035	0.852
Age (year)	36.54±14.15	40.82±13.79	1.557	0.121
CHO (mmol/L)	4.31±1.21	5.07±1.55	3.285	0.001
TG (mmol/L)	1.77±2.22	1.96±0.96	0.703	0.483
HDL-C (mmol/L)	1.44±0.58	1.48±0.55	0.417	0.667
LDL-C (mmol/L)	2.29±0.82	2.79±1.13	2.958	0.004
Lpa	170.81±153.08	294.15±310.48	2.601	0.011
ApoA	1.30±0.36	1.34±0.37	0.454	0.651
ApoB	0.76±0.30	0.90±0.36	2.343	0.021
A/G	1.71±0.38	1.51±0.44	-5.638	0.000
BUN(mmol/L)	4.99±1.57	10.16±8.48	5.303	0.000
CREA (umol/L)	69.63±23.84	135.21±140.41	4.076	0.000
Cys-C (mg/L)	0.91±0.26	1.87±1.22	6.674	0.000
CCR (ml/min)	95.09±19.61	64.85±46.88	-5.267	0.000
UA (umol/L)	305.15±118.00	402.37±162.85	4.421	0.000
mALBU (mg/L)	29.04±68.60	1819.57±3357.35	4.092	0.000
mALBU/UCR(mg/g)	38.44±110.04	2400.72±5912.72	3.066	0.003
FIB	2.87±0.98	3.40±1.23	2.717	0.007
D-Dimer	1.85±1.79	3.22±6.50	1.159	0.250
hs-CRP (mg/L)	0.99 ± 0.98	1.45 ± 2.30	1.617	0.108
CD3 + CD4 + CD8-	32.59±9.92	32.15±8.89	-0.315	0.753
CD3 + CD4-CD8+	33.69±11.95	37.00±10.50	1.968	0.051
CD3 + CD4 + CD8+	0.40±0.35	0.84±0.91	4.012	0.000
CD3 + CD4-CD8-	3.59±2.85	3.55±2.45	-0.096	0.924
T4/T8	1.18±0.77	0.99±0.60	-1.770	0.078

3.2 The proportion of DPT cells is high risk factor for nephropathy in SLE patients

As shown in Table 2, the proportion of DPT cells was an independent risk factor for nephropathy in SLE patients (odds ratio [OR] 5.136, 95% confidence interval [CI] 2.115 ~ 12.473; P < 0.001) by multivariate logistic regression analysis. Hyperuricemia (OR = 3.285, P = 0.001, 95% CI = 1.597 ~ 6.757) and hypertriglyceridemia (OR = 2.617, P = 0.013, 95% CI = 1.288 ~ 5.577) may also contribute to the deterioration of LN in patients with SLE. No significant associations between any of the other factors (hypercholesterolemia, hyperuricemia, Sex) and nephropathy were observed in SLE patients.

Table 2
Multivariate logistic regression analysis of independent risk factor for renal impairment in systemic lupus erythematosus.

Independent risk factor	95% CI	P value	OR value
hypercholesterolemia	0.505 ~ 3.742	0.534	1.375
hypertriglyceridemia	1.288 ~ 5.577	0.013	2.617
hyperuricemia	1.597 ~ 6.757	0.001	3.285
Sex	0.264 ~ 2.065	0.563	0.738
CD4 + CD8 + DPT	2.115 ~ 12.473	0.000	5.136

3.3 The proportion of DPT cell in LN group is obviously higher than that in NS group

To further determine the impact of DPT cells on the Lupus nephritis development process, we compared the clinical test results of the nephritic syndrome group and the lupus nephritis group (Table 3). The proportions of sex and age were significant difference between in the nephritic syndrome group and the lupus nephritis group. As compared with nephritic syndrome group, the levels of HDL-C, LDL-C, ApoA, ApoB and FIB were significantly lower in the lupus nephritis group in blood fatty and coagulative function detection. In liver and renal function biochemical examination, the level of A/G was higher in lupus nephritis group than in the SLE group, but the levels of hs-CRP and mALBU were higher. In lymphocyte subsets examination, the proportion of CD3 + CD4-CD8+, CD3 + CD4 + CD8 + and T4/T8were also significantly higher in lupus nephritis group than in the SLE group. There were no significant associations between 2 groups in the levels of CHO, TG, Lpa, ApoA, BUN, CREA, Cys-C, CCR, mALBU/UCR, D-Dimer, hs-CRP, CD3 + CD4 + CD8- and CD3 + CD4-CD8-.

Table 3
Clinical test information of the nephritic syndrome group and the Lupus nephritis group.

