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慢性阻塞性肺病患者巨噬细胞功能与受体表达的关系研究 *

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摘要 目的:研究慢性阻塞性肺病(COPD)患者气道巨噬细胞功能变化及其与受体表达的相关性。**方法:**将 COPD 患者 84 例按病情分为轻中度组 44 例,重度组 40 例,选取同期健康体检者 40 例作为对照组,获取 3 组诱导痰,分离痰巨噬细胞,检测 3 组吞噬荧光标记曲霉孢子的吞噬指数(PI),采用实时定量反转录 PCR 法检测 3 组吞噬相关受体的表达。**结果:**轻中度组与重度组细胞总数均多于对照组,而巨噬细胞比例却显著下降(P＜0.05);轻中度组与重度组巨噬细胞吞噬功能均受到抑制,3 组 PI 比较差异有统计学意义(P＜0.05);3 组巨噬细胞胶原结构清道夫系统(MARCO)、清道夫受体 A1(SR-A1)表达量比较差异不明显(P＞0.05);轻中度组与对照组 Toll 样受体 4(TLR4)表达量比较差异不明显,但重度组 TLR4 表达上调,与轻中度组、对照组比较差异有统计学意义(P＜0.05);3 组 MUC5A、AQP5 表达量比较差异显著(P＜0.05);巨噬细胞 PI 与 TLR4、黏蛋白 5AC(MUC5A)表达量呈负相关(P＜0.05),与水通道蛋白 5(AQP5)表达量呈正相关(P＜0.05)。**结论:**COPD 患者巨噬细胞占细胞总数的比例下降,其吞噬功能也受到抑制,其机制可能与 TLR4、MUC5A 表达上调及 AQP5 表达下调等有关。

关键词:慢性阻塞性肺病;巨噬细胞;吞噬功能;受体表达

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The Relationship of Macrophage Function with Receptor Expression in Patients with Chronic Obstructive Pulmonary Disease*

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ABSTRACT Objective: To study airway macrophages function changes and its relationship with receptor expression in patients with COPD. **Methods:** 84 patients with COPD in our hospital between January 2015 and March 2016 were divided into the mild-moderate group (44 cases) and the severe group (40 cases), and selected 40 healthy people as the control group. We obtained the induced sputum from the three groups, separated macrophage, and detected the phagocytic index (PI) of fluorescent taged aspergillus spores. Real-time quantitative reverse transcription PCR method was used to detect the expression of related genes of 3 groups. **Results:** The total number of cells in mild-to-moderate group and severe group were more than in the control group, but the macrophages ratio had dropped significantly (P<0.05). All the phagocytosis of macrophage were restrained in mild-to-moderate group and severe group. PI of three groups had statistically significant difference (P<0.05). The difference in expression quantity of Macrophage SR with collagenous structure (MARCO) and Scavenger receptor-A1 (SR-A1) had no statistical significance between the three groups (P>0.05). Toll-like receptors-4 (TLR4) expression quantity in mild-to-moderate group showed no statistically significant difference, when compared with control group, but that in severe group was upregulated and had significant difference with those of the mild-to-moderate group and the control group (P<0.05). MUC5A and AQP5 expression quantity also showed statistically significant differences between three groups (P<0.05). Macrophages PI was negatively correlated with the expression quantity of TLR4 and MUC5A (P<0.05), but positively correlated with the expression quantity of AQP5 (P<0.05). **Conclusion:** Patients with COPD had the proportion of macrophages decreased and the phagocytosis function restrained as more greatly as the disease was aggravating. Its mechanism may be related to the up-regulation of TLR4 and MUC5A expression and down-regulation of AQP5 expression.

Key words: Chronic obstructive pulmonary disease; Macrophages; Phagocytosis; Receptor expression

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前言

巨噬细胞在机体固体免疫及获得性免疫中发挥重要作用。慢性阻塞性肺病(Chronic obstructive pulmonary disease,COPD)一类以肺功能进行性恶化、持续性气道炎症为特征的慢性疾病[1]。巨噬细胞是肺脏防御系统的重要组成部分,受 Toll 样受体(Toll-like receptors,TLRs)、巨噬细胞胶原结构清道夫系统(Macrophage SR with collagenous structure,MARCO)、清道夫受体 A1(Scavenger receptor-A1,SR-A1)、黏蛋白 5AC(Mucin 5AC,MUC5AC)及水通道蛋白 5(Aquaporin 5,AQP5)等受体表达的影响,巨噬细胞吞噬功能受到抑制,这与 COPD 的发生及发展密切相关[2-4]。本文旨在研究不同病情的 COPD 患者气道巨噬细胞吞噬功能的变化及其与受体表达的相关性。

1 资料与方法

1.1 临床资料

选取 84 例 COPD 患者作为研究对象。纳入标准:① 符合《慢性阻塞性肺疾病诊治指南(2015 年修订版)》中关于 COPD 诊断标准及分级标准[1],并经体检、肺功能检查、X 线或 CT 检查确诊;② 年龄≥ 40 岁;③ 病情处于稳定期患者;④ 签署知情同意书。排除标准:① 合并严重心、肝、肾及血液系统疾病;② 伴有支气管扩张、哮喘、肺结核纤维化病变或其他慢性肺部疾病者;③ 近 1 个月有发热或其他感染性疾病者。男 58 例,女 26 例,年龄年龄 44~70 岁,平均(64.07± 8.64)岁;病程 5~17 年,平均(11.64± 3.76)年。根据病情将 84 例患者分为轻中度组 44 例,重度组 40 例。另选取同期在本院体检的健康人群 40 例作为对照组,男 27 例,女 13 例,年龄 43~68 岁,平均(63.66± 9.02)岁。COPD 组与对照一般资料比较差异无统计学意义(P>0.05),具有可比性。

