·论著·

不同剂量胸腺肽-α1在肠毒素B与脂多糖复合诱导小鼠脓毒症模型中的免疫干预作用研究

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【摘要】 目的 模拟革兰阳性球菌和革兰阴性杆菌混合感染，建立金黄色葡萄球菌肠毒素B（SEB）和脂多糖（LPS）复合诱导的小鼠脓毒症模型，观察血清炎症因子及组织蛋白表达水平的变化，探讨不同剂量胸腺肽-α1（Tα1）对脓毒症的免疫干预作用。**方法** 选择SPF级健康雄性BALb/c小鼠90只，按随机数字表法将小鼠分为对照组、Tα1小剂量组和Tα1大剂量组，每组30只。采用向小鼠腹腔注射300 μg/kg SEB和1 000 μg/kg LPS的方法复制小鼠复合感染脓毒症模型。于腹腔注射SEB 2 h后，给所有小鼠腹腔注射LPS 1 000 μg/kg的同时Tα1小剂量组小鼠腹部皮下注射Tα1 100 μg/kg，Tα1大剂量组小鼠腹部皮下注射Tα1 2 000 μg/kg，对照组给予等量磷酸盐缓冲液（PBS）。观察并记录各组小鼠制模后6 h的体征表现。于制模后2、4、6 h取血分离血清，采用酶联免疫吸附试验（ELISA）检测各组小鼠血清白细胞介素（IL-6，IL-10）、γ干扰素（IFN-γ）、肿瘤坏死因子-α（TNF-α）水平；处死动物后留取脾脏组织，光镜下观察脾脏组织的病理学改变，采用蛋白质免疫印迹试验（Western blotting）测定脾脏组织核转录因子-κB p65（NF-κB p65）的蛋白表达水平。**结果** 给药后6 h，Tα1小剂量组和Tα1大剂量组小鼠精神活动、进食、寒战、呼吸、竖毛、大便等体征评分均较对照组明显降低〔精神活动（分）：1.20±0.42、1.10±0.31比2.70±0.48，进食（分）：1.50±0.53、1.40±0.52比2.60±0.70，寒战（分）：1.20±0.42、1.30±0.48比2.30±0.48，呼吸（分）：1.20±0.42、1.30±0.48比2.40±0.70，竖毛（分）：1.40±0.52、1.20±0.42比2.60±0.52，大便（分）：1.40±0.52、1.40±0.52比2.40±0.52，均$P<0.05$〕。随时间延长，各组IL-6、IL-10逐渐降低，IL-10/IL-6比值呈先降低后升高的趋势，IFN-γ呈先升高后降低的趋势；对照组TNF-α持续降低，脾脏组织NF-κB p65的蛋白表达水平持续升高；Tα1小剂量组和Tα1大剂量组TNF-α呈先降低后升高趋势，脾脏组织NF-κB p65的蛋白表达水平呈先升高后降低趋势。给药后2 h，Tα1小剂量组和Tα1大剂量组IL-6、IFN-γ、NF-κB p65的蛋白表达水平均明显高于对照组〔IL-6（μg/L）：2 439.63±3.46、2 442.38±22.53比2 281.47±27.97，IFN-γ（μg/L）：47.37±4.69、50.16±7.50比40.24±8.64，NF-κB p65/β-actin：0.160±0.009、0.155±0.009比0.108±0.005，均$P<0.05$〕，IL-10、IL-10/IL-6比值均明显低于对照组〔IL-10（μg/L）：82.30±17.00、70.89±8.25比214.71±110.43，IL-10/IL-6：0.034±0.007、0.029±0.003比0.094±0.048，均$P<0.05$〕；给药后2 h起Tα1小剂量组和Tα1大剂量组TNF-α较对照组明显降低（μg/L：774.84±136.97、1 068.88±279.99比2 712.68±718.06），持续到给药后6 h（均$P<0.05$）。病理学观察显示，肺组织光镜下可见对照组部分肺泡壁破坏、有肺间质出血现象，Tα1小剂量组及Tα1大剂量组可见肺间质血管充血扩张，未见肺间质出血；肝组织光镜下可见对照组肝细胞水肿，颜色变淡，Tα1小剂量组及Tα1大剂量组病变较轻；肾组织光镜下可见对照组肾间质血管充血，Tα1小剂量组及Tα1大剂量组病变较轻；心肌组织光镜下对照组偶见血管扩张充血，Tα1小剂量组及Tα1大剂量组形态学表现基本正常。**结论** Tα1有利于改善SEB与LPS复合介导的脓毒症小鼠预后，增强脓毒症小鼠的免疫应答，但持续时间有限，提示Tα1在混合感染的研究中应关注使用时机和疗程；Tα1在SEB与LPS复合介导的脓毒症小鼠中的有效剂量范围较大，合适的应用剂量有待进一步研究。

