DOI: 10.13241/j.cnki.pmb.2014.01.015

肺炎支原体感染小鼠体内 IL-6、IFN-γ、IL-5 的水平及克拉霉素的影响

刘海燕[1] 杨旭东[2] 侯 伟[1] 钟 波[1] 陈新平[1]

(1 西安交通大学医学院第二附属医院儿科 陕西 西安 710004;
2 西安交通大学医学院遗传学与分子生物学系 陕西 西安 710061)

摘要 目的:研究肺炎支原体感染小鼠血清及支气管肺泡灌洗液中细胞因子的水平,了解克拉霉素治疗对细胞因子的影响。**方法:**将昆明小鼠分为感染组、正常组及药物治疗组,建立小鼠肺炎支原体感染模型成功后,药物组用克拉霉素(6 g·kg^{-1}·d^{-1})进行治疗,连续 5 天。在肺炎支原体感染模型建立后的第 8 天,检测三组小鼠的血清及支气管肺泡灌洗液中 IL-6、IFN-γ、IL-5 水平。**结果:**相比较于正常组,感染组小鼠支气管灌洗液和血清中的 IL-6、IFN-γ、IL-5 均显著升高 ($P < 0.05$)。而克拉霉素的使用能使 IL-6、IFN-γ 降低($P < 0.05$),但对 IL-5 水平无影响。**结论:**肺炎支原体感染使小鼠 IL-6、IFN-γ、IL-5 水平增高,克拉霉素治疗有一定疗效。

关键词:肺炎支原体;克拉霉素;细胞因子

中图分类号:Q95-3,R56 **文献标识码:**A **文章编号:**1673-6273(2014)01-74-03

Level of the IL-6, IFN-γ、IL-5 in the Mice with Mycoplasma Pneumoniae Infection and the Effect of Clarithromycin Treatment on Cytokine Production

LIU Hai-yan[1], YANG Xu-dong[2], HOU Wei[1], ZHONG Bo[1], CHEN Xin-ping[1]

(1 Department of Paediatrics, The Second Affiliated Hospital of Xi,an Jiaotong University School of Medicine, Xi'an, Shaanxi,710004;
2 Genetics and Molecular Biology, Xi,an Jiaotong University School of Medicine, Xi'an, Shaanxi, 710061, China)

ABSTRACT Objective: To observe the cytokine in the serum and bronchoalveolar lavage fluid of Mycoplasma Pneumonia mice and the effect of clarithromycin treatment on cytokine production. **Methods:** The Kunming mice were randomized into three groups. The three groups were normal group, infection group, treatment group. After infection, clarithromycin treatment group were treated with clarithromycin for five days (6 g·kg^{-1}·d^{-1}). On the 8th day, the IL-6, IFN-γ and IL-5 levels were determined in the serum and bronchoalveolar lavage fluid of each group. **Results:** The IL-6, IFN-γ and IL-5 levels were increased in the Pneumonia Mycoplasma infected group. A decrease of IL-6, IFN-γ levels were observed in the clarithromycin treatment group, but had no effect on the IL-5. **Conclusion:** The IL-6, IFN-γ and IL-5 levels were increased in the Mycoplasma Pneumonia mice. Clarithromycin treatment had effect on Mycoplasma Pneumonia infection.

Key words: Mycoplasma pneumonia; Clarithromycin; Cytokines

Chinese Library Classification(CLC): Q95-3, R56 Document code: A

Article ID: 1673-6273(2014)01-74-03

前言

肺炎支原体(mycoplasma pneumonia)为引起人类支原体肺炎的病原体[1]。肺炎支原体肺炎表现为以上呼吸道感染为主,人群普遍易感,而儿童及青少年为易感人群[2]。在小儿肺炎支原体感染中,可表现为全身脏器的损害,但临床中以呼吸道感染较为多见[3]。IL-6、IFN-γ、IL-5 作为免疫与炎症重要的调节因子,与肺炎支原体感染之间可能存在关联[4,5]。因此本文通过检测血清及支气管肺泡灌洗液中细胞因子的水平,了解肺炎支原体感染小鼠体内 IL-6、IFN-γ、IL-5 的水平变化特点;同时笔者通过对肺炎支原体感染小鼠克拉霉素治疗后细胞因子水平的检测,研究克拉霉素对肺炎支原体感染的影响。