Variable	nephritic syndrome (n = 108)	Lupus nephritis (n = 100)	t/χ2 value	P value
Male (%)	55.56	13.00	20.59	0.000
Age (year)	46.45±18.68	40.82±13.79	-2.895	0.004
CHO (mmol/L)	7.21±3.04	5.07±1.55	-0.631	0.529
TG (mmol/L)	2.48±1.62	1.96±0.96	1.336	0.183
HDL-C (mmol/L)	1.81±0.73	1.48±0.55	-3.250	0.001
LDL-C (mmol/L)	4.18±2.20	2.79±1.13	-4.82	0.000
Lpa	410.88±392.15	294.15±310.48	-1.755	0.081
ApoA	1.57±0.63	1.34±0.37	-2.608	0.010
ApoB	1.37±0.63	0.90±0.36	-5.182	0.000
A/G	1.29±0.47	1.51±0.44	2.534	0.012
BUN(mmol/L)	8.91±6.20	10.16±8.48	1.220	0.224
CREA (umol/L)	109.37±90.96	135.21±140.41	1.582	0.115
Cys-C (mg/L)	1.63±1.17	1.87±1.22	1.365	0.174
CCR (ml/min)	66.95±26.19	64.85±46.88	-0.389	0.698
UA (umol/L)	408.08±122.77	402.37±162.85	-0.285	0.776
mALBU (mg/L)	5212.46±7287.50	1819.57±3357.35	-3.793	0.000
mALBU/UCR(mg/g)	3237.29±3670.86	2400.72±5912.72	-1.138	0.257
FIB	4.18±1.64	3.40±1.23	-3.282	0.001
D-Dimer	3.31±4.19	3.22±6.50	-0.092	0.927
hs-CRP (mg/L)	21.15 ± 57.17	1.45 ± 2.30	-3.373	0.001
CD3 + CD4 + CD8-	34.18±10.47	32.15±8.89	-0.510	0.135
CD3 + CD4-CD8+	27.58±10.31	37.00±10.50	6.525	0.000
CD3 + CD4 + CD8+	0.53±0.36	0.84±0.91	3.240	0.001
CD3 + CD4-CD8-	3.29±2.11	3.55±2.45	0.816	0.415
T4/T8	1.52±1.03	0.99±0.60	-4.446	0.000

3.4 The proportion of DPT cell in LN group is obviously higher than that in nephritis group

To further determine the impact of DPT cells on the Lupus nephritis development process, we compared the clinical test results of the nephritis group and the lupus nephritis group (Table 4). The proportion of sex was significant difference between in the nephritic syndrome group and the lupus nephritis group. In blood fatty and coagulative function detection, there were no significant associations in the nephritis group and the lupus nephritis group. In liver and renal function biochemical examination, the level of BUN and Cys-C was higher in lupus nephritis group than in the nephritis group, and the level of hs-CRP was lower in lupus nephritis group than in the nephritis group. In lymphocyte subsets examination, the proportion of CD3 + CD4-CD8+, CD3 + CD4 + CD8+, CD3 + CD4-CD8- and T4/T8 were significantly higher in lupus nephritis group than in the SLE group. There were no significant associations between 2 groups in the levels of age, CHO, TG, HDL-C, LDL-C, Lpa, ApoA, ApoB, A/G, CCR, UA, mALBU, mALBU/UCR, FIB, D-Dimer and CD3 + CD4 + CD8-.

Table 4
Clinical test information of the nephritis group and the Lupus nephritis group.

Variable	nephritis (n = 108)	Lupus nephritis (n = 100)	t/χ2 value	P value
Male (%)	65.00	13.00	19.018	0.000
Age (year)	40.85±16.46	40.82±13.79	-0.377	0.707
CHO (mmol/L)	4.57±1.41	5.07±1.55	1.630	0.106
TG (mmol/L)	1.64±0.98	1.96±0.96	1.613	0.110
HDL-C (mmol/L)	1.31±0.39	1.48±0.55	1.603	0.112
LDL-C (mmol/L)	2.60±1.06	2.79±1.13	0.856	0.394
Lpa	171.11±136.54	294.15±310.48	1.834	0.070
ApoA	1.29±0.32	1.34±0.37	0.557	0.579
ApoB	0.87±0.37	0.90±0.36	0.430	0.668
A/G	1.57±0.49	1.51±0.44	-0.668	0.505
BUN(mmol/L)	7.12±6.23	10.16±8.48	2.058	0.042
CREA (umol/L)	123.38±139.95	135.21±140.41	0.446	0.657
Cys-C (mg/L)	1.28±0.61	1.87±1.22	2.763	0.007
CCR (ml/min)	77.23±29.46	64.85±46.88	-1.477	0.142
UA (umol/L)	354.91±124.35	402.37±162.85	1.654	0.100
mALBU (mg/L)	1083.72±1485.24	1819.57±3357.35	1.208	0.230
mALBU/UCR(mg/g)	897.33±1537.22	2400.72±5912.72	1.437	0.154
FIB	3.57±1.56	3.40±1.23	-0.598	0.551
D-Dimer	3.86±5.94	3.22±6.50	-0.352	0.726
hs-CRP (mg/L)	19.24 ± 35.04	1.45 ± 2.30	-4.975	0.000
CD3 + CD4 + CD8-	34.32±9.74	32.15±8.89	-1.267	0.207
CD3 + CD4-CD8+	26.02±8.13	37.00±10.50	5.934	0.000
CD3 + CD4 + CD8+	0.46±0.36	0.84±0.91	2.579	0.011
CD3 + CD4-CD8-	4.76±3.99	3.55±2.45	-2.177	0.031
T4/T8	1.53±0.88	0.99±0.60	-4.137	0.000

