·基础研究·

EZH1/2 抑制剂 UNC1999 对外周血免疫细胞表型的影响

刘丹平[1]，池沛冬[2]，梅美华[1]，游 莹[1]，黄俊琪[1]
(1. 中山大学附属第一医院器官移植科//广东省器官捐献与移植免疫重点实验室//广东省器官移植国际科技合作基地，广东 广州 510080；2. 中山大学肿瘤防治中心检验科，广东 广州 510060)

摘 要：【目的】探讨 EZH1/2 抑制剂 UNC1999 对外周血免疫细胞表型的影响。【方法】用 CCK-8 法检测 UNC1999 作用于外周血单个核细胞(PBMC)后的细胞存活率；多色流式细胞术分析免疫细胞表型。【结果】相比对照组，UNC1999 组经典型单核细胞($CD14^{++}CD16^{-}$)比例上调[(19.53±1.79)% *vs.* (66.60±5.02)%，t=13.31，P=0.006]，中间型单核细胞($CD14^{++}CD16^{+}$)和非经典型单核细胞($CD14^{+}CD16^{+}$)比例下调[(35.08±3.97)% *vs.* (15.42±2.89)%，t=6.130，P=0.026；(35.50±3.53)% *vs.* (8.40±3.12)%，t=25.740，P=0.002]；$CD56^{dim}CD16^{+}$、$CD56^{dom}CD16^{+}$ NK 细胞亚群比例下调[(3.39±0.86)% *vs.* (0.27±0.06)%，t=4.882，P=0.040；(80.50±0.64)% *vs.* (0.63±0.23)%，t=133.100，P<0.000 1]；初始 B 细胞比例上调[(10.67±1.76)% *vs.* (37.99±3.76)%，t=17.690，P=0.003]，记忆 B 细胞、过渡 B 细胞、浆细胞比例下调[(23.39±4.20)% *vs.* (11.82±1.90)%，t=7.059，P=0.020；(3.58±0.47)% *vs.* (1.52±0.56)%，t=26.970，P=0.001；(0.18±0.03)% *vs.* (0.00±0.00)%，t=8.647，P=0.013]；DC 比例上调[(0.20±0.05)% *vs.* (1.38±0.13)%，t=16.500，P=0.004]，其中 pDC/DC 下调[(24.90±1.95)% *vs.* (12.70±2.11)%，t=7.566，P=0.017]，mDC/DC 上调[(32.41±13.14)% *vs.* (60.87±8.43)%，t=8.252，P=0.014]；$CD8^{+}$T 细胞亚群中 $CD8^{+}$中枢记忆 T 细胞、$CD8^{+}PD\text{-}1^{+}$比例上调[(5.62±1.24)% *vs.* (18.38±2.34)%，t=15.600，P=0.004；(2.50±1.02)% *vs.* (18.34±2.69)%，t=8.822，P=0.013]，$CD8^{+}$效应记忆 T 细胞比例下调[(28.27±10.15)% *vs.* (15.62±9.48)%，t=19.480，P=0.003]；$CD4^{+}$T 细胞亚群中 $CD4^{+}CD27^{+}$比例下调[(82.77±2.66)% *vs.* (56.00±9.01)%，t=5.715，P=0.029]。其余细胞亚群差异无统计学意义(P>0.05)。【结论】UNC1999 可以改变 PBMC 免疫细胞表型。

关键词：Zeste 增强子同源物 1/2；UNC1999；多色流式细胞术；免疫表型
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Effects of EZH1/2 Inhibitor UNC1999 on Immunophenotypes of Peripheral Immune Cells

LIU Dan-ping[1], CHI Pei-dong[2], MEI Mei-hua[1], YOU Ying[1], HUANG Jun-qi[1]
(1. Department of Transplantation, The First Affiliated Hospital//Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology//Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation), Sun Yat-sen University, Guangdong 510080, China; 2. Department of Clinical Laboratory, Sun Yat-Sen University Cancer Center, Guangzhou 510060, China)
Correspondence to: HUANG Jun-qi; E-mail: huangjq@mail.sysu.edu.cn

Abstract: 【Objective】To investigate the effects of EZH1/2 inhibitor UNC1999 on the immune cell phenotypes in peripheral blood of healthy adults.【Methods】CCK8 assay was used to measure the cell viability of peripheral blood mononuclear cells (PBMC). Multicolor flow cytometry was performed to analyze the immunophenotypes.【Results】Compared with DMSO group, UNC1999 group showed increased classical monocytes ($CD14^{++}CD16^{-}$) [(19.53±1.79)% *vs.* (66.60±5.02)%, t=13.31, P=0.006), decreased intermediate monocytes ($CD14^{++}CD16^{+}$) and non-classical monocytes ($CD14^{+}CD16^{+}$) [(35.08±3.97)% *vs.* (15.42±2.89)%, t=6.130, P=0.026; (35.50±3.53)% *vs.* (8.40±3.12)%, t=25.740, P=0.002]. The proportions of $CD56^{dim}$ $CD16^{+}$, $CD56^{dom}$ $CD16^{+}$ in UNC1999 group were lower [(3.39±0.86)% *vs.* (0.27±

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作者简介：刘丹平，硕士生，E-mail：liudanp@mail2.sysu.edu.cn；黄俊琪，通信作者，教授，研究方向：免疫学，E-mail：huangjq@mail.sysu.edu.cn

