



研究报告

具核梭杆菌脂多糖诱导 THP-1 细胞 M2 极化及对低浓度白介素 6 产生的作用

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摘要:【背景】在结直肠癌等多种肿瘤中普遍存在的具核梭杆菌(*Fusobacterium nucleatum*)与结直肠癌发生、预后不良、复发及化疗耐药等密切相关。其引发炎症、对肿瘤微环境中巨噬细胞等免疫细胞作用与机制尚待阐明。【目的】对比分析 *F. nucleatum* 脂多糖(Lipopolysaccharide, LPS)与嗜黏蛋白阿克曼氏菌(*Akkermansia muciniphila*)、大肠杆菌(*Escherichia coli*)的 LPS 诱导单核细胞极化、炎症细胞因子表达等活性差异,探讨 *F. nucleatum* 在诱发慢性炎症、致癌等过程中的作用与机制。【方法】分别用 *A. muciniphila*、*E. coli*、*F. nucleatum* LPS 或联合干扰素 γ (Interferon- γ , IFN- γ) 处理后,观察 THP-1、THP-1 M0 细胞的细胞形态变化,然后检测 M0 (*CD11B*)、M1 (*CD40*、*CD86*) 和 M2 (*CD163*、*CD206*) 巨噬细胞标志基因、*TLR3*、*TLR4*、*IL-6*、*IL-10* 等基因转录水平,以及 *IL-6*、*IL-10*、C 反应蛋白(C Reactive Protein, CRP)翻译水平的表达变化。【结果】聚丙烯酰胺凝胶电泳(Polyacrylamide Gel Electrophoresis, PAGE)分析显示, *A. muciniphila*、*E. coli*、*F. nucleatum* 这 3 种细菌的 LPS 条带位置、数量存在明显差异。*F. nucleatum* LPS 在具有较强诱导 THP-1 细胞贴壁的同时,对经佛波肉豆蔻醋酸(Phorbol Myristate Acetate, PMA)处理贴壁的 THP-1 细胞,无论是单独或是联合 IFN- γ 处理,诱导形成伪足数、伪足长度及形成梭形细胞比例(M1 型巨噬细胞)等均低于 *A. muciniphila* 和 *E. coli* LPS。进一步转录水平检测巨噬细胞标志基因表达发现, M1 标志基因中, *CD40* 分别上调 5 011.0% ($P<0.001$)、6 048.9% ($P<0.001$)和 1 011.6% ($P=0.009 4$), *CD86* 分别上调 637.3% ($P<0.001$)、657.9% ($P<0.001$)和 194.1% ($P>0.05$); M2 标志基因中, *CD163* 分别下调 39.5% ($P=0.001 1$)、53.7% ($P<0.001$)和 5.9% ($P>0.05$), *CD206* 分别下调 18.6% ($P>0.05$)、88.4% ($P=0.005 5$)和 24.8% ($P>0.05$)。TLR、白介素家族基因转录水平分析发现, *TLR3* 分别下调 32.3% ($P=0.044 7$)、311.5% ($P=0.001 9$)、9.6% ($P>0.05$); *IL-6* 分别上调 17 763.2% ($P<0.001$)、35 458.2% ($P<0.001$)、1 123.6% ($P>0.05$); *IL-10* 分别上调 729.3% ($P<0.001$)、1 223.3% ($P<0.001$)、124.4% ($P>0.05$)。翻译水平上, *A. muciniphila*、*E. coli*、*F. nucleatum* LPS 单独或联合 IFN- γ 处理时, THP-1 细胞产生 *IL-6* 分别为 0.16、6.17、0 pg/mL 与 410.03、1 334.40、46.20 pg/mL。【结论】*F. nucleatum* LPS 不仅具有较强招募单核细胞并诱导其向 M2 极化的作用,同时,具有诱导巨噬细胞分泌低浓度 *IL-6* 的特性,说明其在引发慢性炎症及肿瘤免疫应答、逃逸等过程中发挥重要作用。综合上述信息,对致

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癌、免疫激活及肿瘤治疗相关细菌 LPS 的结构、活性、分子机制等研究将有助于明确革兰氏阴性细菌在慢性炎症、肿瘤发生、免疫调控等中的作用, 以期为相关疾病预防与治疗提供新的策略与靶点。

关键词: 具核梭杆菌, 脂多糖, THP-1, 白介素 6, 慢性炎症, 单核细胞

***Fusobacterium nucleatum* lipopolysaccharide induces THP-1 cells to polarize towards M2 and produce a low concentration of IL-6**