【关键词】 脓毒症； 胸腺肽-α1； 免疫治疗； 金黄色葡萄球菌肠毒素B； 脂多糖； 混合感染

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Research on the immune intervention of different dosages of thymosin α1 on septic mouse model induced by enterotoxin B and lipopolysaccharide

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【Abstract】 Objective To simulate the combined infection of Gram positive coccus and Gram negative bacillus, and establish a mouse model of sepsis induced by *Staphylococcus aureus* enterotoxin B (SEB) and lipopolysaccharide (LPS), observe the expression levels of serum inflammatory factors and hiprotein, and explore the immune intervention effect of different doses of thymosin α1 (Tα1) on sepsis. **Methods** Ninety SPF healthy male BALb/c mice were selected and divided into control group, Tα1 low-dose group, and Tα1 high-dose group according to random number table method, with 30 mice in each group. Mice were intraperitoneally injected with 300 μg/kg SEB and 1 000 μg/kg

LPS to reproduce a mouse model of complex sepsis. After 2 hours intraperitoneal injection of SEB, all mice were intraperitoneally injected with LPS 1 000 μg/kg, while mice in low-dose group were subcutaneously injected with Tα1 100 μg/kg, and mice in high-dose group were subcutaneously injected with Tα1 2 000 μg/kg, the control group was given the same amount of phosphate buffer salt solution (PBS). The physical manifestations of mice in each group were observed and recorded 6 hours after modeling. Blood samples were collected at 2, 4, and 6 hours after modeling, and serum levels of interleukins (IL-6, IL-10), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were detected by enzyme-linked immunosorbent assay (ELISA). After death, spleen tissue was taken, and histopathological changes of spleen were observed under light microscope. Protein expression level of nuclear transcription factor-κB p65 (NF-κB p65) in spleen tissue was determined by Western blotting. **Results** Six hours after administration, the scores of mental activity, feeding, shivering, breathing, hair erectomia, and stool in Tα1 low-dose group and Tα1 high-dose group were significantly lower than those in control group (mental activity: 1.20±0.42, 1.10±0.31 vs. 2.70±0.48, feeding: 1.50±0.53, 1.40±0.52 vs. 2.60±0.70, shivering: 1.20±0.42, 1.30±0.48 vs. 2.30±0.48, breathing: 1.20±0.42, 1.30±0.48 vs. 2.40±0.70, hair erectomia: 1.40±0.52, 1.20±0.42 vs. 2.60±0.52, stool: 1.40±0.52, 1.40±0.52 vs. 2.40±0.52, all $P<0.05$). With the extension of time, IL-6 and IL-10 decreased gradually, IL-10/IL-6 ratio first decreased and then increased, and IFN-γ first increased and then decreased, TNF-α decreased continuously in control group, the expression level of NF-κB p65 protein in the spleen of the control group was increased. The TNF-α in Tα1 low-dose group and Tα1 high-dose group were first decreased and then increased, and the protein expression level of NF-κB p65 in spleen tissue was first increased and then decreased. Two hours after administration, the protein expression levels of IL-6, IFN-γ and NF-κB p65 in Tα1 low-dose group and Tα1 high-dose group were significantly higher than those in control group [IL-6 (μg/L): 2 439.63±3.46, 2 442.38±22.53 vs. 2 281.47±27.97, IFN-γ (μg/L): 47.37±4.69, 50.16±7.50 vs. 40.24±8.64, NF-κB p65/β-actin: 0.160±0.009, 0.155±0.009 vs. 0.108±0.005, all $P<0.05$], IL-10 and IL-10/IL-6 ratios were significantly lower than those in the control group [IL-10 (μg/L): 82.30±17.00, 70.89±8.25 vs. 214.71±110.43, IL-10/ IL-6: 034±0.007, 0.029±0.003 vs. 0.094±0.048, both $P<0.05$]. TNF-α in Tα1 low-dose group and Tα1 high-dose group were significantly decreased from 2 hours after administration compared with control group (μg/L: 774.84±136.97, 1 068.88±279.99 vs. 2 712.68±718.06), and continued to 6 hours after administration (all $P<0.05$). Pathological observation: partial alveolar wall destruction and pulmonary interstitial hemorrhage were observed in the control group under light microscope, while vascular congestion and dilatation were observed in the low-dose and high-dose Tα1 groups, but no pulmonary interstitial hemorrhage was observed. Under light microscope: hepatocyte edema and color were observed in the control group, and the lesions in the low-dose and high-dose Tα1 groups were less severe. The vascular congestion in the renal interstitial was observed in the control group under light microscope, and the lesions in the low-dose Tα1 group and the high-dose Tα1 group were mild. Vascular dilatation and hyperemia were occasionally observed in the control group under light microscope, while morphological findings in the low-dose and high-dose Tα1 groups were normal. **Conclusions** Tα1 can improve the prognosis of SEB and LPS-mediated septic mice and enhance the immune response of septic mice, but the duration is limited, suggesting that the time and course of Tα1 should be paid attention to in the study of mixed infection. The effective dose range of Tα1 in SEB and LPS-mediated septic mice is large, and the appropriate dose needs to be further studied.

