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I 型干扰素在脂多糖所致树突状细胞免疫
麻痹中的作用机制研究

The Mechanism Studies of I -IFN in LPS-induced
Dendritic Cells Immune Paralysis

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缩略语索引

缩略语	英文全名	中文全名
AEC	3-mino-9-ethylcarbozole	3-氨基-9-乙基卡唑
APC	antigen presenting cell	抗原提呈细胞
APC	allophycocyanin	别藻蓝蛋白
BCA	bicinchoninic acid	二喹啉甲酸
BrdU	5-bromo-2-deoxyuridine	5-溴尿嘧啶
BM-DC	bone marrow-derived dendritic cell	骨髓衍生树突状细胞
BSA	bovine serum albumin	牛血清白蛋白
CTL	cytotoxic T lymphocyte	杀伤性 T 淋巴细胞
DC	dendritic cell	树突状细胞
ELISA	enzyme linked immunosorbent assay	酶联免疫吸附实验
ELISPOT	enzyme linked immunospot	酶联免疫斑点试验
ERK	extracellular receptor-activated kinase	细胞外受体活化激酶
FBS	fetal bovine serum	胎牛血清
FCM	flow cytometry	流式细胞术
FITC	fluorescein isothiocyanate	异硫氰酸荧光素
GITR	glucocorticoid-induced tumor necrosis factor receptor family-related gene	糖皮质激素诱导肿瘤坏死因子相关受体
GM-CSF	granulocyte-microphage colony-stimulating factor	粒细胞-巨噬细胞集落刺激因子
ICAM-1	intercellular adhesion molecule-1	细胞间黏附分子-1
IFN- β	interferon- β	干扰素- β
IL-6	interleukin-6	白细胞介素-6
JAK1	janus kinase signal transducers 1	蛋白酪氨酸激酶 1
LBP	LPS binding protein	脂多糖结合蛋白

LCMV	lymphocytic choriomeningitis virus	淋巴细胞脉络丛脑膜炎病毒
LPS	lipopolysaccharide	脂多糖
LY	lucifer yellow	荧光黄
MAPK	mitogen activated protein kinase	丝裂原活化蛋白激酶
MFI	mean fluorescent intensity	平均荧光密度
MHC	major histocompatibility complex	主要组织相容性复合体
MLR	mixed lymphocyte reaction	混合淋巴细胞反应
NF- κ B	nuclear factor-kappa B	核因子 kappa B
OVA	ovalbumin	鸡卵白蛋白
PBS	phosphate buffered saline	磷酸盐缓冲液
PD-1	programmed cell death-1	程序性细胞死亡受体-1
PE	phycoerythrin	藻红蛋白
PI3K	phosphoinositide 3-kinase	磷脂酰肌醇 3-激酶
Real-time PCR	real-time polymerase chain reaction	实时定量聚合酶链反应
TCR	T cell receptor	T 细胞受体
Th	T helper cell	T 辅助细胞
TLR	Toll like receptor	Toll 样受体
TMB	tetramethylbenzidine	四甲基联苯胺
TNF	tumor necrosis factor	肿瘤坏死因子
Treg	regulatory T cell	调节性 T 细胞
VCAM-1	vascular cell adhesion molecule-1	血管细胞黏附分子-1

摘要

树突状细胞是目前已知功能最强的抗原提呈细胞,然而在细菌性感染以及某些病理条件下,其介导的免疫反应将会受到严重抑制,呈现免疫麻痹状态。I型干扰素是一种多功能的细胞因子,可能参与细菌性感染所致免疫麻痹过程,但其作用机制不甚明了。为此,我们以脂多糖(LPS)刺激树突状细胞(DCs)建立抗原交叉提呈免疫麻痹体系,对I型干扰素(I-IFN)在脂多糖所致树突状细胞免疫麻痹中的作用及所涉及细胞因子和细胞亚群功能转化进行了探讨。对小鼠骨髓来源的DCs给予一定剂量的LPS刺激,联合抗原负载(SIINFEKL),检测LPS刺激对BM-DCs胞饮及交叉提呈抗原能力的影响;继而行体外混合淋巴细胞反应和体内CTL诱生动物实验,检测DCs介导的OVA抗原肽特异性T淋巴细胞增殖反应和CTL效应;在此基础上,通过IFN- β 重组细胞因子以及IFN- β 封闭抗体处理,探究IFN- β 在此免疫麻痹中的可能作用及相关机制。