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Abstract: [Background] *Fusobacterium nucleatum* identified in many kinds of tumors is closely related to tumor initiation, poor prognosis, recurrence, and chemotherapy resistance in colorectal tumor. However, the mechanisms of *F. nucleatum* inducing inflammation and affecting immune cells such as macrophages in tumor microenvironment remain to be elucidated. **[Objective]** We explored the role and mechanism of *F. nucleatum* in the process of inducing chronic inflammation and cancer by comparing the monocyte polarization and inflammatory cytokine expression induced by *Fusobacterium nucleatum*-, *Akkermansia muciniphila*-, and *Escherichia coli*-derived lipopolysaccharides (LPSs). **[Methods]** After the treatment with *A. muciniphila* LPS, *E. coli* LPS, *F. nucleatum* LPS alone or combined with interferon- γ (IFN- γ), we observed the morphological changes of THP-1 and THP-1 (M0) cells. Further, we determined the mRNA levels of macrophage marker genes [including M0 (*CD11B*), M1 (*CD40*, *CD86*), and M2 (*CD163*, *CD206*)], *TLR3*, *TLR4*, *IL-6*, and *IL-10* as well as the protein levels of IL-6, IL-10, and C-reactive protein. **[Results]** PAGE results showed that the LPSs from the three microbial species were significantly different in position and number of bands. *F. nucleatum* LPS possessed stronger activity of inducing adhesion of THP-1 cells. Meanwhile, the group treated with *F. nucleatum* LPS alone or in combination with IFN- γ had shorter pseudopodia and lower proportion of cells with pseudopodia and spindle-shaped cells (M1 cells) than the groups treated with *A. muciniphila* LPS and *E. coli* LPS. The LPSs from *A. muciniphila*, *E. coli*, and *F. nucleatum* up-regulated the mRNA level of *CD40* by 5 011.0% ($P<0.001$), 6 048.9% ($P<0.001$), and 1 011.6% ($P=0.009 4$) and that of *CD86* by 637.3% ($P<0.001$), 657.9% ($P<0.001$), and 194.1% ($P>0.05$), respectively. The LPSs down-regulated the mRNA level of *CD163* by 39.5% ($P=0.001 1$), 53.7% ($P<0.001$), and 5.9% ($P>0.05$) and that of *CD206* by 18.6% ($P>0.05$), 88.4% ($P=0.005 5$), and 24.8% ($P>0.05$), respectively. They down-regulated the mRNA level of *TLR3* by 32.3% ($P=0.044 7$), 311.5% ($P=0.001 9$), and 9.6% ($P>0.05$), up-regulated that of *IL-6* by 17 763.2% ($P<0.001$), 35 458.2% ($P<0.001$), and 1 123.6% ($P>0.05$), and up-regulated that of *IL-10* by 729.3% ($P<0.001$), 1 223.3% ($P<0.001$), and 124.4% ($P>0.05$), respectively. The THP-1 cells treated with the LPSs of *A. muciniphila*, *E. coli*, and *F. nucleatum* alone produced IL-6 at 0.16 pg/mL, 6.17 pg/mL, and 0 pg/mL, and those treated with the LPSs in combination with IFN- γ produced IL-6 at 410.03 pg/mL, 1 334.40 pg/mL, and 46.20 pg/mL, respectively. **[Conclusion]** *F. nucleatum* LPS possessed a strong activity of recruiting monocytes and inducing them to polarize toward M2. It induced macrophages to produce a much lower amount of IL-6 than the LPSs of *A. muciniphila* and *E. coli*, which may play a role in triggering chronic inflammation and tumor immune response and escape. These findings suggest that studying the structure, activity, and mechanism of LPS from carcinogenic, immunomodulating or tumor therapy-associated bacteria will facilitate the elucidation of the role of these bacteria in chronic inflammation and tumorigenesis, which

will provide new targets and strategies for the prevention and treatment of these diseases.

Keywords: *Fusobacterium nucleatum*, lipopolysaccharide, THP-1, IL-6, chronic inflammation, monocyte

据世界卫生组织(World Health Organization, WHO)《2020 全球癌症报告》显示, 癌症成为全球第二大死因, 而且呈逐年上升趋势, 2020 年全球癌症死亡人数已达 996 万, 其中中国癌症死亡人数达 300 万, 居全球首位, 占全球癌症死亡总人数的 30%^[1]。

由于炎症会刺激正常细胞产生氧化应激反应, 导致细胞损伤和DNA损伤并刺激细胞增殖, 因此, 目前普遍认为慢性炎症是诱发和促进肿瘤发展的重要因素^[2]。例如, 在炎症性肠道疾病患者中, 长期、严重肠炎患者的结直肠癌发病率显著高于正常人群^[2-3]。

中性粒细胞、单核细胞等非特异性免疫细胞与炎症有着复杂的关系^[4], 骨髓瘤等肿瘤中, 骨髓单核细胞会被慢性炎症招募到肿瘤部位^[5-7]并分化、发展为肿瘤相关巨噬细胞(Tumor-Associated Macrophage, TAM), 但具体机制尚不清楚^[4]。TAM主要分为M1型和M2型, 由脂多糖(Lipopolysaccharide, LPS)与干扰素 γ (Interferon- γ , IFN- γ)、粒细胞-巨噬细胞集落刺激因子等辅助性T (T Helper, Th) 1 细胞因子联合诱导形成的M1型巨噬细胞多以长梭形为主, 伪足较多; 由白介素(Interleukin, IL) 4 等Th2细胞因子诱导极化形成的M2细胞以圆形为主, 伪足相对较少^[8]; M1型细胞通过分泌IL-1 β 、IL-6等促炎细胞因子来促进炎症反应^[9], 具有抗病原微生物、抗肿瘤等活性; M2型细胞分泌IL-10等抗炎细胞因子, 具有较强的组织修复与促进肿瘤进展作用^[10-11]。

近年来, 多种细菌的致癌或免疫调控作用被发现, 如幽门螺杆菌(*Helicobacter pylori*)等细菌被证明与胃癌发生、发展等密切相关^[12]; 嗜黏蛋白阿克曼氏菌(*Akkermansia muciniphila*)丰度高的患者往往PD-1 治疗预后更好^[13]; 小鼠实验进一步证明两者的相关性^[14]。越来越多的研究表

明细菌对肿瘤、人体免疫系统功能有重要影响, 但机制不明。

与牙周炎等口腔疾病相关的革兰氏阴性厌氧菌具核梭杆菌(*Fusobacterium nucleatum*)^[15], 自2012年被发现在结直肠肿瘤组织中丰度异常增高以来^[16-17], 该菌与结直肠癌发病^[18]、预后不良^[19]、复发^[20]及化疗耐药^[20]等相关性被一一证明^[21]。近年来在乳腺癌^[22]、口腔癌、胰腺癌^[23]等多种肿瘤中也发现了具核梭杆菌, 因此, 其在肿瘤中的作用与机制受到广泛关注。