0.06)%, t=4.882, P=0.040; (80.50±0.64)% *vs.* (0.63±0.23)%, t=133.100, P<0.000 1]. UNC1999 group exhibited higher frequency of naive B cells [(10.67±1.76)% *vs.* (37.99±3.76)%, t=17.690, P=0.003], lower frequency of memory B cells, transitional B cells, plasmablasts B cells [(23.39±4.20)% *vs.* (11.82±1.90)%, t=7.059, P=0.020; (3.58±0.47)% *vs.* (1.52±0.56)%, t=26.970, P=0.001; (0.18±0.03)% *vs.* (0.00±0.00)%, t=8.647, P=0.013]. The percentage of DC and mDC/DC was significantly elevated in UNC1999 group [(0.20±0.05)%vs.(1.38±0.13)%, t=16.500, P=0.004; (32.41±13.14)% *vs.* (60.87±8.43)%, t=8.252, P=0.014], with a significantly decreased percentage of pDC/DC [(24.90±1.95)% *vs.* (12.70±2.11)%, t=7.566, P=0.017]. Higher proportions of $CD8^+$ central memory T cells (TCM) and $CD8^+PD\text{-}1^+$ [(5.62±1.24)% *vs.* (18.38±2.34)%, t=15.600, P=0.004; (2.50±1.02)% *vs.* (18.34±2.69)%, t=8.822, P=0.013], but lower proportions of $CD8^+$ effective memory T cells (TEM) and $CD4^+CD27^+$were observed in UNC1999 [(28.27±10.15)% *vs.* (15.62±9.48)%, t=19.480, P=0.003; (82.77±2.66)% *vs.* (56.00±9.01)%, t=5.715, P=0.029]. No statistical difference was found in other cell subsets (P>0.05).【Conclusion】UNC1999 can lead to changes in PBMC immunophenotypes.

Key words: EZH1/2; UNC1999; multicolor flow cytometry; immunophenotype

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Zeste增强子同源物1(enhancer Zeste of homolog 1, EZH1)和Zeste增强子同源物2(enhancer Zeste of homolog 2, EZH2)是多梳蛋白抑制复合物2(polycomb repressive complex 2, PRC2)的两个功能亚基,可以催化组蛋白3赖氨酸27位点(H3K27)甲基化至其三甲基化形式(H3K27me3),诱导转录阻断和基因沉默;EZH1还可通过与参与RNA聚合酶介导的转录起始和/或延伸的因子的赖氨酸残基甲基化促进转录激活[1-2]。UNC1999是一个EZH1/2双重抑制剂,通过与S-腺苷-L-甲硫氨酸(S-adenosyl-L-methionine,SAM)竞争发挥作用,可口服生物利用,细胞毒性低[3-4],已被报道能抑制套细胞淋巴瘤、多发性骨髓瘤、胃癌等多种肿瘤[5-7],为多种癌症治疗带来了新的希望。同时有研究发现UNC1999具有潜在的抑制病毒复制的作用[8]。本研究利用多色流式细胞术对用药后外周血免疫细胞亚群表型进行分析,为UNC1999的临床应用提供参考。

1　材料与方法

1.1　材料

选取2020年12月至2021年1月中山大学附属第一医院成年健康体检者,签署知情同意书后,使用其检测剩余血液标本,研究方案已通过中山大学附属第一医院伦理委员会审批(伦理审查批件号:[2018]259)。实验使用12份健康者血液样本(2 mL/份),为减少个体差异带来的误差,每4份血液样本提取PBMC后混合均匀,获得PBMC细胞数为(1.57±0.27)×10^7个,其中1/4细胞用于CCK8试验,3/4细胞经处理后用于流式细胞术检测。实验所用的1640培养基、PBS缓冲液、青链霉素、胎牛血清均为Gibco产品;CCK-8试剂盒购自日本同仁;Ficoll分离液(17-1440-03);流式细胞仪(Beckman Coulter);DuraClone IM表型检测管(B53309、B53318、B53328、B53351、B53340、B53346;BeckmanCoulter)、固定液(8546859;Beckman Coulter)、PerFix- nc Kit(B31168;Beckman Coulter)。

1.2　方法

1.2.1　外周血PBMC提取及细胞培养　外周血(EDTA抗凝)500 ×*g*离心5 min后,弃血浆,加入两倍血液体积的PBS,混匀后,在15 mL离心管加入Ficoll分离液(Ficoll分离液:血液:PBS=1:1:2),管子倾斜45 ℃,缓慢加入混了PBS的血,形成明显分层,2 300 r/min离心20 min(离心机半径r=9.5 cm),升速为6,降速为0,离心完毕后中间白色层细胞为PBMC,分离出PBMC后用PBS洗涤细胞3次。使用含体积分数10 g/L胎牛血清及体积分数1%青链霉素的1640培养基培养PBMC(培养条件为37 ℃、体积分数5% CO_2)。

1.2.2　CCK-8实验　将细胞悬液以100 000个/孔的密度铺于96孔板,每孔100 μL,二氧化碳孵育箱培养(37 ℃、体积分数5% CO_2)。分别加入终浓度梯度为0、5、10、20和40 μmol/L的UNC1999(溶剂为DMSO),作用12 h后每孔加入10 μL CCK-8试剂,37 ℃孵育4 h,于450 nm下检测OD值,计算细胞存活率,细胞存活率=[(实验孔-空白孔)/(对照孔-空白孔)]×100%。