【Key words】 Sepsis; Thymosin α1; Immunotherapy; *Staphylococcal aureus* enterotoxin B; Lipopolysaccharide; Complex infection

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脓毒症促炎和抗炎反应失衡所造成的免疫功能紊乱是导致器官功能障碍及病情恶化的重要原因[1-3]。每年全球新增脓毒症患者数以百万计，其中约16.7%～33.3%的患者死亡[4-6]。针对脓毒症的免疫治疗是目前研究的热点，但脓毒症的免疫状态在病程早、中、晚期表现各异，机体对不同病原体的反应模式也不尽相同，新的脓毒症指南对免疫治疗仍无明确规定[7]。胸腺肽-α1（thymosin α1，Tα1）是目前临床应用较多的免疫调理药物[8]，在脓毒症中应用的相关研究众多，但Tα1的应用时机、应用剂量、疗程等尚无定论[9]。脓毒症患者中革兰阳性菌与革兰阴性菌的复合感染大量存在[10-13]，外毒素与内毒素之间存在复杂的协同和交互作用，而且会共同影响感染进展和持续时间，不同病原菌种类的合并感染往往会增加疾病的严重程度[14-16]。由于临床上尚缺乏对免疫状态及时可靠的监测手段，使得脓毒症炎症免疫亢进与抑制的界限常难以界定[17-18]，免疫调理药物特别是Tα1对混合感染所致脓毒症炎症反应及预后影响的研究甚少。本实验通过复制金黄色葡萄球菌肠毒素B（*Staphylococcus aureus* enterotoxin B，SEB）与脂多糖（lipopolysaccharide，LPS）复合感染小鼠脓毒症模型，旨在模拟革兰阳性球菌与革兰阴性杆菌的混合感染，探讨不同剂量Tα1对小鼠脓毒症模型的免疫干预作用，以期为脓毒症的免疫治疗提供参考。