3.5 Individuals' biochemical and immunologic results difference in different CCR stages

To further illustrate the differences of the above significant indexes in different kidney damage degree, LN, NS and nephritis groups, the trend chart was plotted among the LN, NS and nephritis groups in Fig. 2. We analyzed individuals' biochemical and immunologic results difference including mALBU/UCR, A/G, CD3 + CD4 + CD8 + cells (DPT cells) and CHO in different CCR stages. The mALBU/UCR values increases with CCR decreases. When CCR < 30, the mALBU/UCR values of NS group were significantly higher than LN group and nephritis group (Fig. 2A). The A/G values decreases with CCR decreases, and there was no significant difference in three groups (Fig. 2B). AS Fig. 2C showed, the proportion of DPT cell in LN group were significantly higher than in NS group and nephritis group. In addition, the proportion of DPT cell increases with CCR decreases. In NS group, the levels of CHO were no significant difference indifferent CCR stages, but the CHO results of NS group were significantly higher than LN group and nephritis group when CCR values over 30. The above results indicate that mALBU/UCR and A/G were highly dependent on kidney damage. In LN group, the proportions of DPT cell increased in Creatinine clearance rate dependent manner. When CCR value range from 30 to 70, the CHO concentration in NS group was significantly higher than in LN group and nephritis group. Besides, the degree of renal injury was positively correlated with the concentration of CHO.

3.6 Comparative analysis and distribution map of CD4 + CD8 + DPT in three groups

There were significant differences between SLE group and LN group by using t-test analysis of the proportion of DPT cell ($t = 4.012$, $p < 0.001$). As compared with NS group and nephritis groups, the proportions of DPT cell were significantly higher in the lupus nephritis group ($t = 3.240$, $p = 0.001$; $t = 2.57$, $p = 0.011$)(Fig. 3).

Discussion

Systemic lupus erythematosus is an autoimmune disease with a worldwide distribution and always accompanied by the production of autoantibodies. Lupus nephritis remains a major cause of morbidity and mortality in patients with systemic lupus erythematosus[7]. Although the renal prognosis has improved, there are still major challenges remain in the management of disease progression and treatment of the disease due to the lack of early diagnosis method[8].

Immature T cells expressed both CD4 and CD8 were traditionally thought to be the ancestor of T lymphocytes and to undergo thymic development[9]. After T cells had transitioned to naive T cells and exited the thymus, the CD4 or CD8could no longer be expressed. Research has shown that healthy humans exhibit low proportions DPT cells in peripheral blood[10]. At present, the origin and function of DPT cells are not clear. However, several mainstream views hold that DPT cells might trace back to immature CD4 + CD8 + thymocytes, mature CD4 + single positive T cells, or mature CD8 + single positive T cells[11]. Therefore, peripheral blood CD4 + CD8 + DPT cells are regarded as extrathymic cells. In the present study, an increase in the proportion of DPT cell are associated with kidney damage during SLE. The proportion of DPT cell in LN group were significantly higher than those in NS group and nephritis

group. So, when assessing the risk of kidney damage during SLE with DPT cell proportion, we can effectively exclude the interference of NS and nephritis.

In recent years, there has been more and more research on DPT cells, and it has been found that DPT cells plays an important role in the pathogenesis of autoimmune diseases and infectious diseases[12]. Wu *et al* in 2014 have reported that the DPT cells play a key suppressive role in the production of autoantibodies in SLE[13]. In infectious diseases, DPT cells are a group of cells with rapid response during acute HIV infection, which is different from the conventional T cell compartments[14].

In our study, $CD4CD8^{dim}$ was found to be the most common in patients with lupus nephritis, and $CD4CD8^{bright}$ was extremely rare (Fig. 4). However, $CD4CD8^{bright}$ was found in groups of SLE, NS and nephritis, but there was no renal injury. The results suggest that $CD4CD8^{dim}$ DPT may play a more important role in the development of renal injury in SLE. The specific reasons and mechanisms need to be further studied.

Conclusion

To conclude, the association between LN and the proportion of DPT cells is extremely significant, which suggests that the proportion of DPT cells is related to the LN susceptibility in SLE patients. High proportion of DPT cells is an independent risk factor for LN, and the risk of LN is 5.136 times higher than normal proportion in SLE patients. The proportion of DPT cells is a potential maker to evaluate LN susceptibility in SLE patients. Furthermore, the deteriorative degree of nephropathy is more and more obvious as the proportion of DPT cells raised. On the basis of our current results, we speculate that DPT cells are a rapid response to the production of autoantibodies in SLE. The increased proportion of DPT cells reflects the increase of autoantibodies, the severity of disease and the destruction of renal tissue caused by immune complex.