1.2.3　流式细胞术检测细胞亚群　以1×10^6/mL将细胞接种于12孔板中,置于37 ℃,体积分数5% CO_2培养箱中,静置培养12 h加入药物,使终浓

度为10 μmol/L,对照组加入DMSO,终浓度为2 μL/mL,作用36 h后终止,离心去培养基,加PBS洗涤1次,留下100 μL(包含1×10⁶细胞),混匀后,各加100 μL样本至贝克曼干粉管,Treg管加50 μL样本;常温孵育15 min(避光),各加2 mL PBS,离心后吸净上清,各加400 μL PBS重悬(Treg管除外),上机检测;Treg管尽量吸干净上清,加入5 μL固定液,混匀,避光放置10 min;加入300 μL的破膜液,室温避光孵育1 h,加入2 mL的PBS,离心5 min去上清,加入500 mL PBS重悬,上机检测(表1)。

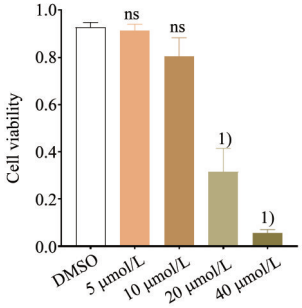
1.3 统计学分析

实验数据均使用Graphpad Prism 8.0软件进行统计分析。计量资料符合正态分布,采用均数±标准差,不符合正态分布,采用中位数(四分位间距)描述。配对数据两组间比较,差值数据呈正态分布且方差齐,采用配对t检验;否则采用校正t检验或配对资料的符号秩和检验。多组均数比较,各组定量资料都呈正态分布并且方差齐性采用单因素方差进行分析,反之用Kruskal Wallis H检验,有统计学意义时再进行两两比较。均采用双侧检验,$P<0.05$为差异具有统计学意义。

2 结 果

2.1 UNC1999的细胞存活率检测

不同浓度的UNC1999与PBMC作用12 h后,CCK-8法检测细胞OD值计算其存活率。经检验5组差异有统计学意义(F=134.953, P<0.001),5、10 μmol/L浓度与对照组相比差异无统计学意义(P=0.992, 0.087),且细胞的存活率保持在80%以上,遂选择UNC1999 10 μmol/L作为后续实验的处理条件(图1)。



CCK-8 kit was used to detect the cell viability after UNC1999 treatment with the indicated concentrations. The cell viability was above 80% at the concentration of 5 μmol/L and 10 μmol/L. Data were analyzed using the one-way ANOVA followed by the Dunnett's t-test. 1) compared control, P <0.001. n=3.

图1 UNC1999作用后PBMC的存活率

Fig.1 The cell viability of PBMC after UNC1999 treatment

2.2 流式细胞术检测结果

2.2.1 用药组与对照组单核细胞亚群的比较 相比对照组,用药组单核细胞占PBMC比例差异没有统计学意义(P>0.05),经典型单核细胞(CD14^{++}CD16^{-})比例上调,中间型单核细胞(CD14^{++}CD16^{+})和非经典型单核细胞(CD14^{+}CD16^{+})比例下调(P<0.05;图2)。

2.2.2 用药组与对照组NK亚群的比较 相比对照组,用药组NK细胞占淋巴细胞比例差异没有统计学意义(P>0.05),CD56^{dim}CD16^{+},CD56^{dom}CD16^{+}细胞亚群比例明显下调,差异有统计学意义(P<0.05;图3)。

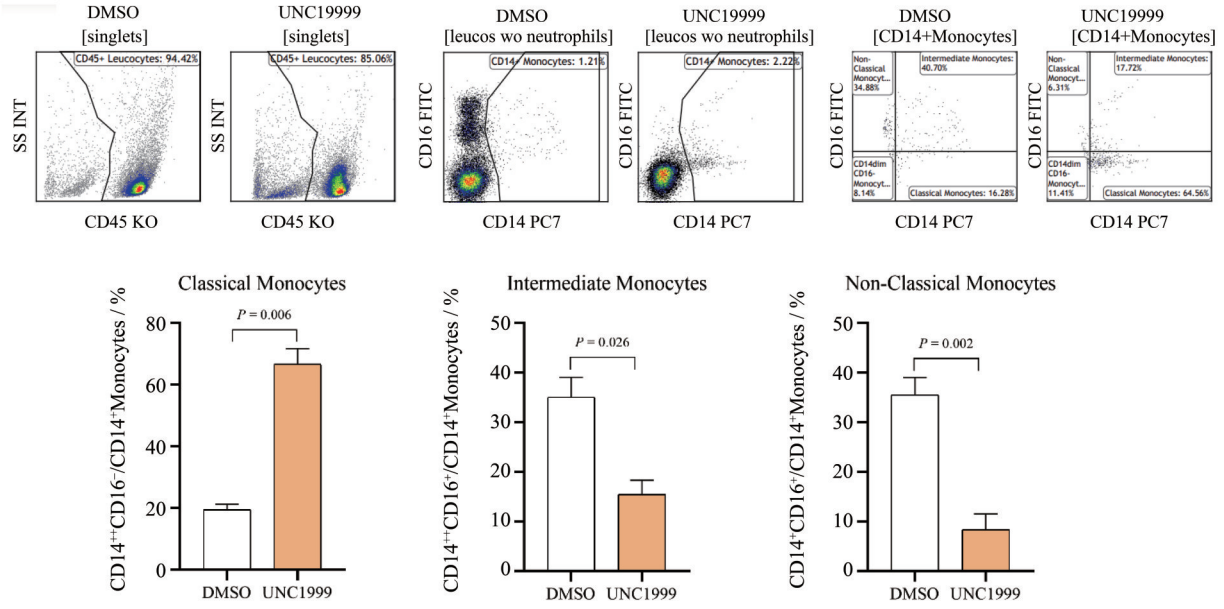
2.2.3 用药组与对照组B细胞的比较 相比对照组,UNC1999组B细胞比例差异没有统计学意义(P>0.05),初始B细胞(naïve B)比例明显上调,记忆B细胞(non switched memory B cells, class switched memory B cells, memory B Cells)、过渡B细胞(transitional B cell)、浆细胞(plasmablasts)比例下调,差异有统计学意义(P<0.05;图4)。