作者简介:刘海燕(1971-),女,本科,主治医师,研究方向:儿童呼吸、过敏性疾病,E-mail:liuhaiyan_2232@163.com

(收稿日期:2013-08-06 接受日期:2013-08-26)

1 材料与方法

1.1 实验材料及分组

病原肺炎支原体 MP 国际标准株,购自首都儿科研究所。

SPF 级 8-11 周的昆明小鼠(17~20 g),雌雄各半,分开饲养。购于中山大学动物实验中心。每组 20 只,按随机数字表随机分为三组:正常组(未感染 MP),肺炎支原体感染组,治疗组给予克拉霉素(克拉霉素分散片,国药准字 H20020148),除正常组外,其余两组均鼻内接种同等剂量的 MP 菌液。

1.2 实验方法

1.2.1 接种及治疗 以乙醚将小鼠轻度麻醉后,小鼠鼻内接种 50 μL 含有 1 × 10^6 cfu/mL 的肺炎支原体菌液,正常对照组以等量生理盐水接种。克拉霉素治疗组按 6 g·kg^{-1}·d^{-1} 剂量灌胃给药,并在肺炎支原体感染模型建立后 1 天后给药,连续 5 天。进行

分离培养及 PCR 检测肺炎支原体，以确认肺炎支原体感染模型的建立。各组小鼠每天称重并观察。

1.2.2 **支气管肺泡灌洗液** 在肺炎支原体感染模型建立后的第 8 天，三组（各 20 只）分别采用支气管肺泡灌洗收集支气管肺泡灌洗液，具体方法为：通过气管插管，用 1.0 mL 的盐水对肺组织进行灌洗 2 次，并回收灌洗液，记录灌洗液的回收率。

1.2.3 **血清的采集** 各组小鼠摘眼球取血后，2500 转 / 分低速离心 30 min，取上层血清保存于 -20℃ 备用。

1.2.4 **流式细胞术对细胞因子进行检测** 检测各组支气管肺泡灌洗液和血清中的 IFN-γ，IL-6，IL-5 的浓度，采用流式细胞术，所使用仪器为美国 BD 公司 FACSCalibur 型流式细胞仪，使用试剂盒为 ebioscience 公司所产。比较各组小鼠的支气管肺泡灌洗液中的 IFN-γ，IL-6，IL-5 水平是否有差异。用流式细胞仪检测空白孔管和标准品共 6 管，绘制标准曲线，然后分别用流式细胞仪检测待测血清和支气管肺泡灌洗液样本。

1.3 统计学分析

采用 SPSS 14.0 统计软件对数据进行分析，三组间的比较进行单因素方差分析或 Kruskal-Wallis 检验。结果以 P＜0.05 表示有统计学意义。

2 结果

2.1 临床表现

肺炎支原体感染组小鼠活动减少，食量减少，体重减轻，部分小鼠在感染第 5 天呼吸急促。正常组小鼠正常觅食，体重持续增加。克拉霉素治疗组较肺炎支原体感染组情况稍好。

2.2 血清中细胞因子水平的测定

相对于正常组，感染组血清中 IL-6、IFN-γ、IL-5 水平的变化有统计学意义(P<0.01)。小鼠感染后使用克拉霉素能使 IL-6、IFN-γ 降低(P<0.05)，但对 IL-5 水平无影响(P>0.05)。具体见表 1、图 1。