Abbreviations

SLE: Systemic lupus erythematosus

LN: Lupus nephritis

DPT: CD4+CD8+ double-positive T lymphocytes

NS: nephrotic syndrome

CHO: total cholesterol

TG: triglyceride

HDL-C: High-density lipoprotein cholesterol

LDL-C: Low-density lipoprotein cholesterol

Lpa: Lipoprotein a

ApoA: Apolipoprotein a

ApoB: Apolipoprotein b

Cys-C: cystatin C

BUN: blood urea nitrogen

CREA: Creatinine serum

CCR: Creatinine clearance rate

UA: Uric acid

mALBU: Microalbuminuria

UCR: urine creatinine

FIB: fibrinogen

hs-CRP: High-sensitivity C-reactive protein

FITC: fluorescein isothiocyanate

PE: phycoerythrin

PerCP: peridinin chlorophy protein

APC: allophycocyanin

Declarations

Ethics approval and consent to participate

The protocol for this study was approved by The General Hospital of Western Theater Command Review Board, Chengdu, China. Consent to participate was not necessary because the study was retrospective.

Consent for publication

Not applicable.

Availability of supporting data

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

The conception and design were performed by KC and ZYJ. The methodology was developed by KC and YYW. The acquisition of data was conducted by KC, YYW and CXL. The analysis and interpretation of data were performed by WLN and HXX. The writing, review, and/or revision of the manuscript were completed by KC, HXX and ZYJ. Administrative, technical, or material support was given by CXL. Study supervision was performed by KC and ZYJ. All authors read and approved the final manuscript.

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Figures

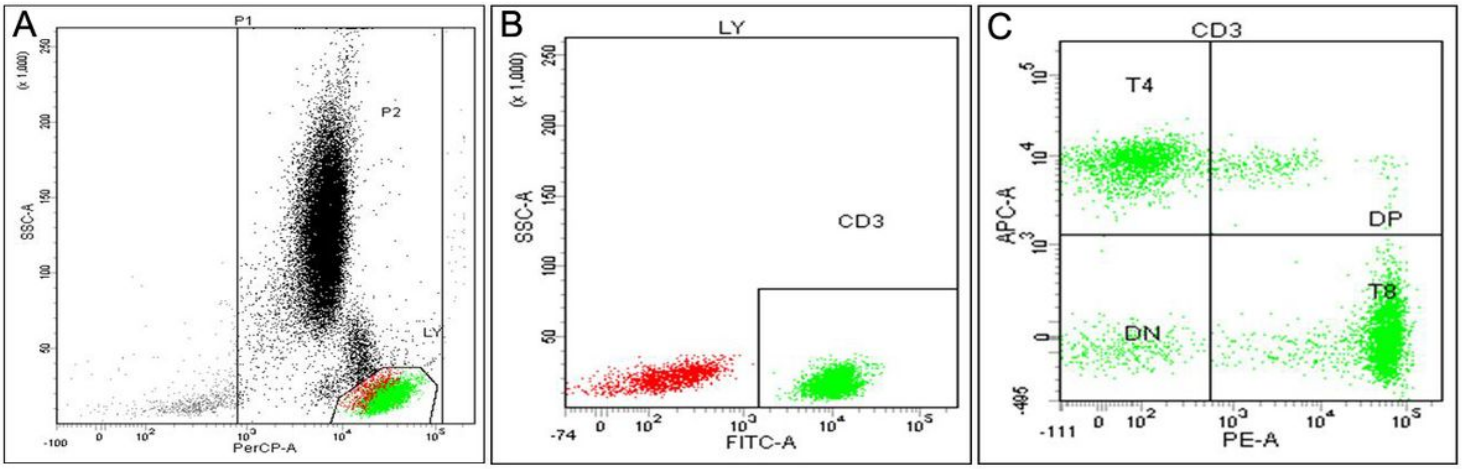
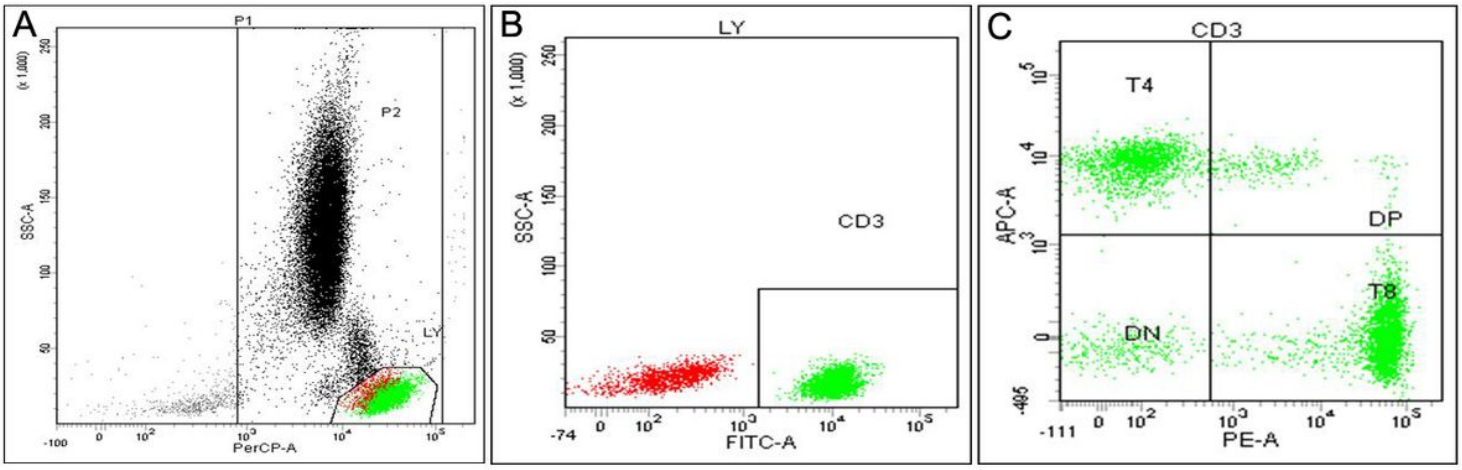