位于革兰氏阴性菌细胞表面的脂多糖^[24]具有诱发内毒素血症、炎症等作用^[25], 研究发现大肠杆菌(*Escherichia coli*) LPS具有诱导THP-1 等单核细胞瞬时贴壁^[26]作用, 其与IFN- γ 等Th1 细胞因子联合作用时, 通过上调CD86 等M1 标志基因表达诱导THP-1 细胞向M1 型极化^[27-28]。

通过对比分析 *A. muciniphila*、*E. coli*、*F. nucleatum*的LPS的电泳结果以及 3 种LPS对THP-1 细胞的形态, M0、M1、M2 等巨噬细胞标志基因, *TLR3*、*TLR4* 及*IL-6* 等细胞因子表达的影响等, 明确致癌菌*F. nucleatum* LPS的独特活性及在肿瘤发生、发展中的可能作用。

1 材料与方法

1.1 材料

1.1.1 菌株

A. muciniphila BNCC 341917 (DSM22959)、*Escherichia coli* ATCC 25922 由本实验室保存; *F. nucleatum* ATCC 25586, 上海交通大学附属仁济医院洪洁教授惠赠。

1.1.2 细胞株

THP-1 细胞株购自中国科学院细胞库。

1.1.3 主要试剂和仪器

RPMI-1640 培养基、磷酸缓冲液(PBS), HyClone公司; 胎牛血清, BI公司; 佛波肉豆蔻醋酸

(Phorbol Myristate Acetate, PMA)、Hieff UNICON[®] qPCR SYBR Green Master Mix、SDS-PAGE凝胶配置试剂盒, 翌圣生物科技(上海)股份有限公司; IFN- γ , 北京达科为生物技术有限公司; 总RNA提取试剂盒, 广州美基生物科技有限公司; FastKing cDNA第一链合成试剂盒, 天根生化科技(北京)有限公司; TureColor三色预染蛋白Marker, 生工生物工程(上海)股份有限公司; LPS提取试剂盒, iNtRON Biotechnology公司; Trypsin-EDTA (0.25%), Gibco公司; IL-6、IL-10 检测试剂盒, 爱必信(上海)生物科技有限公司; CRP检测试剂盒, Novus Biologicals公司。多功能酶标仪, Tecan公司; 荧光定量PCR仪, ThermoFisher公司; 荧光正置显微镜, 蔡司公司; 微孔板成像检测系统, PerkinElmer公司。

1.2 方法

1.2.1 菌株培养

*A. muciniphila*采用BHI培养基(+4%瘤胃液)在 85% N₂、5% CO₂、10% H₂、37 °C厌氧培养; *E. coli* ATCC 25922采用LB培养基在 37 °C有氧培养; *F. nucleatum*采用BHI培养基在 85% N₂、5% CO₂、10% H₂、37 °C厌氧培养。

1.2.2 细胞培养

THP-1 细胞采用含 10%胎牛血清、0.05 mmol/L的 β -巯基乙醇的RPMI-1640 培养基培养。细胞在 37 °C、5% CO₂、饱和湿度的细胞培养箱中培养。细胞传代、细胞冻存等实验操作按照常规方法进行。

1.2.3 脂多糖的提取和电泳分析

(1) 收集培养至OD₆₀₀为 0.8–1.0 左右的菌体, 按照LPS提取试剂盒的说明书操作, 干燥、称重后溶于 10 mmol/L Tris-HCl (pH 8.0)。

(2) LPS经SDS-PAGE、考马斯亮蓝染色等检测后, 采用相应的细胞培养液稀释至指定浓度, 用于后续细胞实验。

(3) SDS-PAGE分析、银染按文献[29]报道方法进行。

1.2.4 LPS处理THP-1 细胞贴壁及形态观察

设置 96 孔板中THP-1 细胞密度为 5 000 个/孔, 分别用终浓度为 0、0.01、0.1、1、10、20、40 μ g/mL的LPS处理 1 h后, 弃上清, 用培养基温和冲洗 3 次, 用显微镜观察拍照、计数, 4 h后再观察并计数。

THP-1 细胞贴壁: 采用 10 ng/mL PMA处理细胞 24 h后, 用于后续实验。

贴壁的THP-1 细胞分别用 20 μ g/mL的 3 种LPS进行单独或联合IFN- γ (20 ng/mL)处理, 采用微孔板成像检测系统观察各实验组、对照组的细胞形态变化, 每 3 h拍照一次, 连续观察 24 h。

1.2.5 总RNA提取、反转录、定量PCR检测

贴壁THP-1 细胞分别用 20 μ g/mL的 3 种LPS处理 3 h后, 采用RNA提取试剂盒提取总RNA, 具体操作按说明书进行。然后以 500 ng总RNA为模板进行反转录(FastKing cDNA第一链合成试剂盒)后, 进行定量PCR (Hieff UNICON[®] qPCR SYBR Green Master Mix), *GAPDH* (内参)等基因qPCR引物采用Primer 3 网站设计, 由擎科公司合成, 引物序列见表 1。qPCR反应体系(20 μ L): 模板DNA 0.5 μ L, 正、反向引物(10 μ mol/L)各 0.5 μ L, Hieff UNICON[®] qPCR SYBR Green Master Mix (25 μ mol/L) 10 μ L, ddH₂O补足 20 μ L。qPCR反应条件: 95 °C 5 min; 95 °C 10 s, 60 °C 20 s, 72 °C 20 s, 共 40 个循环; 最后进行溶解曲线分析。采用 2^{- $\Delta\Delta C_t$} 进行表达相对定量。