2.2.4 用药组与对照组树突状细胞亚群的比较 相比对照组,UNC1999组DC比例上调,其中pDC/DC下调,mDC/DC上调,差异有统计学意义(P<0.05;图5)。

2.2.5 用药组与对照组T淋巴细胞亚群的比较 相比对照组,UNC1999组CD8^{+}T细胞亚群中CD8^{+}中枢记忆T细胞(central memory T cell, TCM)、CD8^{+}PD-1^{+}比例上调,CD8^{+}效应记忆T细胞(effective memory T cell, TEM)比例下调;CD4^{+}T细胞亚群中CD4^{+}CD27^{+}比例下调(P<0.05),其余淋巴细胞亚群在两组间差异没有统计学意义(P>0.05;图6)。

3 讨 论

UNC1999处理后CD16表达下调,CD16是Ⅲ型免疫球蛋白Fc受体,分为FcγRⅢA和FcγRⅢB,FcγRIIIB仅中性粒细胞表达,FcγRⅢA与FcRγ链结合,根据免疫受体酪氨酸激活基序(immunoreceptor tyrosine-based activation motif, ITAM)或免疫受体酪氨酸抑制基序(immunoreceptor tyrosine-based inhibitory motif, ITIM)介导细胞激活和抑制,起双向调节作用。同时,ITAM还存在抑制性ITAM(inhibitory ITAM, ITAMi)构型,可阻断炎症引起的钙内流、ROS产生、内吞作用、白细胞浸润等作用,这使得FcγRⅢA具有靶向治疗炎症性疾病的潜力[9-10]。



In the UNC1999 group, the proportion of Classical Monocytes ($CD14^{++}CD16^{-}$) was higher than those in the DMSO group; the proportions of Intermediate Monocytes ($CD14^{++}CD16^{+}$), Non-Classical Monocytes ($CD14^{+}CD16^{+}$) were lower than those in the DMSO group ($P<0.05$). Data were analyzed using the paired t-test. $n=3$.

图2　用药组与对照组单核细胞亚群的比较

Fig.2　Comparison of monocyte subsets in UNC1999 and DMSO groups

表1　UNC1999组与对照组外周血免疫细胞表型的比较

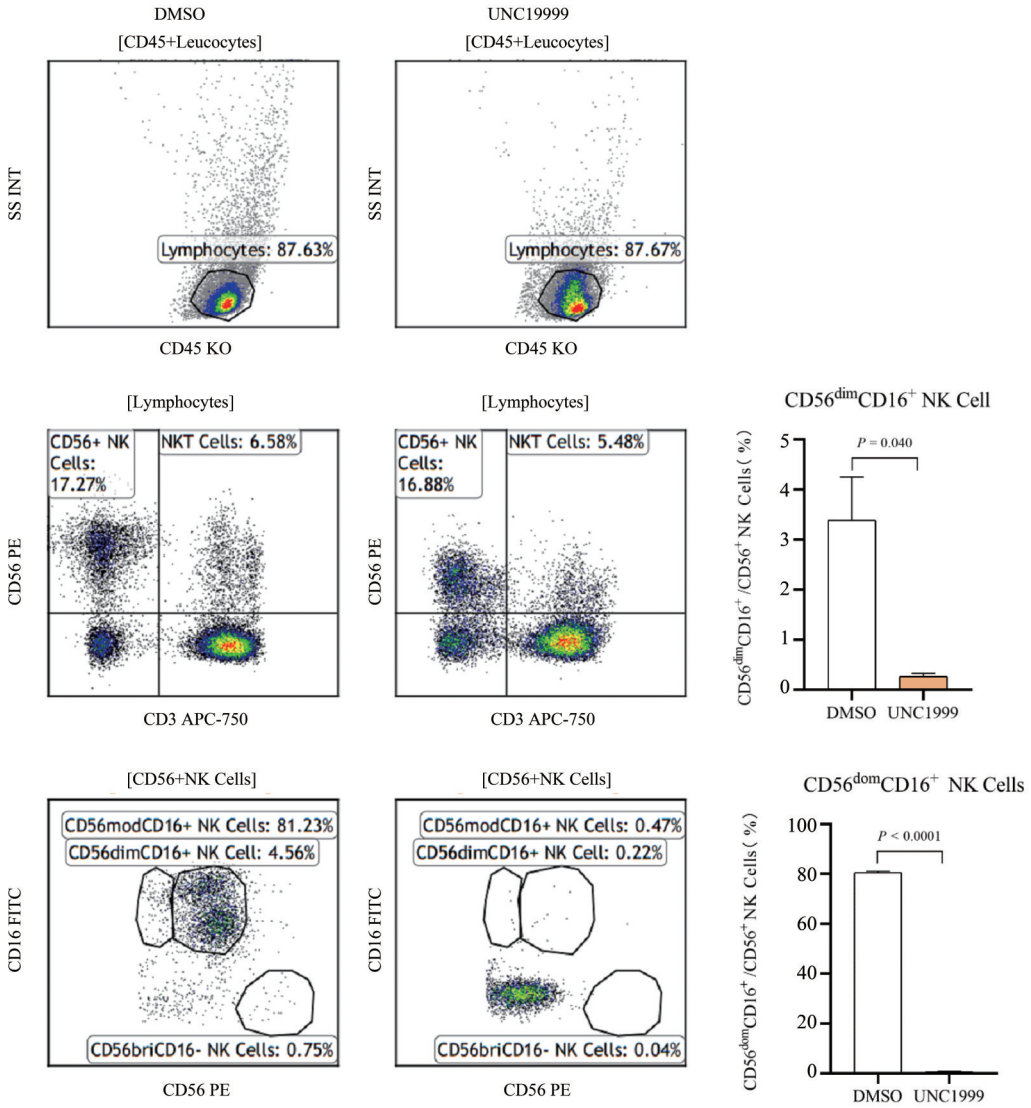
Table 1　Comparison of immunophenotype of peripheral immune cells in UNC1999 and DMSO groups　($\bar{x} \pm s$)