1　材料与方法

1.1　实验动物及分组：选择90只健康雄性SPF级BALb/c小鼠，体质量19～21 g，由河北医科大学实验动物中心提供，动物合格证号：SCXK（冀）2013-1-003。将小鼠按随机数字表法分为对照组、Tα1小剂量

组、Tα1大剂量组，每组30只。再将每组小鼠分为制模后2、4、6 h 3个时间点进行观察，每组10只。向所有小鼠腹腔内注射SEB 300 μg/kg，2 h后腹腔注射LPS 1 000 μg/kg，同时Tα1小剂量组腹部皮下注射Tα1 100 μg/kg，Tα1大剂量组腹部皮下注射Tα1 2 000 μg/kg，对照组小鼠腹部皮下注射等量磷酸盐缓冲液（phosphate buffer saline，PBS）。

1.2 复合感染模型复制：采用向小鼠腹腔内注射300 μg/kg SEB 2 h后再注射1 000 μg/kg LPS诱导小鼠复合感染脓毒症模型。

1.3 伦理学：本研究中对动物的处置方法符合动物伦理学标准，并经本院科研伦理委员会批准（审批号：2015214）。

1.4 动物体征表现量化评分：于制模过程中给予LPS后5.5～6 h观察3组小鼠的精神活动、进食、寒战、呼吸、竖毛、大便情况。

1.5 检测指标及方法

1.5.1 炎症因子水平测定：于制模后2、4、6 h取小鼠腹腔静脉血分离血清，采用酶联免疫吸附试验（enzyme linked immunosorbent assay，ELISA）检测小鼠血清白细胞介素（interleukins，IL-6，IL-10）、γ干扰素（interferon-γ，IFN-γ）、肿瘤坏死因子-α（tumor necrosis factor-α，TNF-α）水平。

1.5.2 核转录因子-κB p65（nuclear factor-κB p65，NF-κB p65）的蛋白表达水平测定：制模后2、4、6 h采血后处死小鼠取脾组组，采用蛋白质免疫印迹试验（Western blotting）测定小鼠脾脏组织NF-κB p65的蛋白表达水平。具体方法为：将脾脏组织匀浆，提取蛋白，用Lowry蛋白定量试剂盒测定蛋白含量，十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（sodium dodecylsulfate-sodium dodecylsulfatepolyacrylamide gel electrophoresis，SDS-PAGE），转膜，封闭，加一抗孵育过夜，再加荧光素标记的二抗孵育，双色红外激光扫描仪扫膜后，以β-肌动蛋白（β-actin）作为内参计算NF-κB p65的相对表达量。

1.5.3 组织病理学观察：于制模后6 h用戊巴比妥钠麻醉小鼠后，留取心、肝、肺、肾组织，行苏木素-伊红（hematoxylin-eosin，HE）染色，光镜下观察各组器官组织的病理学改变。

1.6 统计学方法：使用SPSS 19.0统计软件分析数据。计量数据均符合正态分布以均数±标准差（$\bar{x}\pm s$）表示，正态性检验后进行方差分析，方差齐采用单因素方差分析，方差不齐的组间比较采用多个独立样本的秩和检验。$P<0.05$为差异有统计学意义。

2 结 果

2.1 各组小鼠体征表现量化评分比较（表1）：制模后6 h，不同剂量Tα1组大鼠精神活动、进食、寒战、呼吸、竖毛、大便评分均较对照组明显降低（均$P<0.05$）。

2.2 各组小鼠血清IL-6、IL-10、IL-10/IL-6比值、IFN-γ、TNF-α水平的比较（表2）：3组IL-6、IL-10水平随时间延长呈逐渐降低趋势，IL-10/IL-6呈先降低后升高趋势；对照组TNF-α持续降低，Tα1小剂量组和Tα1大剂量组先降低后升高；IFN-γ呈先升高后降低趋势。给药后2 h起Tα1小剂量组和Tα1大剂量组TNF-α均明显低于对照组，持续到给药后6 h（均$P<0.01$）。给药后2 h，Tα1小剂量组和Tα1大剂量组IL-6、TNF-α水平均明显高于对照组，IL-10/IL-6明显低于对照组（均$P<0.05$），但Tα1小剂量组和Tα1大剂量组IL-6、IL-10、IL-10/IL-6比值比较差异均无统计学意义（均$P>0.05$）。