1.2.6 ELISA检测炎症细胞因子IL-6、IL10、CRP

THP-1 细胞以每孔 5 \times 10⁵ 个细胞/mL密度加入 24 孔板, PMA处理贴壁后, 换成 5%胎牛血清培养基, 用 20 μ g/mL LPS (或联合 20 ng/mL IFN- γ) 处理 24 h。

培养液 4 °C、3 000 r/min离心 10 min后, 上清用ELISA分别检测IL-6、IL-10 和CRP, 具体操作按试剂盒说明书进行。

表 1 基因定量PCR引物序列

Table 1 Primers sequence of RT-qPCR

基因Genes	引物Primers	引物序列Primers sequence (5'→3')
GAPDH	正向Forward	TCCTGCACCACCAACTGCTT
	反向Reverse	GGGGCCATCCACAGTCTTCT
TLR3	正向Forward	CATGACCCTGGAATGAGAAATATCC
	反向Reverse	GGTGGCAAATAACATGAAAGTGACA
TLR4	正向Forward	CATATCAGAGCCTAAGCCACCTCTC
	反向Reverse	AGCCACCAGCTTCTGTAAACTTGAT
IL6	正向Forward	CCCAGGAGAAGATTCCAAAGATGTA
	反向Reverse	GTCGAGGATGTACCGAATTTGTTTG
IL10	正向Forward	CCTGACCACGCTTTCTAGCTGTT
	反向Reverse	GGCTCCCTGGTTTCTTCTCCTAAG
CD11B	正向Forward	TTTGTCTCAACTGTGATGGAGCAAT
	反向Reverse	GTGATCTTGGGTTAGGGTTGTTCTG
CD40	正向Forward	TTAACTGTCCATCAGCAGGAGACTG
	反向Reverse	AAGTTCCATACCCATCATTCCAGCAG
CD86	正向Forward	AGTGAATAGCCTCCCTGTAAGTCC
	反向Reverse	CCCATAAGTGTGCTCTGAAGTGAAA
CD163	正向Forward	GCAAACCTCAGAATGGTGCTACTTGA
	反向Reverse	CAGTAATGGTGAAGGGACTCAGGTT
CD206	正向Forward	TTGAATACTGTGGTGAGCTGAAAGG
	反向Reverse	GGCAAATCCAGTTGTTAAGGTGTTCT

1.2.7 统计学分析

结果为 3 次独立实验的平均值, 采用 GraphPad 8.4.0 的 ANOVA 方差分析进行统计分析, $P < 0.05$ 时认为差异有统计学意义。

2 结果与分析

2.1 3 种 LPS SDS-PAGE 分析

A. muciniphila、*E. coli*、*F. nucleatum* LPS 经 SDS-PAGE 与银染后, 可以在 10–70 kD 范围内分别观察到 8、13、7 条带。其中, *A. muciniphila* LPS 有一条约 40 kD 的特有条带(图 1 中 b); *E. coli* LPS 在 53 kD 附近有 3 条(图 1 中 a)、28 kD (图 1 中 c)、17 kD (图 1 中 e)和 36 kD (图 1 中 f)附近一条特有条带; *F. nucleatum* LPS 有一条约 20 kD 的特有条带(图 1 中 d)。上述结果表明 3 种 LPS 可能在结构上存在差异。

2.2 3 种 LPS 诱导 THP-1 细胞贴壁作用

分别用不同浓度的 3 种 LPS 处理 THP-1 细胞 1 h 后, *A. muciniphila*、*F. nucleatum* LPS 浓度高于

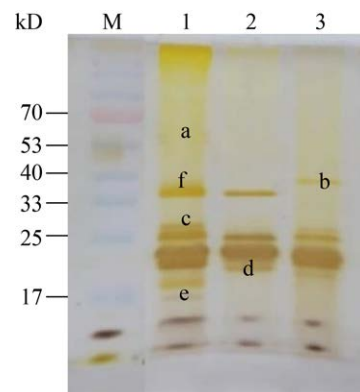


图 1 3 种 LPS SDS-PAGE 分析

Figure 1 Three kinds of LPS analyzed by SDS-PAGE

注: M: TureColor 三色预染蛋白 Marker (17–70 kD); 1: *E. coli* ATCC 25922; 2: *F. nucleatum* ATCC 25586; 3: *A. muciniphila* BNCC 341917; *E. coli* LPS 特有条带: a、c、e、f; *A. muciniphila* LPS 特有条带: b; *F. nucleatum* LPS 特有条带: d

Note: M: TureColor three-color pre-stained protein Marker (17–70 kD); 1: *E. coli* ATCC 25922; 2: *F. nucleatum* ATCC 25586; 3: *A. muciniphila* BNCC 341917; Specific bands of *E. coli* LPS: a, c, e, f; Specific band of *A. muciniphila* LPS: b; Specific band of *F. nucleatum* LPS: d

0.1 $\mu\text{g/mL}$ 的处理组贴壁细胞数显著多于对照组;当*E. coli* LPS浓度高于1 $\mu\text{g/mL}$ 时各处理组才具有显著性差异;去除LPS后继续培养4 h后细胞则基本恢复悬浮状态。结果表明*A. muciniphila*、*F. nucleatum* LPS具有与文献报道类似的诱导THP-1细胞瞬时贴壁作用^[26],但是,*A. muciniphila*、*F. nucleatum* LPS诱导贴壁作用强于*E. coli* LPS(图2)。

2.3 3种LPS诱导THP-1细胞形态变化

如图3-5所示,贴壁THP-1细胞分别用3种LPS处理24 h后,对各种形态细胞进行统计分析发现,LPS单独处理时,*F. nucleatum*处理组有伪足细胞比例分别是*A. muciniphila*和*E. coli* LPS处理组的67%和77%,平均伪足长度分别是另两组的77%和83%,形成梭形细胞比例分别是另两组的53%和67%;与IFN- γ 联合处理时,*F. nucleatum*组有