Cell types	DMSO	UNC1999	t	P
$CD14^{+}$ Monocytes	1.07±0.18	3.18±1.03	2.484	0.131
Classical Monocytes	19.53±1.79	66.60±5.02	13.310	0.006
Intermediate Monocytes	35.08±3.97	15.42±2.89	6.130	0.026
Non-Classical Monocytes	35.50±3.53	8.40±3.12	25.740	0.002
Lymphocytes	86.78±0.83	83.92±3.15	1.324	0.317
$CD3^{+}$ T Cells/Lym	71.63±0.55	68.29±1.53	2.984	0.096
$CD3^{+}CD4^{+}$ T Cells/Lym	45.72±2.88	37.67±3.96	2.037	0.179
$CD3^{+}CD8^{+}$ T Cells/Lym	19.43±2.33	21.62±1.09	1.070	0.397
$CD56^{+}$ NK Cells	16.74±0.37	16.89±2.14	0.096	0.933
NKT Cells	5.76±0.78	5.56±1.35	0.270	0.813
$CD56dimCD16^{+}$ NK Cells	3.39±0.86	0.27±0.06	4.882	0.040
$CD56domCD16^{+}$ NK Cells	80.50±0.64	0.63±0.23	133.100	<0.0001
$CD56briCD16^{-}$ NK Cells	1.11±0.29	0.31±0.22	4.270	0.051
$CD19^{+}$ B cells	10.48±0.31	12.58±0.92	2.789	0.108
Transitional B cell	3.58±0.47	1.52±0.56	26.970	0.001
Naive B cells	10.67±1.76	37.99±3.76	17.690	0.003
Marginal zone B cells	0.78±0.18	0.95±0.09	1.084	0.392
Non switched memory B cells	7.80±0.75	3.86±0.54	11.080	0.008
Class switched memory B cells	15.79±3.59	8.17±1.33	4.752	0.042
Plasmablasts	0.18±0.03	0.00±0.00	8.647	0.013

续表

Cell types	DMSO	UNC1999	t	P
Memory B Cells	23.39±4.20	11.82±1.90	7.059	0.020
CD21low CD38low	5.96±0.75	6.99±0.44	1.261	0.335
DC Cells	0.20±0.05	1.38±0.13	16.500	0.004
pDCs/Leu	0.05±0.01	0.17±0.02	13.980	0.005
mDCs/Leu	0.07±0.05	0.85±0.19	7.636	0.017
pDCs/DC	24.90±1.95	12.70±2.11	7.566	0.017
mDCs/DC	32.41±13.14	60.87±8.43	8.252	0.014
mDC1	43.71±14.90	63.83±6.01	3.131	0.089
mDC2	0.57±0.81	0.04±0.05	0.905	0.461
$CD16^+$ mDC	3.12±2.45	0.02±0.03	1.789	0.216
$CD4^+CD25^+/CD4^+$	5.63±2.44	6.40±0.77	0.511	0.660
Treg	3.22±2.11	2.30±1.85	2.386	0.140
Naive Treg	7.02±1.72	30.96±25.54	1.315	0.319
Memory Treg	75.92±23.43	49.04±14.62	1.692	0.233
$CD39^+$ Treg	32.26±7.70	31.81±11.41	0.169	0.882
$CD39^-$ Treg	60.20±7.97	61.52±7.84	0.384	0.738
TCRab T cells/$CD3^+$	89.21±0.83	87.99±1.70	0.710	0.551
TCRrd T cells/$CD3^+$	8.71±0.93	7.43±1.06	1.829	0.209
$TCRVd1^+$ T cells/$TCRrd^+$	8.38±2.71	6.43±2.79	2.112	0.169
$TCRVd2^+$ T cells/$TCRrd^+$	75.90±7.23	69.02±8.30	1.348	0.310
$CD4^+HLA-DR^+/CD3^+$	2.88±0.68	2.57±0.70	0.969	0.435
$CD8^+HLA-DR^+/CD3^+$	2.16±0.35	2.60±0.68	0.605	0.607
TCR ab $CD4^+$ T cells	69.31±5.05	62.84±0.74	1.673	0.236
TCR ab $CD8^+$ T cells	26.63±7.04	29.89±1.07	0.748	0.532
$CD4^+$ Naive	48.12±6.22	46.68±2.28	0.441	0.702
$CD4^+$ Central Memory	49.58±7.70	52.79±2.34	0.775	0.519
$CD4^+$ Effector Memory	1.85±1.32	0.47±0.23	1.743	0.223
$CD4^+$ TEMRA	0.46±0.20	0.06±0.03	2.992	0.096
$CD8^+$ Naive	42.36±9.34	43.39±18.03	0.093	0.934
$CD8^+$ Central Memory	5.62±1.24	18.38±2.34	15.600	0.004
$CD8^+$ Effector Memory	28.27±10.15	15.62±9.48	19.480	0.003
$CD8^+$ TEMRA	23.75±8.13	22.62±10.64	0.096	0.933
$CD4^+CD27^+$	82.77±2.66	56.00±9.01	5.715	0.029
$CD4^+CD28^+$	96.84±0.67	94.25±0.42	3.873	0.061
$CD4^+PD-1^+$	7.26±6.31	9.69±10.13	0.895	0.466
$CD4^+CD57^+$	2.31±1.49	2.11±1.86	0.121	0.915
$CD8^+CD27^+$	56.01±5.52	44.62±10.27	1.643	0.242
$CD8^+CD28^+$	63.44±4.97	76.08±2.40	3.222	0.084
$CD8^+PD-1^+$	2.50±1.02	18.34±2.69	8.822	0.013
$CD8^+CD57^+$	21.92±8.84	17.46±3.38	0.561	0.631

Data were analyzed using the paired t-test.