2.3 各组小鼠脾脏组织NF-κB p65蛋白表达水平的比较（表2）：Tα1小剂量组和Tα1大剂量组脾脏组织NF-κB p65蛋白表达水平均随时间延长呈先升高后降低的趋势，对照组脾脏组织NF-κB p65的蛋白表达随时间延长持续升高；给药后2 h，Tα1小剂量组和Tα1大剂量组NF-κB p65的蛋白表达水平均明显高于对照组（均$P<0.05$），其余各时间点NF-κB p65蛋白表达水平比较差异均无统计学意义（均$P>0.05$）。

2.4 病理学观察：给药后6 h，光镜下可见，对照组小鼠肺组织部分肺泡壁破坏，有肺间质出血现象，

表1 制模后6 h各组小鼠体征表现量化评分比较（$\bar{x}\pm s$）

组别	动物数（只）	精神活动（分）	进食（分）	寒战（分）	呼吸（分）	竖毛（分）	大便（分）
对照组	30	2.70±0.48	2.60±0.70	2.30±0.48	2.40±0.70	2.60±0.52	2.40±0.52
Tα1小剂量组	30	1.20±0.42[a]	1.50±0.53[a]	1.20±0.42[a]	1.20±0.42[a]	1.40±0.52[a]	1.40±0.52[a]
Tα1大剂量组	30	1.10±0.31[a]	1.40±0.52[a]	1.30±0.48[a]	1.30±0.48[a]	1.20±0.42[a]	1.40±0.52[a]

注：与对照组比较，[a]$P<0.05$

表2　制模后不同时间点各组小鼠血清炎症因子及NF-κB p65蛋白表达水平的比较（$\bar{x}\pm s$）

组别	时间	动物数（只）	IL-6（μg/L）	IL-10（μg/L）	IL-10/IL-6	IFN-γ（μg/L）	TNF-α（μg/L）	NF-κB/β-actin
对照组	给药后2 h	10	2 281.47±27.97	214.71±110.43	0.094±0.048	40.24±8.64	2 712.68±718.06	0.108±0.005
	给药后4 h	10	2 280.82±31.34	87.92±57.83	0.038±0.025	86.52±45.02	1 220.36±598.56	0.158±0.068
	给药后6 h	10	1 363.72±786.48	51.68±29.36	0.043±0.019	46.55±14.13	898.22±588.28	0.192±0.007
Tα1小剂量组	给药后2 h	10	2 439.63±3.46[a]	82.30±17.00[a]	0.034±0.007[a]	47.37±4.69[a]	774.84±136.97[a]	0.160±0.009[a]
	给药后4 h	10	2 264.88±38.75	33.83±7.91	0.015±0.004	88.11±16.18	128.75±32.58[a]	0.340±0.087
	给药后6 h	10	825.44±210.06	31.91±5.59	0.040±0.009	54.21±2.98	191.75±70.80[a]	0.232±0.031
Tα1大剂量组	给药后2 h	10	2 442.38±22.53[a]	70.89±8.25[a]	0.029±0.003[a]	50.16±7.50[a]	1 068.88±279.99[ab]	0.155±0.009[a]
	给药后4 h	10	2 310.88±76.61	37.23±6.67	0.016±0.003	107.53±39.40	159.56±71.27[a]	0.244±0.057
	给药后6 h	10	1 232.32±405.16	38.77±11.40	0.033±0.008	55.16±6.36	246.90±76.43[a]	0.223±0.011