伪足细胞比例分别是*A. muciniphila*和*E. coli* LPS组的71%和91%;梭形细胞比例分别约为另两组的32%和63%,而且*A. muciniphila*和*E. coli*联合处理梭形细胞比例均显著高于IFN- γ 单独处理,仅*F. nucleatum*联合处理与IFN- γ 单独处理无显著差异($P>0.05$)(图5);此外,*F. nucleatum*联合处理组细胞平均伪足长度比*A. muciniphila*和*E. coli*联合处理均低约17%。

2.4 LPS处理对THP-1细胞基因表达变化的影响

2.4.1 3种LPS对PMA处理前后THP-1细胞TLR家族、白介素家族部分基因表达的影响

A. muciniphila、*E. coli*、*F. nucleatum* LPS直接处理THP-1细胞3 h,*IL-6*表达分别显著上调700.8% ($P=0.0395$)、1378.0% ($P=0.0004$)和782.9% ($P=0.0031$);但未检测到*TLR3*、*TLR4*和*IL-10*的表达(图6A)。

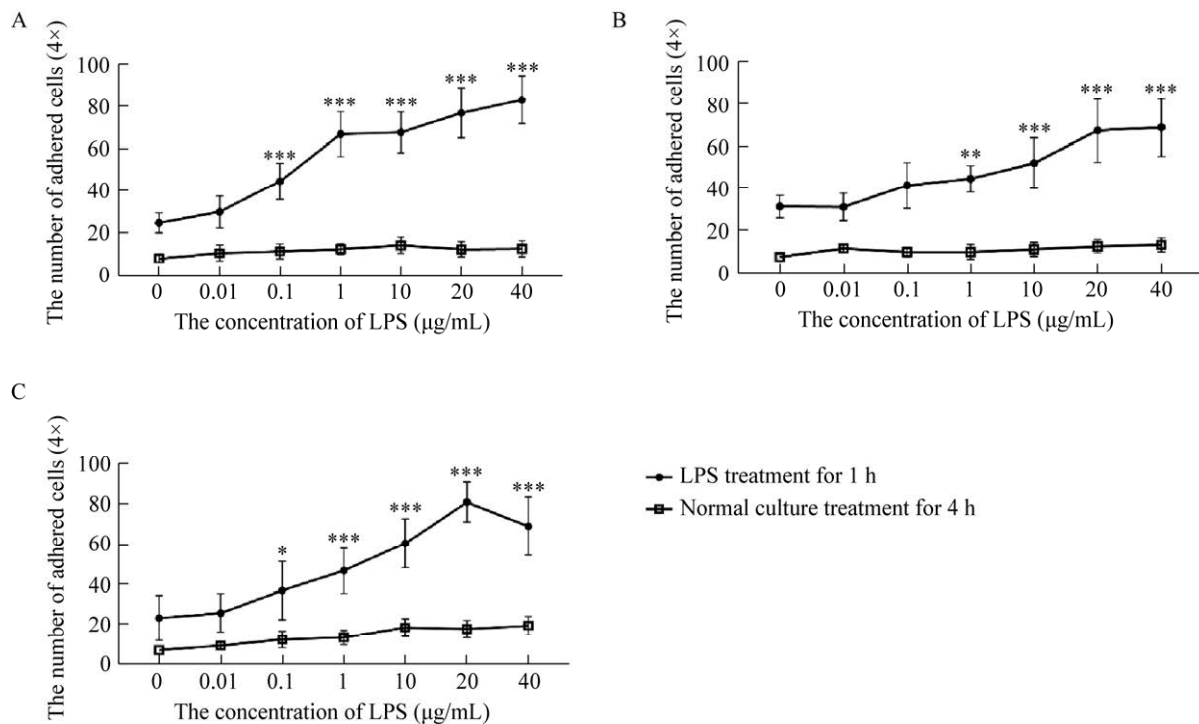


图2 3种LPS诱导THP-1细胞贴壁作用

Figure 2 The inducing adhesion of THP-1 after treated with three kinds of LPS

Note: A: *A. muciniphila* LPS; B: *E. coli* LPS; C: *F. nucleatum* LPS; ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$