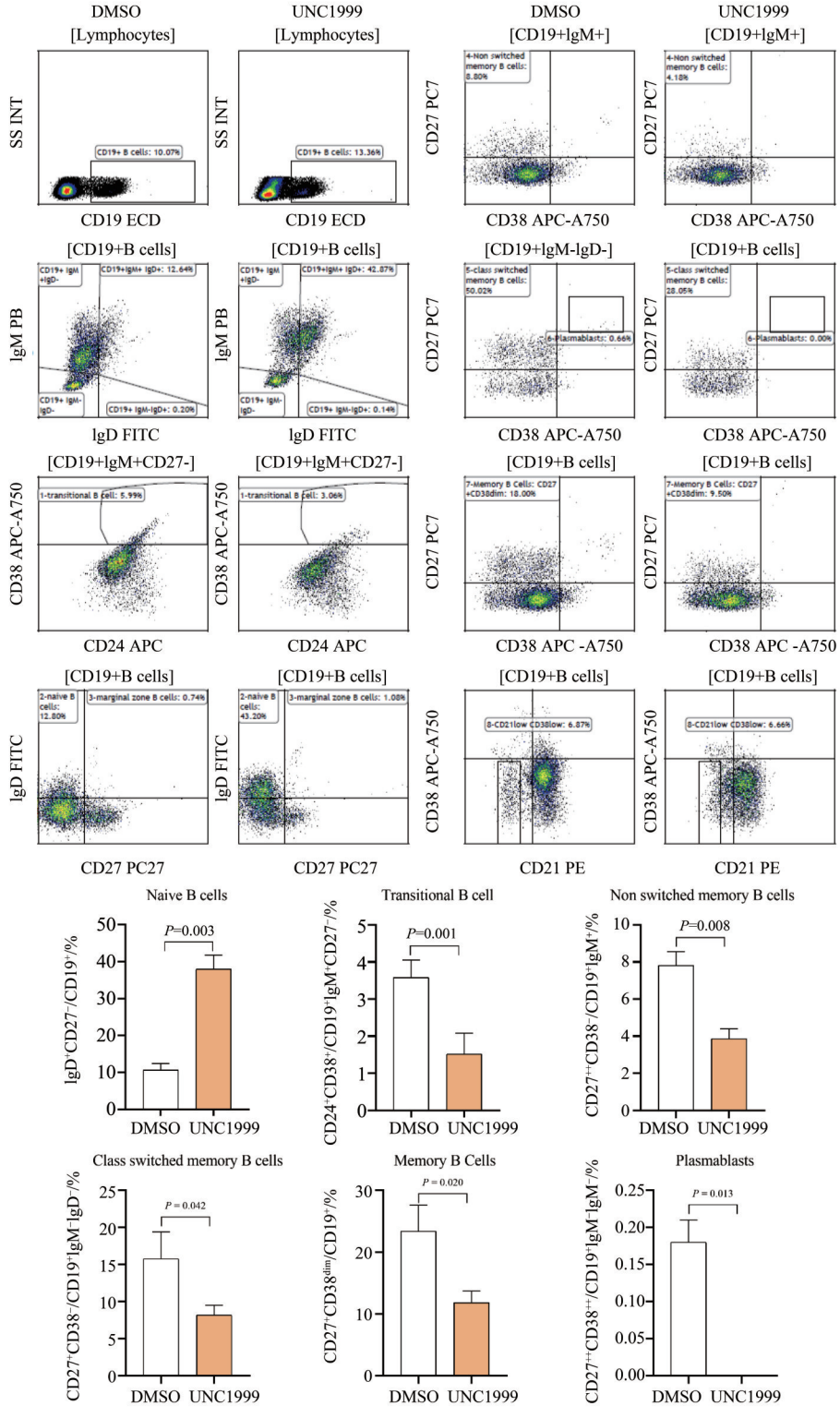
The proportion of total NK cells was no statistical difference between the two groups ($P>0.05$), but the proportions of CD56^{dim} CD16^{+} and CD56^{dom} CD16^{+} in UNC1999 group were all lower than those in the DMSO group ($P<0.05$). Data were analyzed using the paired t-test. n=3.