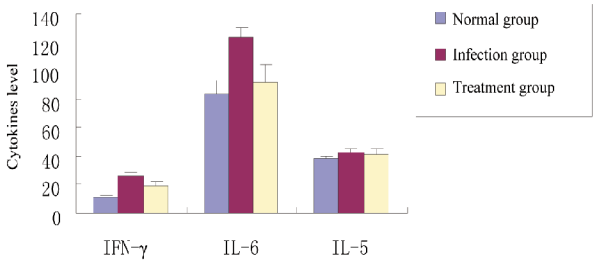

图 1 小鼠血清中细胞因子水平

Fig.1 Cytokines level in the serum of mice

2.3 支气管肺泡灌洗液中细胞因子水平的测定

相对于正常组，感染组小鼠支气管灌洗液中 IL-6、IFN-γ、IL-5 显著升高(P<0.01)。而克拉霉素的使用能使 IL-6、IFN-γ 降低(P<0.05)，但对 IL-5 水平无影响(P>0.05)。具体见表 2。

表 1 小鼠血清中细胞因子检测值（n=20）

Table1 Testing value of cytokines in the mice serum

Group	IFN-γ	IL-6	IL-5
Normal group	10.7± 2.3**	83.4± 10.6* *	38.1± 1.9*
Infection group	25.6± 3.5	123.0± 6.8	42.6± 3.0
Treatment group	19.1± 2.9**	91.4± 12.7* *	41.7± 4.2

注：与感染组相比，* P ＜0.05，* * P ＜0.01。

Note:Compare with the infection group, * P ＜0.05,* * P ＜0.01.

表 2 小鼠支气管肺泡灌洗液中细胞因子检测值（n=20）

Table 2 Testing value of cytokines in the bronchoalveolar lavage fluid of mice

Group	IFN-γ	IL-6	IL-5
Normal group	17.5± 4.1* *	101.7± 9.9* *	49.5± 5.4* *
Infection group	30.2± 1.3	143.6± 10.8	55.8± 4.9
Treatment group	29.0± 4.0*	118.4± 6.7* *	54.2± 1.5

注：与感染组相比，* P ＜0.05，* * P ＜0.01。

Note: Compare with the infection group, * P ＜0.05,* * P ＜0.01.

3 讨论

肺炎支原体 MP 感染近年来越来越引起关注，它是导致社区获得性肺炎的主要病原体之一，学龄儿童为肺炎支原体感染的易感人群[6]。MP 感染后临床表现不一，可对呼吸系统等多个系统造成损伤。肺炎支原体感染后的症状包括咽炎、声音嘶哑、高热等，在严重感染病例中可能会出现呼吸困难[7,8]。由于其临床症状与其它病原体感染无显著差异，故肺炎支原体感染的诊断有一定难度。临床上肺炎支原体感染治疗的首选药物为大环内酯类抗生素，但其对该类药物的耐药性问题及疗效问题常常导致治疗效果不佳甚至是无效[9,10]。肺炎支原体如何作用于免疫系统还有待于研究。

肺炎支原体感染时的病理改变主要为间质性肺炎、支气管炎。关于肺炎支原体致病机制的研究，目前认为除了与体液免疫有关外，还与机体的细胞免疫反应有关[11,12]。研究[13,14]发现，很多细胞因子参与了 MP 感染。IL-6、IL-4、IL-18、IL-10、IL-5、

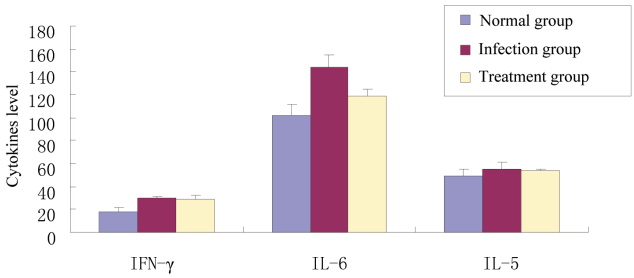

图 2 小鼠支气管肺泡灌洗液中细胞因子水平

Fig.2 Cytokines level in the bronchoalveolar lavage fluid of mice

IFN-γ、TNF-α 等是肺炎支原体感染时其刺激机体产生的主要细胞因子，细胞因子水平在不同患者及患者感染后的不同时期均不同 [15,16]。IL-6 水平是支原体肺炎感染严重与否的标志[17]，IL-5 可能与喘息等症状有关[18]，而 IFN-γ 则与体液免疫应答密切相关[19]。而这些因子的产生与肺炎支原体感染的临床表现有关，因此对血清或肺泡灌洗液中细胞因子的水平的检测对于了解疾病的严重程度和预后有重要意义[20]。而克拉霉素治疗支原体肺炎时是否对细胞因子的产生及对哪些细胞因子起作用，仍有待进一步研究。