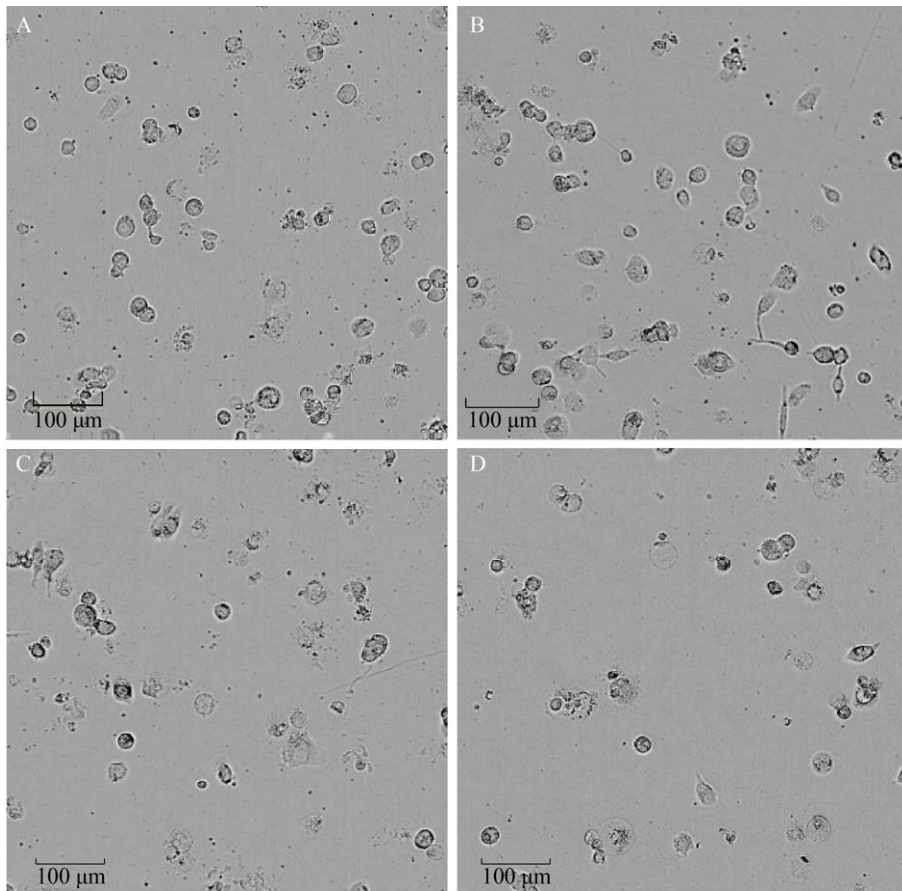


图 3 3 种LPS处理对THP-1 细胞形态影响

Figure 3 The morphology changes of THP-1 cells treated with LPS

Note: A: Control; B: *A. muciniphila* LPS; C: *E. coli* LPS; D: *F. nucleatum* LPS

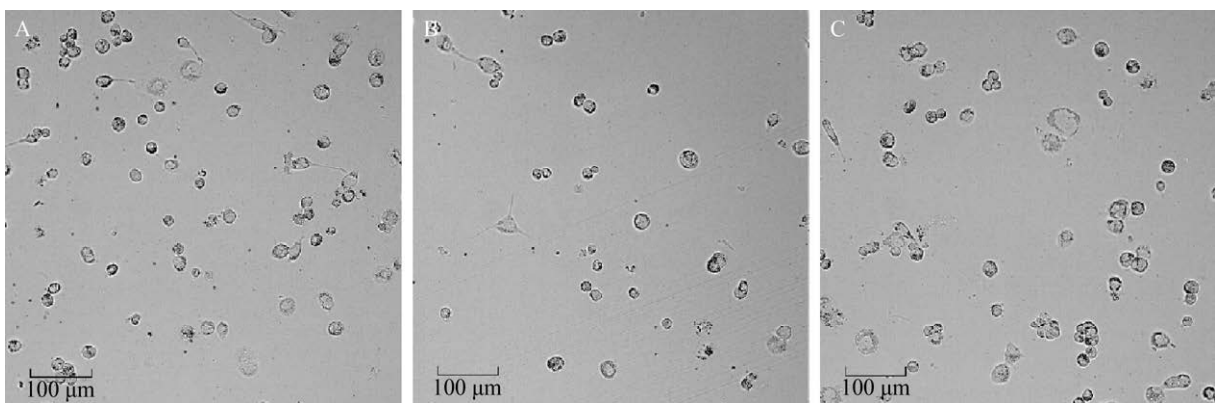


图 4 LPS与IFN- γ 联合处理对THP-1 细胞形态影响

Figure 4 The morphological change of THP-1 cells treated with LPS combining with IFN- γ