图3　用药组与对照组NK细胞亚群的比较

Fig.3　Comparison of NK subsets in UNC1999 and DMSO groups

外周单核细胞根据CD14和CD16分为经典型单核细胞（CD14^{++}CD16^{-}），中间型单核细胞（CD14^{++}CD16^{+}）以及非经典型单核细胞（CD14^{+}CD16^{+}）。储备在髓外部位的经典型单核细胞可聚集到感染组织中，在控制感染、限制炎症损伤方面起重要作用；非典型单核细胞被招募到非炎症组织中，负责清除细胞碎片和修复内皮[11]。在败血症、艾滋病毒、炎症性肠病以及代谢性疾病及心血管疾病中，中间和/或非经典型单核细胞亚群的细胞数或比例增加，CD16^{+}单核细胞可产生大量TNF-a和IL-1b导致促炎环境[12]，并维持炎症因子的高水平状态，被称为“促炎症亚群CD16^{+}单核细胞”。研究[13]显示，CD16^{+}单核细胞在SARS-CoV-2感染患者中与疾病严重程度相关，可能与该细胞群在COVID-19患者肺部富集及其在维持血管内环境稳定方面的功能等相关。本研究发现用药后经典型单核细胞比例明显上调，CD16^{+}单核细胞群比例下调，提示UNC1999可能抑制CD16表达，可能有利于减缓细胞因子的产生，减轻炎症反应。

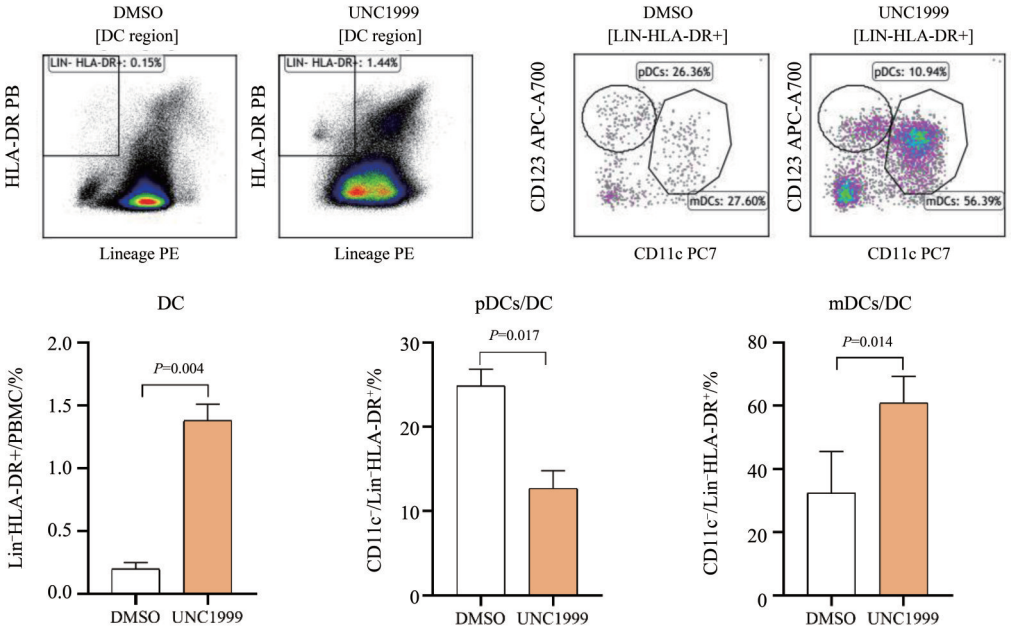
NK细胞通过多个方面发挥免疫调节作用，抑制过度的免疫应答以避免病理损伤，一些肿瘤也可利用这些生理调节功能导致免疫耐受和肿瘤逃逸[14]。NK细胞分成CD56^{bri}和CD56^{dim}NK细胞群，CD56^{bri}NK细胞代表相对年轻的、不成熟的NK细



In the UNC1999 group, the proportion of Naive B cells was higher than those in the DMSO group; the proportions of Non switched memory B cells, Class switched memory B cells, Transitional B cell, Plasmablasts, Memory B Cells were lower than those in the DMSO group ($P<0.05$). Data were analyzed using the paired t-test. $n=3$.

图4 用药组与对照组B细胞亚群的比较

Fig.4 Comparison of B cells subsets in UNC1999 and DMSO groups



In the UNC1999 group, the proportions of DCs, mDC/DC were higher than those in the DMSO group; the proportion of pDC/DC was lower than those in the DMSO group ($P<0.05$). Data were analyzed using the paired t-test. $n=3$.