结果显示:第一,LPS(10 ng/ml)刺激能显著增强BM-DCs的胞饮作用和抗原交叉提呈能力;第二,LPS刺激能明显提高BM-DCs表面共刺激分子CD80、CD86、CD40、OX40L和4-1BBL的表达,并且此上调作用可能与PI3K、Akt、Erk1/2和p38信号相关;第三,LPS刺激抑制DCs介导的T细胞增殖反应和CTL的诱生;第四,IFN- β 是介导LPS所致DCs免疫麻痹的重要细胞因子,并且可能通过上调DCs表面共抑制分子B7-H1(PD-L1)的表达,与T细胞表面PD-1结合,抑制T细胞增殖;第五,调节性T细胞是介导LPS所致DCs免疫麻痹的重要细胞亚群,LPS刺激上调DCs表面GITRL,通过与调节性T细胞表面受体GITR作用,促进后者的增殖和功能发挥,介导免疫麻痹。

上述结果提示:细菌性成分LPS抑制DCs介导的免疫反应,引起免疫麻痹。IFN- β 通过上调B7-H1表达介导此LPS所致免疫麻痹现象,同时CD4⁺CD25⁺Foxp3⁺Treg细胞通过GITRL/GITR信号在此免疫麻痹中发挥调节作用,为临床上针对革兰氏阴性细菌感染所致免疫麻痹及继发性感染的治疗提供理论依据及潜在分子靶点。

关键词: 树突状细胞 免疫麻痹 I型干扰素 B7-H1 调节性T细胞 GITR

Abstract

Dendritic cells (DCs) are the most powerful APCs which has unique ability to initiate naïve T cells priming. However, during bacterial infections or in some pathological conditions, DCs-mediated immune response rendered immune paralysis, which characterized by the failure of mounting adaptive immunity towards secondary microbial infections. Although Type I Interferon (I-IFN) has been reported to be involved in bacterial infections induced immune paralysis, the exact effect of I-IFN in immune paralysis is still uncertain. To address this object, bone marrow-derived DCs (BM-DCs) were treated with lipopolysaccharide (LPS) and the effects of LPS on cross-presentation, surface co-stimulator molecules expression, pinocytosis ability and pro-inflammatory cytokines release were firstly determined by flow cytometry; Then, the effects of LPS on DCs-dependent SIINFEKL-specific T cell proliferation in vitro and CTL priming in vivo were explored by mixed lymphocyte reactions and intraperitoneal transfer Elispot respectively; Using recombinant mouse IFN- β or IFN- β blocking Ab, the role of IFN- β in LPS-induced immune paralysis were further confirmed; Importantly, the mechanism of IFN- β induced immune paralysis was investigated by in vivo B7H1/PD-1 and GITRL/GITR expression exploration.

The results showed that: firstly, LPS treatment could augment BM-DCs' pinocytosis ability and antigenic cross-presentation; Secondly, LPS stimulation could increase the expressions of co-stimulator molecules in BM-DCs by activating PI3K-AKT and p38-ERK1/2 pathway; Thirdly, LPS treatment obviously impaired imDCs-dependent SIINFEKL-specific T cell proliferation in vitro and CTL priming in vivo; Interestingly, LPS induced IFN- β up-regulation on DCs was due to LPS-induced DCs' impairment; Most importantly, B7H1/PD-1 and GITRL/GITR signaling contributed to IFN- β induced DCs' impairment.

All these data presented here indicated that bacterial component LPS suppress immune response leading to immune paralysis. IFN- β , B7-H1/PD-1 signaling, Foxp3⁺

Treg and GITRL/GITR signaling were involved in this immune paralysis, which provide a theoretical basis and potential target molecules to overcome Gram-negative bacterial infections induced immune paralysis and facilitate secondary immune response to microbial infection.

Key words: dendritic cells; immune paralysis; type I interferon; B7-H1; Treg; GITR

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