Figure 1

The maps of target lymphocytes and DPT cells by flow cytometry. A: Differentiation of lymphocytes by CD45-PerCP and SSC; B: Differentiation of T lymphocytes by CD3-FITC; C: Differentiation of DPT lymphocytes by CD8-PE and CD4-APC.



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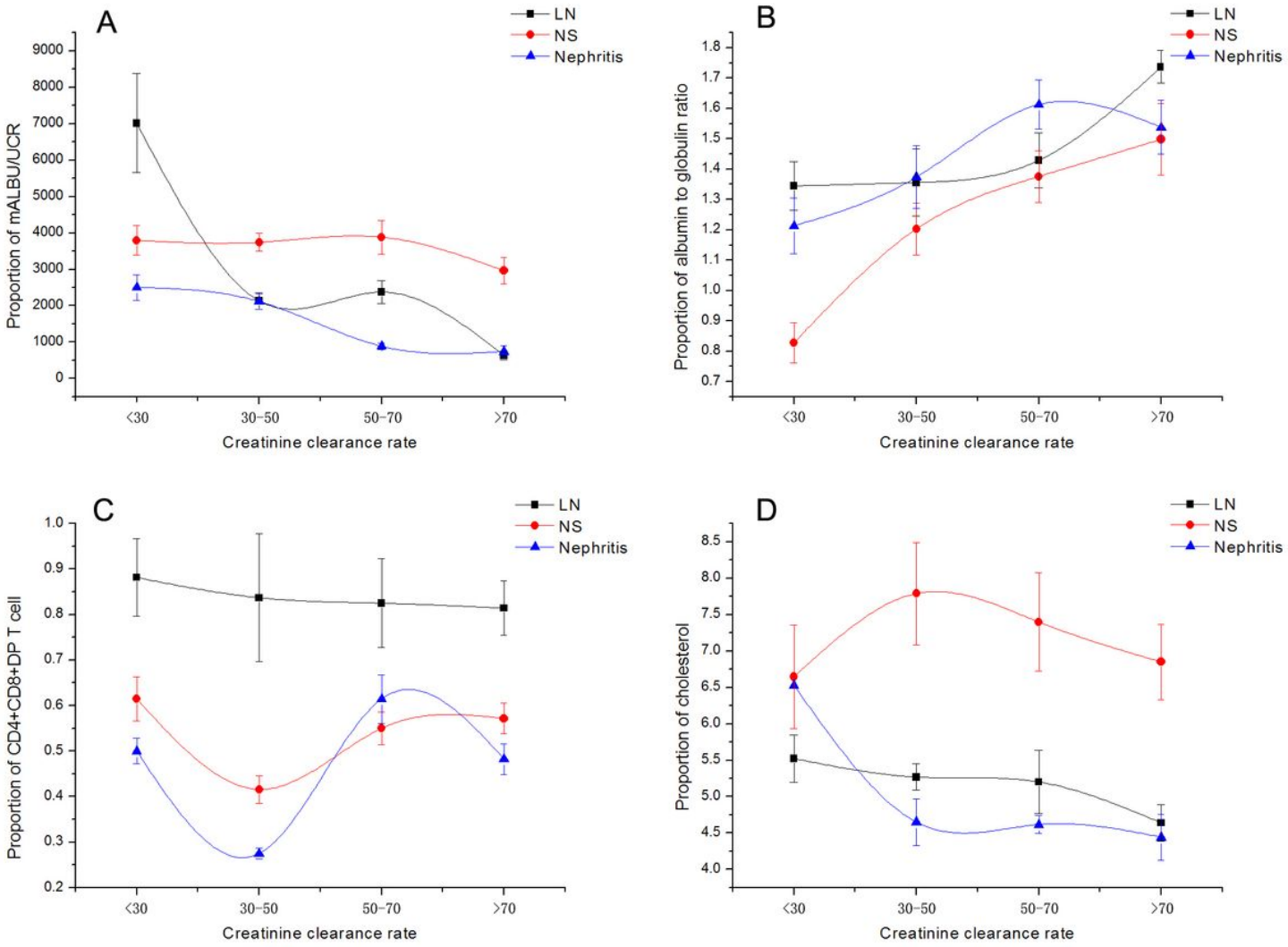
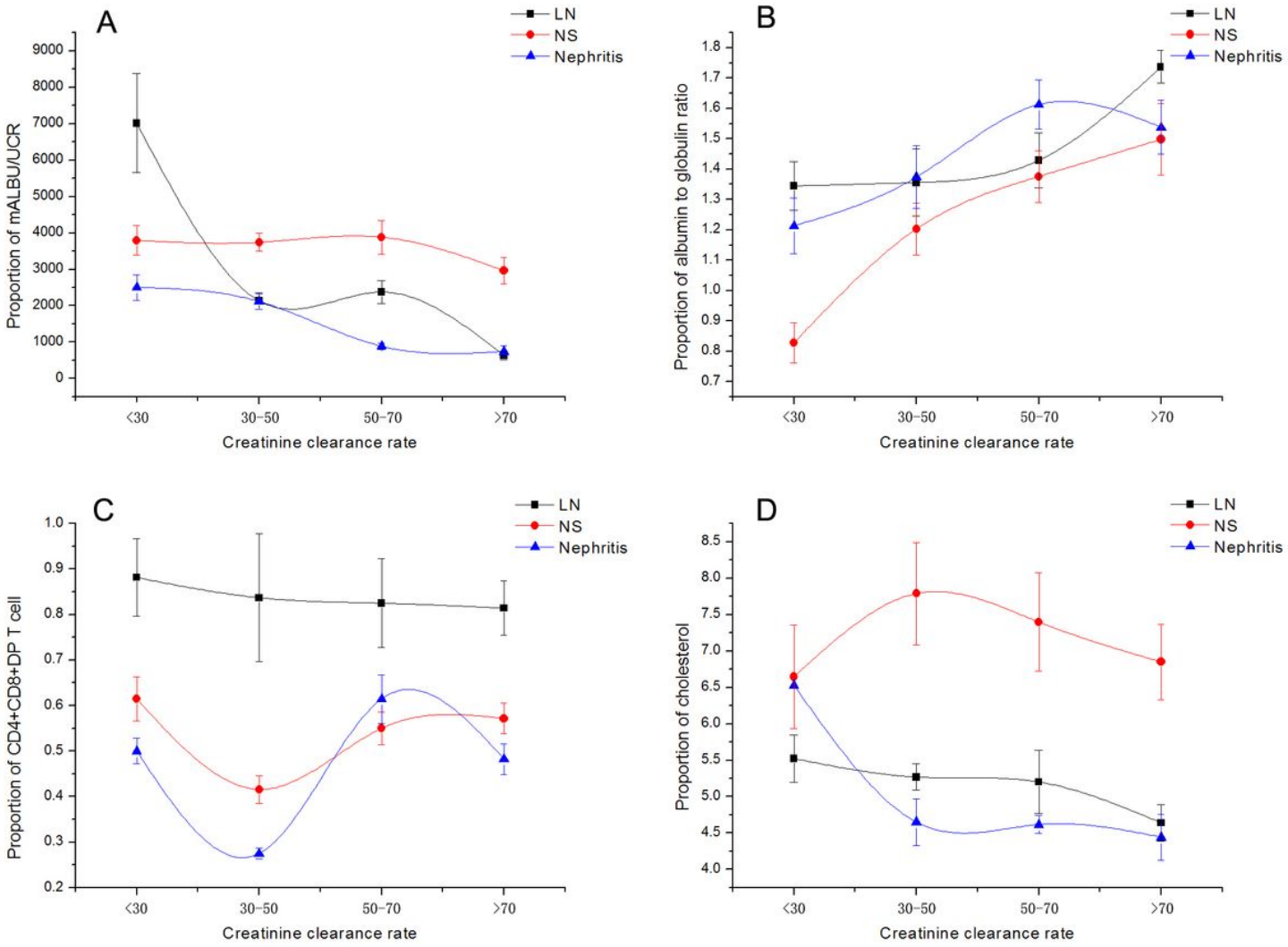