注：与对照组同期比较，[a]$P<0.05$；与Tα1小剂量组同期比较，[b]$P<0.01$

Tα1小剂量组及Tα1大剂量组可见肺间质血管充血扩张，未见肺间质出血；对照组肝组织肝细胞水肿，颜色变淡，Tα1小剂量组及Tα1大剂量组病变较轻；对照组肾组织肾间质血管充血，Tα1小剂量组及Tα1大剂量组病变较轻；对照组心肌组织偶见血管扩张充血，Tα1小剂量组及Tα1大剂量组心肌组织形态学表现基本正常。

3　讨　论

脓毒症的最新定义为机体对感染的反应失调而导致危及生命的器官功能障碍，而机体对感染反应失调与同时发生的失衡的过度炎症和免疫抑制有关[1]。随着人们对脓毒症研究的不断深入，全身炎症反应综合征（systemic inflammatory response syndrome，SIRS）[19]、代偿性抗炎反应综合征（compensatory anti-inflammatory response syndrome，CARS）[20]、混合性拮抗反应综合征（mixed antagonism response syndrome，MARS）[21]、前抗炎反应综合征[22]、持续炎症-免疫抑制-分解代谢综合征（persistent inflammation-immunosuppression catabolism syndrome，PICS）[23]等概念被相继提出，研究者不断打破对脓毒症本质的传统认识，试图揭开这个复杂而庞大的非线性免疫网络系统，治疗上则从免疫抑制走向免疫增强或免疫调理，脓毒症的精准免疫治疗备受关注[24-25]。早期研究者们从炎症网络的上游、下游、感受器和效应器等不同级别进行干预，试图通过免疫手段阻断单个环节，包括抗内毒素抗体、拮抗单一炎症介质、抗Toll样受体（Toll-like receptor，TLR）、靶向补体、抑制程序性死亡受体-1（programmed death-1，PD-1）和（或）PD配体-1（PD-ligand 1，PD-L1）途径等，尽管近几十年来人们对脓毒症免疫学的认识有所提高，但目前仍未发现有效的治疗方法[26-27]。Tα1是可以在健康人体内合成和分泌的一种多肽，具有多重免疫调节作用，能刺激淋巴细胞（主要为T细胞）的增殖、分化和成熟，激活树突状细胞，调节T淋巴细胞亚群的比例等，具有增强人体细胞免疫功能的作用[28-31]。目前Tα1在脓毒症治疗中的应用时机、应用剂量、疗程等尚无定论[32]，模拟革兰阳性菌与革兰阴性菌混合感染的免疫干预研究尚未见报道。

IL-6是一个具有复合功能的细胞因子，具有促炎和抗感染的双重作用[33-34]。IL-10是重要的抗炎细胞因子，能抑制促炎因子TNF-α、IL-6等的过度表达，防止过度“炎症瀑布”反应对机体造成的损害[35]；但是过度的IL-10抗炎介质也可引起免疫抑制，减弱对致病菌的清除能力[36-38]，甚至导致“免疫麻痹状态”，极大增加“二次感染”的风险[39]，过度和不足的抗炎反应均会对机体产生不利影响。IL-10/IL-6反映了脓毒症中促炎/抗炎细胞因子的平衡关系即免疫平衡状态，比IL-6或IL-10单独作为评估脓毒症预后和严重程度的标志物更有意义[35, 40-41]。IL-10/IL-6升高表示抗炎反应逐渐占优势，提示预后良好[42]；但对严重脓毒症或脓毒性休克时处于免疫抑制状态的患者，IL-10/IL-6升高说明抗炎反应继续增强，免疫抑制状态进行性加重，对患者预后不利[43]。IFN-γ是一种多功能细胞因子，能改善细胞功能，调节细胞生长和分化，影响细胞间信号交流[44-45]。IFN-γ可改善脓毒症患者的免疫功能低下状态，逆转辅助性T细胞2（T helper cell 2，Th2）细胞反应，有利于患者清除感染，从而提高生存率[46-47]。NF-κB是普遍存在于细胞质中的快反应转录因子，参与免疫反应及细胞增殖和分化等多个过程[48]。在正常情况下，p65是NF-κB重要的亚单位之一，内毒素、TNF-α、IL-6等多种因素均可使其激活，进而高效诱导多种促炎