图5　用药组与对照组DC细胞亚群的比较

Fig.5　Comparison of DC subsets in UNC1999 and DMSO groups

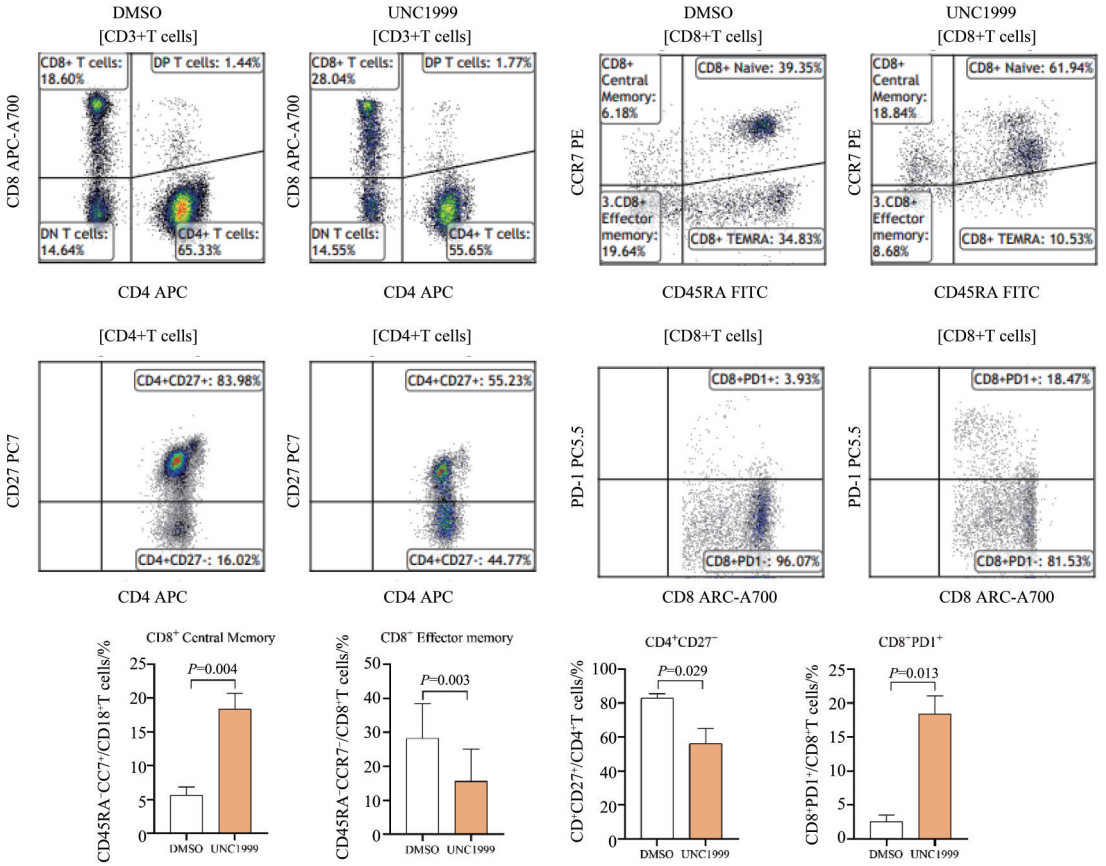
胞，在成熟的过程中获得表达CD16的能力，成为$CD56^{dim}$NK细胞，CD16受体可介导直接细胞毒作用和抗体依赖性细胞毒作用[15]。在体外，PBMC分离出来的$CD56^{bri}$NK细胞与滑膜成纤维细胞接触后上调CD16，分化为$CD56^{dim}$NK细胞，产生大量的细胞因子，具有更强的细胞溶解能力[16]。淋巴结和扁桃体中的$CD56^{bri}$NK细胞能够在IL-2刺激下上调CD16分化为$CD56^{dim}$NK细胞[17]。有研究[18]表明EZH1/2抑制剂促进$CD56^{+}$前体细胞增殖，使前体细胞谱系向3型固有淋巴样细胞倾斜，揭示了参与NK细胞成熟调节的表观遗传机制。本实验用药组$CD56^{dim}CD16^{+}$，$CD56^{dom}CD16^{+}$细胞亚群比例下调，但该药物改变NK细胞表型的作用机制及对感染免疫的影响仍需进一步的深入研究。

B细胞中的EZH2失活将B细胞阻断在增殖和不成熟阶段，构成淋巴瘤发展的关键因素[19-20]。套细胞淋巴瘤中EZH1表达下调，EZH2/EZH1比值的失衡可能在淋巴瘤的发病机制中起作用[21]。相比对照组，用药后初始B细胞比例上调，记忆B细胞、过渡B细胞、浆细胞比例下调，药物可能阻滞了B细胞的分化，其中EZH1或者EZH1/EZH2比值的失衡是否也影响了外周B细胞的分化，值得深入探讨。

树突状细胞（dendritic cells，DC）分为浆细胞样DC（plasmacytoid DC，pDC）和髓样DC（myeloid/conventional DC，mDC）。pDC通过感知病毒感染快速产生大量干扰素和细胞因子，可减缓病毒复制并导致炎症环境；mDC可限制病毒复制，促进T细胞免疫[22-23]。实验结果显示用药组DC总比例以及mDC/DC上调，UNC1999可能促进DC的表达，有利于发挥抗原提呈作用。

T细胞根据CD45RA和CCR7分成初始T细胞（$CD45RA^{+}CCR7^{+}$naïve，TN），中枢记忆T细胞（$CD45RA^{-}CCR7^{+}$Central memory，TCM），效应记忆T细胞（$CD45RA^{-}CCR7^{-}$effector memory，TEM），终末效应记忆T细胞（$CD45RA^{+}CCR7^{-}$effector memory re-expressing CD45RA，TEMRA）。$CD8^{+}$TN，$CD8^{+}$TCM具有自我更新能力，在慢性感染中，TCM可被重新激活以清除或抑制病原体[24]。程序性死亡受体1（programmed cell death protein 1，PD-1）是活化T细胞的负调节因子，是T细胞耗竭的标志[25-27]。CD27属于肿瘤坏死因子受体超家族，其在癌症细胞的表达使其成为抗癌选择之一，抗CD27单克隆抗体的激动剂与阻断PD-1配体的协同作用可增强衰竭的$CD8^{+}$T细胞的增殖和功能[28]。本研究显示该药物影响了T细胞的部分亚群比例，但其对T细胞功能的影响需进一步探索。

UNC1999是具有潜力的抗肿瘤药物，研究发现该药物可能具有抗病毒作用[8]，因此研究UNC1999



CD8⁺T subsets were characterized by higher proportions of CD8⁺TCM, CD8⁺PD-1⁺ but lower proportion of CD8⁺TEM in the UNC1999 group compared with the DMSO group. The UNC1999 group contained lower proportion of CD4⁺CD27⁺ than the DMSO group. Data were analyzed using the paired *t*-test. n=3.

图6 用药组与对照组T淋巴细胞亚群的比较

Fig.6 Comparison of T lymphocyte subsets in UNC1999 and DMSO group

对免疫系统影响十分必要。我们的结果显示UNC1999改变了PBMC的细胞表型,在合并其他病原体感染的情况下,使用UNC1999导致上述细胞表型的改变可能影响正常的免疫功能,导致免疫应答的低效或延迟反应。

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