Note: A: *A. muciniphila* LPS; B: *E. coli* LPS; C: *F. nucleatum* LPS

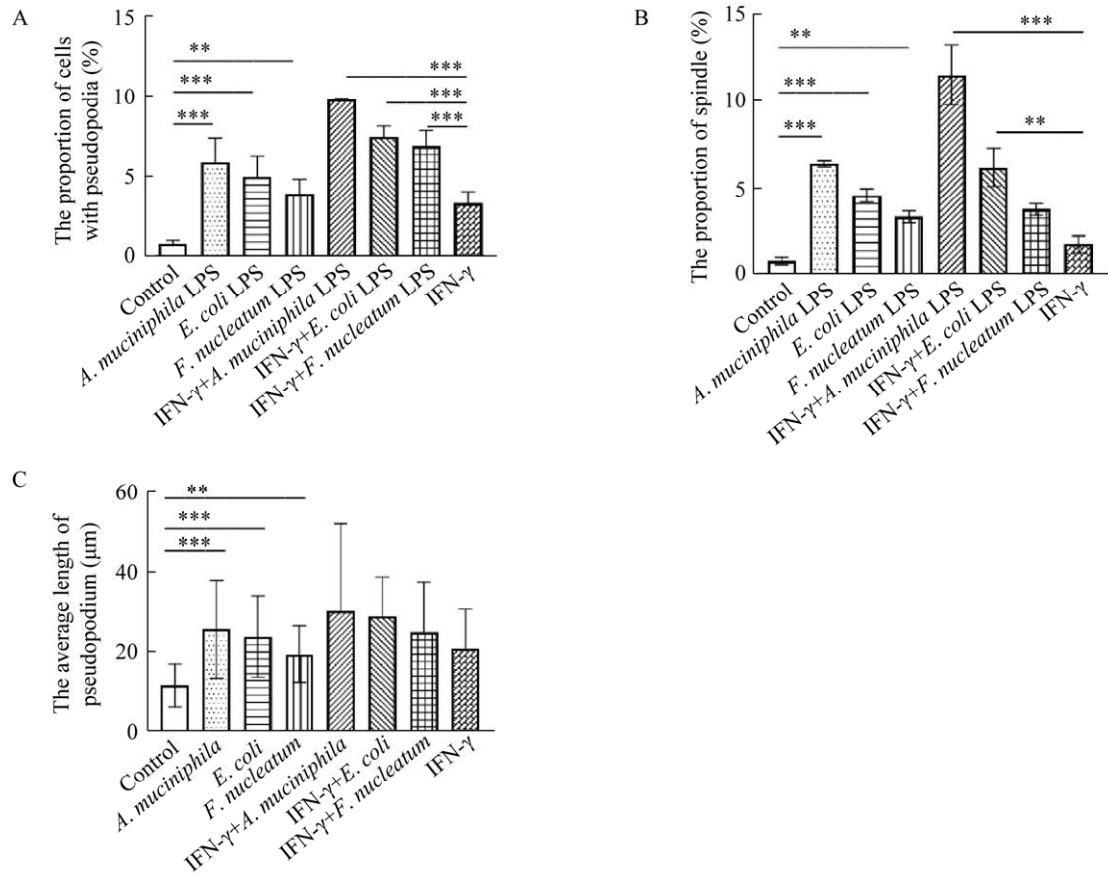


图 5 THP-1 经 3 种 LPS 单独或与 IFN- γ 联合处理后重要细胞特征变化

Figure 5 Changes of important cell characteristics of THP-1 treated with three kinds of LPS or combination with IFN- γ

注: A: 有伪足细胞比例; B: 梭形细胞比例; C: 平均伪足长度。***: $P < 0.001$; **: $P < 0.01$

Note: A: The proportion of cells with pseudopodia; B: The proportion of spindle cells; C: The average length of pseudopodium. ***: $P < 0.001$; **: $P < 0.01$

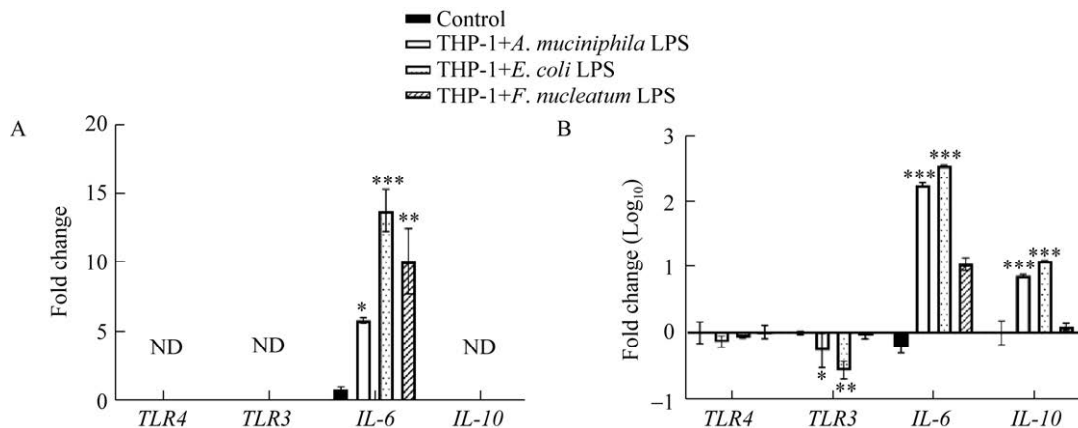


图 6 LPS 处理对 THP-1 细胞 *TLR3*、*TLR4* 以及 *IL-6*、*IL-10* 表达的影响

Figure 6 The effect of LPS treatment on the expression of *TLR3*, *TLR4* and *IL-6*, *IL-10* in THP-1 cells

注: A: THP-1; B: PMA 处理 24 h 的 THP-1。ND: 未检测到; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$

Note: A: THP-1; B: THP-1 treated with PMA for 24 h. ND: Not detected; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$

贴壁THP-1 细胞用相同浓度*A. muciniphila*、*E. coli*、*F. nucleatum* LPS处理, *IL-6* 分别上调 17 763.2% ($P<0.001$)、35 458.2% ($P<0.001$)、1 123.6% ($P>0.05$); *TLR3* 分别下调了 32.3% ($P=0.044$ 7)、311.5% ($P=0.001$ 9)、9.6% ($P>0.05$); *TLR4* 分别下调 35.1% ($P>0.05$)、17.0% ($P>0.05$) 和 10.3% ($P>0.05$); *IL-10* 分别显著上调 729.3% ($P<0.001$)、1 223.3% ($P<0.001$)、124.4% ($P>0.05$) (图 6B), 上述数据表明M0 细胞对LPS敏感性显著上升, 同时, 显示不同LPS作用呈现显著差异。

2.4.2 LPS对贴壁THP-1 细胞巨噬细胞表面标志基因表达的影响

贴壁THP-1 用 3 种LPS处理后, 检测巨噬细胞标志基因表达发现, *A. muciniphila*、*E. coli*、*F. nucleatum* LPS分别略微下调M0 标志基因表达(均无统计学意义); M1 标志基因中: *CD40* 分别上调 5 011.0% ($P<0.001$)、6 048.9% ($P<0.001$)和 1 011.6% ($P=0.009$ 4), *CD86* 分别上调 637.3% ($P<0.001$)、657.9% ($P<0.001$)和 194.1% ($P>0.05$); M2标志基因中: *CD163* 分别下调 39.5% ($P=0.001$ 1)、53.7% ($P<0.001$)和 5.9% ($P>0.05$), *CD206* 分别下调 18.6% ($P>0.05$)、88.4% ($P=0.005$ 5)和 24.8% ($P>0.05$), 详见图 7。