本研究分别对小鼠血清和支气管肺泡灌洗液中几种细胞因子的水平进行检测，主要针对 IL-6、IFN-γ、IL-5。结果表明，在小鼠感染支原体肺炎模型中，相比较于正常组，感染组小鼠支气管灌洗液和血清中的 IL-6、IFN-γ、IL-5 均显著升高。而克拉霉素的使用能使 IL-6、IFN-γ 降低，但对 IL-5 水平无影响。可见，克拉霉素用于治疗支原体肺炎有一定疗效，可能与其能抑制部分细胞因子的水平有关。各组小鼠连续几天的肉眼观察发现，克拉霉素药物治疗组对于支原体肺炎的感染有缓解作用。但更多与 MP 感染有关的细胞因子的表达水平有待阐明。

参考文献(References)

[1] 严峻,易静波,肖雪花.阿奇霉素联合痰热清治疗小儿肺炎支原体肺炎疗效观察[J]. 现代生物医学进展, 2010, 10(12): 2339-2345
Yan Jun, Yi Jing-bo, Xiao Xue-hua. Observation of the Curative Effect of Azithromycin Combined with Tanreqing in treating Child with Mycoplasma Pneumoniae Pneumonia[J]. Progress in Modern Biomedicine, 2010, 10(12): 2339-2345

[2] Surender Kashyap, Malay Sarkar. Mycoplasma pneumonia: Clinical features and management[J]. Lung India, 2010, 27(2): 75-85

[3] Liu Y, Ye X, Zhang H, et al. Antimicrobial susceptibility of Mycoplasma pneumoniae isolates and molecular analysis of macrolide-resistant strains from Shanghai, China [J]. Antimicrob Agents Chemother, 2009, 53(5): 2160-2162

[4] 陈良东, 周建波, 张廷, 等. 肺炎支原体肺炎患儿 TNF-α、IL-6、IL-8 及 T 细胞亚群变化意义[J]. 临床肺科杂志, 2011, 16(9): 1374-1376
Chen Liang-dong Zhou Jian-bo, Zhang Ting, et al. Study on the clinical significance of serum TNF-α, IL-6, IL-8 concentration and T cell subsets in children patients with mycoplasma pneumonia[J]. Journal of Clinical Oulmonary Medicine, 2011, 16(9): 1374-1376

[5] 刘文彬, 赵文利, 汤雪琴, 等. 肺炎支原体肺炎 IL-2、Sil-2R、IL-6、IL-8 检测[J]. 四川医学, 2012, 33(9): 1671-1672
Liu Wen-bin, Zhao Wen-li, Tang Xue-qin, et al. Dynamic changes of IL-2, Sil-2R, IL-6 and IL-8 in children with mycoplasma pneumonia and their clinical significance [J]. Sichuan Medical Journal, 2012, 33 (9): 1671-1672

[6] 李师燕. 克拉霉素转换治疗呼吸道肺炎支原体感染的临床观察[J]. 中国医药导报, 2009, 6(18): 72-73
Li Shi-yan. The clinical observation about Clarithromycin conversion treatment of respiratory tract mycoplasma pneumoniae infection [J]. China Medical Herald, 2009, 6(18): 72-73

[7] Choi IS, Byeon JH, Yoo Y,et al. Increased serum interleukin-5 and vascular endothelial growth factor in children with acute mycoplasma pneumonia and wheeze[J]. Pediatr Pulmonol, 2009, 44(5): 423-428

[8] Salvatore CM, Techasaensiri C, Tagliabue C, et al. Tigecycline therapy significantly reduces the concentrations of inflammatory pulmonary cytokines and chemokines in a murine model of Mycoplasma pneumoniae pneumonia[J]. Antimicrob Agents Chemother, 2009, 53(4): 1546-1551