因子和抗炎因子释放，包括IL-6和IL-10[49]。研究表明，NF-κB可作为脓毒症后炎症反应的评估指标[50]。TNF-α主要是由单核/巨噬细胞产生的多功能细胞因子，是炎症反应的始动因子和重要因子之一[51-52]。低水平的TNF-α可提高细胞免疫反应，激活机体防御机能，降低感染发生率，但TNF-α持续而大量地释放可对器官组织产生损伤[53-54]。

本研究显示，在给LPS的同时即给予Tα1，给药后2 h，Tα1小剂量组和Tα1大剂量组血清IL-6和IFN-γ水平明显高于对照组，IL-10水平明显低于对照组，血清IL-10/IL-6比值明显低于对照组，NF-κB p65的蛋白表达水平明显高于对照组，说明在用药后2 h，Tα1使SEB和LPS复合感染脓毒症小鼠的免疫功能增强，促进了炎症反应；给药后4 h和6 h，Tα1小剂量组和Tα1大剂量组血清IL-6、IL-10、IL-10/IL-6、IFN-γ和脾脏组织NF-κB p65的蛋白表达水平与对照组比较差异均无统计学意义，说明Tα1在给药后2 h一过性地增强了SEB和LPS复合感染脓毒症小鼠的免疫应答，促进了炎症反应，通过降低IL-10水平缓解了免疫抑制状态，有利于改善预后[55]，这与过去仅通过降低炎症因子水平改善预后的免疫治疗是不同的[56]，这种情况与脓毒症机体是处于“免疫亢进”或“免疫抑制”状态有关；随后这种促炎反应不再显著，而TNF-α从给药2 h即持续降低直至给药后6 h，提示Tα1增强细胞免疫持续时间较长，整体上促进免疫应答的持续时间有限，提示Tα1在混合感染的研究中应关注应用时机和疗程；而临床上Tα1在脓毒症中的应用频次为每周2次，尚待进一步研究探讨。此外，反映各器官损害的组织病理学观察显示，应用Tα1后小鼠肺、心、肝、肾组织损害较轻，与对照组比较症状相对较轻，症状缓解出现较早；症状量化评分显示，应用Tα1后小鼠精神活动、进食、寒战、呼吸、竖毛、大便性状等表现与对照组比较差异均有统计学意义。综上，Tα1应用有利于改善SEB与LPS复合介导的脓毒症小鼠预后。

本实验中应用不同剂量Tα1给药后2 h，Tα1大剂量组TNF-α水平明显高于Tα1小剂量组，给药后4 h和6 h，Tα1大剂量组TNF-α水平仍高于Tα1小剂量组，但差异无统计学意义，其他炎症因子在此时段Tα1小剂量组和Tα1大剂量组差异亦均无统计学意义；同时，临床症状量化评分及器官组织病理学分析也提示，Tα1大剂量和Tα1小剂量组间差异无统计学意义。Tα1从100 μg/kg的剂量增大至2 000 μg/kg，最小有效剂量和极量之间的范围比较大。Tα1的安全性良好，但增大Tα1的用量并未使疗效更好，Tα1在混合感染治疗中的合适应用剂量有待探讨。这一结论与其他有关Tα1治疗脓毒症研究的结果一致[57-58]。

综上所述，本研究表明，Tα1有利于改善SEB与LPS复合介导的脓毒症小鼠预后；Tα1可增强SEB与LPS复合介导的脓毒症小鼠的免疫应答，但持续时间有限，提示Tα1在混合感染的研究中使用应关注用药时机和疗程；Tα1在SEB与LPS复合介导的脓毒症小鼠中的有效剂量范围较大，合适的应用剂量有待进一步研究。

利益冲突 所有作者均声明不存在利益冲突

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