2.5 3种LPS对THP-1细胞IL-6、IL-10、CRP表达的影响

检测不同LPS诱导THP-1 细胞分泌IL-6 作用

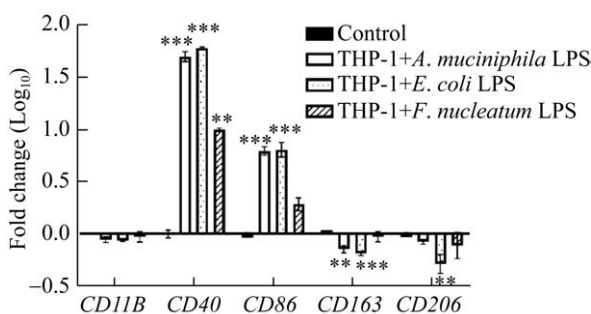


图 7 3种LPS对巨噬细胞表面标志基因表达的影响

Figure 7 The effect of three kinds of LPS on the expression of marker genes of macrophage

注: ***: $P<0.001$; **: $P<0.01$

Note: ***: $P<0.001$; **: $P<0.01$

发现, *A. muciniphila*、*E. coli* LPS可单独诱导THP-1 细胞分泌IL-6, 分别为 0.16 pg/mL和 6.17 pg/mL, *F. nucleatum*组却未检测到; 同时, 当与 20 ng/mL IFN- γ 联合处理时, *A. muciniphila*、*E. coli* 组 IL-6 分别达到 410.03 pg/mL和 1 334.40 pg/mL, *F. nucleatum*仅为 46.20 pg/mL (图 8)。IL-10、CRP在各实验组、对照组均未检测到。

3 讨论与结论

随着*H. pylori*、*F. nucleatum*、*A. muciniphila* 等致癌或免疫调控作用被发现, 导致细菌对肿瘤、免疫影响相关机制研究得到越来越多的关注。

实验结果发现, *F. nucleatum*、*A. muciniphila* LPS具有与文献[26]报道类似、但强于*E. coli* LPS的诱导THP-1 瞬时贴壁的作用, 换言之, 这 2 种LPS具有较强招募单核细胞至细菌存在或炎症部位的活性。综合有伪足细胞比例、平均伪足长度、长梭形细胞(M1)等形态比例以及M1、M2 标志基因表达分析等结果信息, *F. nucleatum* LPS

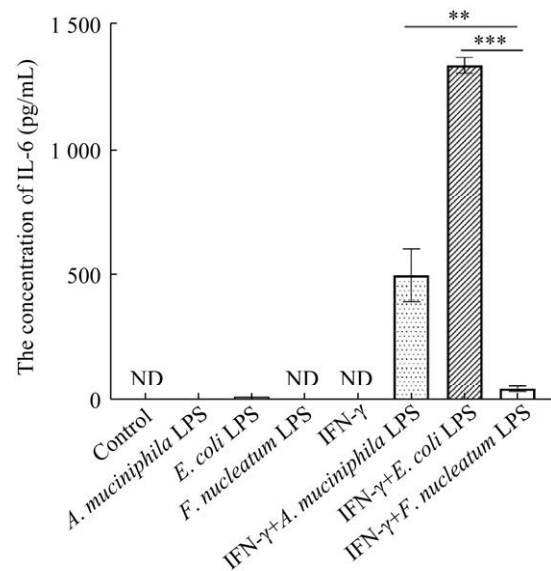


图 8 3种LPS诱导THP-1 细胞分泌IL-6 作用

Figure 8 The effect of three kinds of LPS inducing THP-1 protein level expression of IL-6

注: ND: 未检测到; ***: $P<0.001$; **: $P<0.01$

Note: ND: Not detected; ***: $P<0.001$; **: $P<0.01$

具有较强的诱导单核细胞向M2极化的活性, *A. muciniphila*、*E. coli* LPS具有较强的M1极化的活性。

转录水平基因表达分析发现, *A. muciniphila*、*F. nucleatum* LPS诱导IL-6表达比*E. coli* LPS低约50%; ELISA分析发现, *F. nucleatum* LPS无法单独诱导THP-1分泌可检测水平的IL-6; 与IFN- γ 的协同作用发现, *F. nucleatum* LPS诱导THP-1分泌IL-6的量仅为*A. muciniphila*和*E. coli* LPS组的11%和4%, 即*F. nucleatum* LPS即便与IFN- γ 联合作用, 也仅能诱导THP-1产生少量的IL-6。在肿瘤微环境中, 主要由T细胞和自然杀伤(Natural Killer, NK)细胞分泌的IFN- γ 兼具促进免疫效应细胞杀伤肿瘤与通过抑制免疫系统活性而促进肿瘤进展的双重作用^[30]; 有研究报道, 淋巴因子活化杀伤细胞(Lymphokine-Activated Killer Cell, LAK)杀伤癌细胞活性可被肿瘤细胞分泌的转化生长因子- β 1 (Transforming Growth Factor- β 1, TGF- β 1)抑制, 但该抑制作用会被高浓度的IL-6拮抗^[31]; 鉴于*F. nucleatum*广泛存在于结直肠、乳腺、胰腺等多种肿瘤中^[22-23], 因此*F. nucleatum* LPS诱导巨噬细胞分泌低浓度IL-6在引发慢性炎症及肿瘤免疫应答、逃逸等过程中可能发挥重要作用, 其具体机制值得深入开展研究, 例如, *F. nucleatum* LPS独特生物活性的结构基础。相关研究有助于深化对慢性炎症、肿瘤发生等机制认识, 并为相关疾病预防与治疗提供新的思路与方向。

同时, 综合*A. muciniphila*、*E. coli*、*F. nucleatum* LPS诱导THP-1极化、IL-6细胞因子表达等方面的生物活性差异及3种LPS结构差异等信息, LPS的结构、活性等研究应当由目前主要集中研究的*E. coli* LPS拓展到各种革兰氏阴性菌的LPS, 尤其是有明确致癌、提高免疫或肿瘤治疗作用的革兰氏阴性菌。

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