[9] Hirao S, Wada H, Nakagaki K, et al. Inflammation provoked by Mycoplasma pneumoniae extract: implications for combination treatment with clarithromycin and dexamethasone[J]. FEMS Immunol Med Microbiol, 2011, 62(2): 182-189

[10] Tagliabue C, Techasaensiri C, Torres JP, et al. Efficacy of increasing dosages of clarithromycin for treatment of experimental Mycoplasma pneumoniae pneumonia [J]. J Antimicrob Chemother, 2011, 66(10): 2323-2329

[11] Zeitlinger M, Wagner CC, Heinisch B. Ketolides-the modern relatives of macrolides: the pharmacokinetic perspective[J]. Clin Pharmacokinet, 2009, 48(1): 23-38

[12] 蒋德林, 万荣文, 周巧芳, 等. 分析阿奇霉素联合利巴韦林治疗小儿支原体肺炎的疗效 [J]. 现代生物医学进展, 2010, 10(10): 1923-1925
Jiang De-lin, Wan Rong-wen, Zhou Qiao-fang, et al. Azithromycin Combined with Ribovirin for Child Mycoplasmal Pneumonia[J]. Progress in Modern Biomedicine, 2010, 10(10): 1923-1925

[13] Shimizu T, Kida Y, Kuwano K. Cytoadherence-dependent induction of inflammatory responses by Mycoplasma pneumonia[J]. Immunology, 2011, 133(1): 51-61

[14] 谭笑. 克拉霉素经 NF-κB 途径抑制肺炎支原体抗原诱导 A549 细胞产生 IL-8[J]. 实用预防医学, 2012, 19(1): 119-121
Tan Xiao. Effect of Clarithromycin on IL-8 Production in A549 Cells Induced by Mycoplasma pneumonia Antigen[J]. Practical Preventive Medicine, 2012, 19(1): 119-121

[15] Ahn KK, Kwon D, Jung K, et al. Identification of interleukin-1, tumor necrosis factor-alpha, and interleukin-6 expression in lungs from pigs naturally infected with Mycoplasma hyopneumoniae by in situ hybridization[J]. J Vet Med Sci, 2009, 71(4): 441-445

[16] Kurata S, Taguchi H, Sasaki T. Antimicrobial and immunomodulatory effect of clarithromycin on macrolide-resistant Mycoplasma pneumonia[J]. J Med Microbiol, 2010, 59(Pt 6): 693-701

[17] Pang HX, Qiao HM, Cheng HJ, et al. Levels of TNF-α, IL-6 and IL-10 in bronchoalveolar lavage fluid in children with Mycoplasma pneumoniae pneumonia [J]. Chinese Journal of Contemporary Pediatrics, 2011, 13(10): 808-810

（下转第 22 页）

是一个重要的基础理论问题,也是肿瘤诊断、治疗及预后等具有应用前景的临床问题。本研究结果为下一步筛选针对突变 Kras 基因的靶向药物奠定了基础。

参考文献(References)

[1] Castellano E, Santos E. Functional specificity of ras isoforms: so similar but so different[J]. Genes Cancer, 2011, 2: 216-231

[2] Karnoub A E, Weinberg R A. Ras oncogenes: split personalities [J]. Nat Rev Mol Cell Biol, 2008, 9(7): 517-531

[3] Kobayashi Y, Kawaoi A, Katon R. Mutation of Ras oncogene indiisopropanolnitrosamine induced rat thyroid carcinogenesis [J]. Virchows Arch, 2002, 44: 289-295

[4] Benn J, Schneider RJ. Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAPKinase signaling cascade[J]. Proc Natl Acad Sci USA, 1994, 91(22): 10350-10354

[5] Nassar N, Singh K, Garcia-Diaz M. Structure of the dominant negative S17N mutant of Ras[J]. Biechemistry, 2010, 49: 1970-1974

[6] Luo D, Liu QF, Gove C, et al. Analysis of N-ras gene mutation and p53 gene expression in human hepatocellualr carcinomas [J]. World J Gastroenterol, 1998, 4: 97-99