Figure 2

the trend chart of mALBU/UCR, A/G, DPT cells and CHO among the LN, NS and nephritis groups. Results represented means±standard deviation; A: the trend chart of mALBU/UCR in different creatinine clearance rate (CCR); B: the trend chart of A/G in different CCR; C: the trend chart of DPT cell in different CCR; D: the trend chart of CHO in different CCR.



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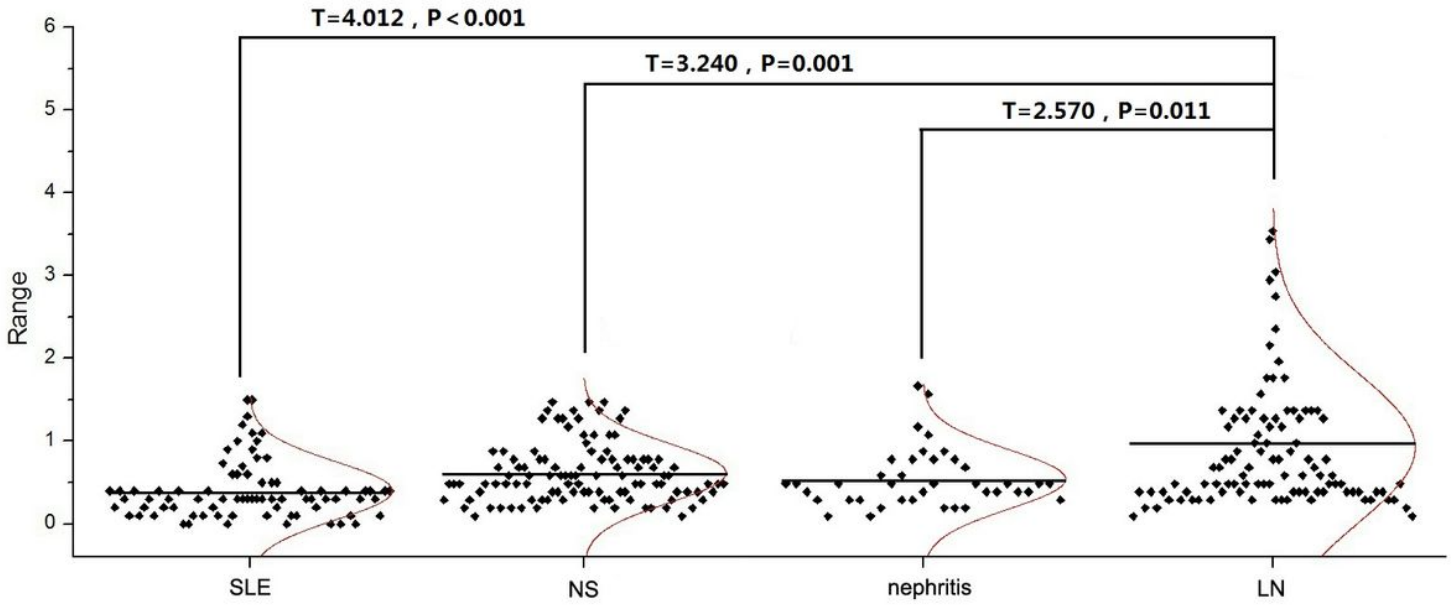
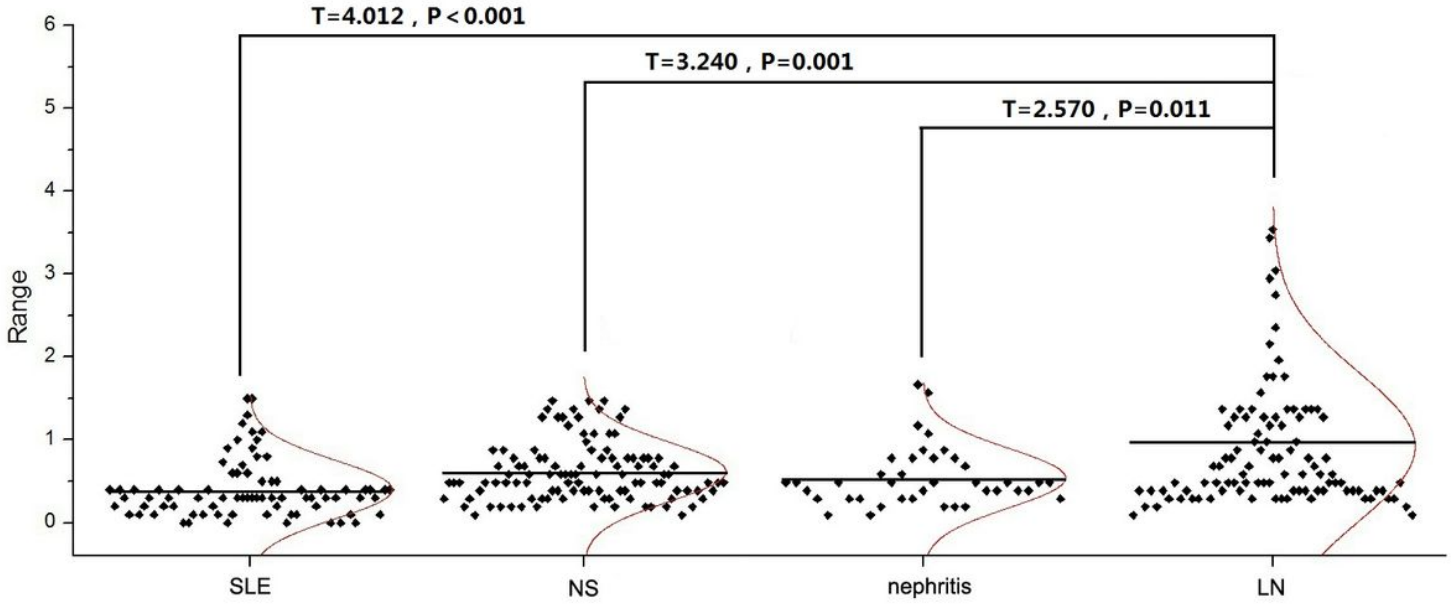


Figure 3

Comparative analysis and distribution map of DPT in three groups.