[7] Richards CA, Short SA, Thorgeirsson SS, et al. Characterization of transforming N-ras gene in the human hepatoma cell line HepG2: additional evidence for the importance of c-myc and ras cooperation in hepatocarcinogenesis[J]. Cancer Res, 1990, 50: 1521-1527

[8] Sun B, Gao Y, Deng L, et al. The level of oncogene H-Ras correlates with tumorigenicity and malignancy[J]. Cell Cycle, 2008, 7: 934-939

[9] Newell P, Toffanin S, Villanueva A, et a1. Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo[J]. J Hepatol, 2009, 51: 725-733

[10] Chalfie M, Tu Y, Fuskirchen G, et al. Green fluorescent protein as a marker for gene expression[J]. Science, 1994, 263(5148): 802-805

[11] Cheng L, Fu J, Tsukamoto A, et al. Use of green fluorescent protein variants to monitor gene transfer and expression in mammalian cells [J]. Nat Biotechnol, 1996, 14(5): 606-609

[12] Chishima T, Miyagi Y, Wang X, et al. Cancer invasion and micrometastasis visulized in liver tissue by green fluorescent protein expression[J]. Cancer Res, 1997, 57(10): 2042-2047

[13] Takada T, Iida K, Awaji T, et al. Selective production of transgenic mice using green fluorescent protein as a marker[J]. Nat Biotechnol, 1997, 15(5): 458-461

[14] Tuveson DA, Zhu L, Gopinathan A, et al. Mist1-KrasG12D knock-in mice develop mixed differentiation metastatic exocrine pancreatic carcinoma and hepatocellular carcinoma [J]. Cancer Res, 2006, 66(1): 242-247

[15] Weihrauch M, Benicke M, Lehnert GFrequent k-ras-2 mutations and p16 (INK4A) methylation in hepatocellular carcinomas in workers exposed to vinyl chloride[J]. Br J Cancer, 2001, 84(7): 982-989

[16] Tannapfel A, Sommerer F, Benicke M, et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma[J]. Gut, 2003, 52(5): 706-712

[17] Marguardt JU, Galle PR, TeufeI A. Hepatocellular carcinoma: molecular pathogenesis and novel targets for therapy [J]. Dtsch Med Wochenschr, 2012, 137(16): 855-860

[18] Zhang C, He H, Zhang H, et al. The blockage of Ras/ERK pathway augments the sensitivity of SphK1 inhibitor SKI II in human hepatoma HepG2 cells [J]. Biochem Biophys Res Commun, 2013, 434(1): 35-41

[19] Man JH, Liang B, Gu YX, et al. Gankyrin plays an essential role in Ras-induced tumorigenesis through regulation of the RhoA / ROCK pathway in mammalian cells[J]. J Clin Invest, 2010, 120:2829-2841

[20] Qin D, Li K, Qu J, et al. HBx and HBs regulate RhoC expression by upregulating transcription factor Ets-1[J]. Arch Virol, 2013, 158: 1773-1781

(上接第 76 页)

[18] Jeong YC, Yeo MS, Kim JH, et al. Mycoplasma pneumoniae Infection Affects the Serum Levels of Vascular Endothelial Growth Factor and Interleukin-5 in Atopic Children [J]. Allergy Asthma Immunol Res, 2012, 4(2): 92-97

[19] 冯金燕, 陈志敏. 过敏状态对肺炎支原体感染后血清白细胞介素-4、5 及 γ 干扰素的影响[J]. 中国小儿急救医学, 2012, 19(3): 241-244

Feng Jin-yan, Chen Zhi-min. Effect of atopic status on interleukin-4, interferon-γ levels in serum of children with mycoplasma pneumonia infection[J]. Chinese Pediatric Emergency Medicine, 2012, 19(3): 241-244

[20] Huang H, Zhong LL, Peng L, et al. Levels of interleukin-4 and interferon-gamma in the bronchoalveolar lavage fluid of children with refractory Mycoplasma pneumoniae pneumonia [J]. Chinese Journal of Contemporary Pediatrics, 2012, 14(8): 581-584