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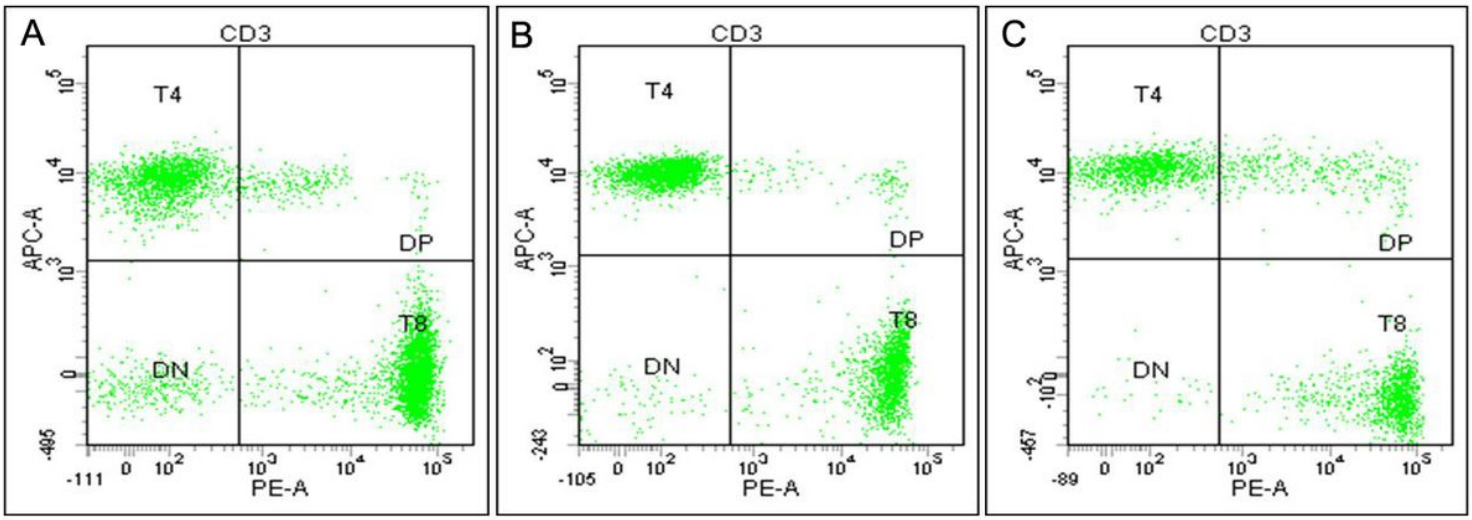
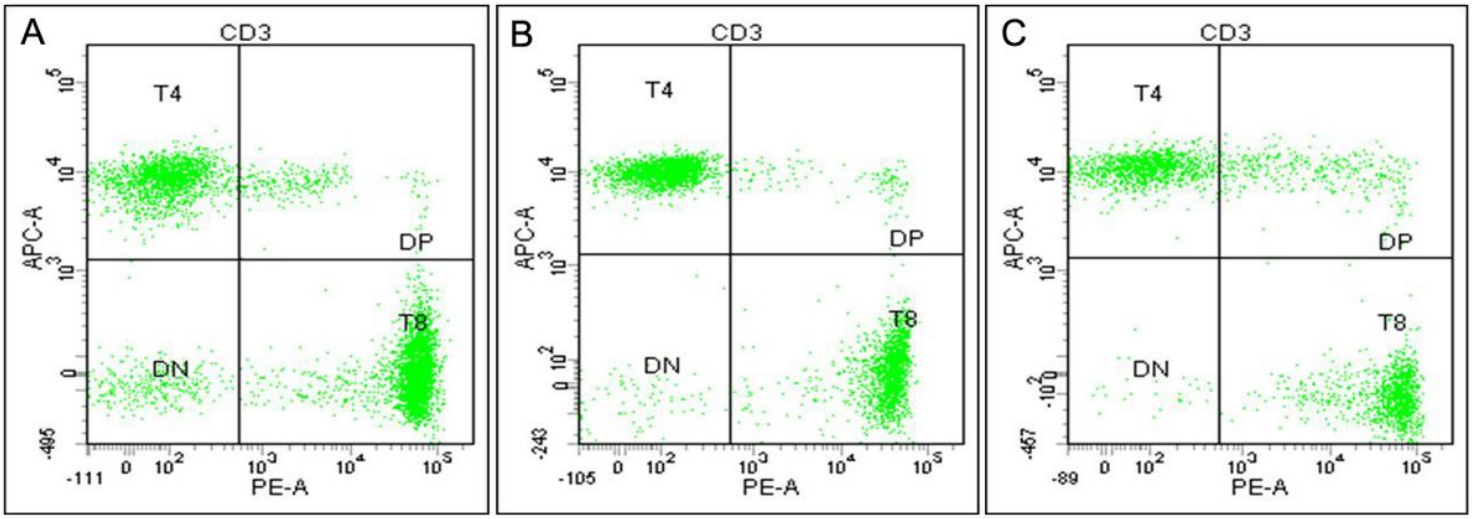


Figure 4

Flow cytometry maps of DPT cells with different fluorescence intensity. A: Flow cytometry maps of CD4CD8dim; B: Flow cytometry maps of CD4CD8bright; C: Flow cytometry maps of CD4CD8bright+dim;



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