1.2 方法

1.2.1 诱导痰获取 3 组均雾化吸入生理盐水,COPD 组吸入 1%~3%,对照组吸入 1%~5%,随后用力用鼻吹气,洗净口腔,对准培养皿用力咳痰,对咳痰进行冷冻处理;将痰液称重,加入 4 倍痰液体积的 0.1%二硫苏糖醇,水浴摇匀 25 min 至痰液粘液溶解,过滤,将不溶物去除,对痰液进行 3000 r/min 离心 10 min,离心半径 13.5 cm;收集上清液检测蛋白,采用台盼兰染色排除法[5]分析粒细胞存活力。

1.2.2 痰巨噬细胞分离 采用 RosetteSep 细胞分离法[6]分离痰巨噬细胞。在细胞沉淀中加入 1 mL 含 20%小牛血清的 PRMI-1640 培养皿中重悬细胞,重于 5%CO_2 细胞培养箱内 37℃培养 2 h,弃上清液;采用 PBS 冲洗细胞 2 次,将为贴壁细胞去除;重新加入 20%小牛血清 PRMI-1640 培养皿中培养,置于 CO_2 细胞培养箱内备用,隔离的痰液细胞再次通过流式细胞仪分析检查总纯度。

1.2.3 吞噬功能检测 离心后获得的细胞以 1× 10^6/mL 接种在六孔板,5%CO_2 细胞培养箱内 37℃培养 15 h,随后每孔中加入 1~10× 10^6 荧光标记曲霉孢子混悬液,孵育 2 h,PBS 清洗,70%乙醇固定 10 min,避光孵育。荧光显微镜下观察吞噬曲霉孢子的巨噬细胞比例及吞噬个数,计算吞噬指数(Phagocytic index,PI)=被吞噬的曲霉孢子总数 / 巨噬细胞总数。

1.2.4 受体表达检测 采用直接裂解法[7]提取总 RNA。随后采用相对定量法[8]分别测定目的基因与内参基因β-Actin 的 Ct。参考文献[6-9]设计引物,由南京金思瑞生物科技有限公司合成,引物序列见表 1。随后将 PCR 反应管用离心机瞬时离心后进行实时 PCR 反应,记录并打印实验结果。吞噬受体表达以相对于看家基因β-actin(内参基因)的倍数表示。

表 1 吞噬受体引物序列及扩增条件

Table 1 The related receptor primers and amplification conditions

Locus	Primer sequences (5 '~ 3')	Amplification conditions
β-Actin	CTCCATCCTGGCCTCGCTGT GCTCTCACCTTCACCGTTCC	-
TLR4	GGATGATGTCTGCCTCGCGCC TTAGGAACCACCTCCACGCAGGG	50℃ 30 min, 94℃ 2 min, 94℃ 20 s, 60℃ 20 s(47cycles), 68℃20 s
MARCO	ATCCTGCTCACGGCAGGTACT GCACATCTCTAGCATCTGGAGCT	94℃ 40 s, 58℃ 40 s, 72℃ 2 min(28 cycles), 72℃10 min
SR-A1	TCCTTGCAGAGTCTGAATATGACT CCTCCTGTTGCTTTGCTGTAGATT	50℃ 30 min, 94℃ 2 min, 94℃ 20 s, 60℃ 20 s (47cycles), 68℃20 s
MUC5A	TGTTCTATGAGGGCTGCGTCT	94℃ 3min, 94℃ 20 s, 57℃ 20 s, 72℃ 20 s (35 cycles), 72℃5 min
C	ATGTCGTGGGACGCACAGA	
AQP5	GCGCTCAGCAACAACACAAC GTGTGACCGACAAGCCAATG	94℃ 40 s,58℃ 40 s,72℃ 2 min(28 cycles), 72℃10 min

1.3 统计学方法

计量资料用($\bar{x}\pm s$)表示,采用 t 或 F 检验;巨噬细胞 PI 与受体的相关性分析采用 Spearman 相关分析,P<0.05 为差异有统计学意义。

2 结果

2.1 巨噬细胞吞噬功能

轻中度组与重度组细胞总数均多于对照组，而巨噬细胞比例却显著下降，差异比较有统计学意义（P＜0.05），但轻中度组与重度组细胞总数及巨噬细胞比例比较差异无统计学意义（P＞0.05）；轻中度组与重度组巨噬细胞吞噬功能均受到抑制，重度组受抑制作用较明显，3 组 PI 比较差异有统计学意义（P＜0.05）（表 2）。

表 2 3 组巨噬细胞 PI 比较（$\bar{x}\pm s$）

Table 2 Comparison of PI of macrophage between three groups（$\bar{x}\pm s$）

Groups	Cases	Total number of cells（× 10^5 cell/g）	Macrophage（%）	PI
Control group	40	5.43± 2.15	60.73± 4.29	85.47± 9.23
Mild - moderate group	44	15.53± 4.03*	54.34± 6.04*	78.41± 10.05*
Severe group	40	17.43± 5.15$^{\#}$	52.09± 5.96$^{\#}$	72.57± 9.34$^{\#\triangle}$
F	-	13.60	7.44	6.21
P	-	＜0.05	＜0.05	＜0.05

Note: comparison between the control group and mild - moderate group, *P＜0.05; comparison between the control group and severe group, $^{\#}$P＜0.05; comparison between the mild - moderate group and severe group, $^{\triangle}$ P＜0.05.

2.2 受体的表达

3 组 MARCO、SR-A1 表达量比较差异无统计学意义（P＞0.05）；轻中度组与对照组 TLR4 表达量比较差异无统计学意义，但重度组 TLR4 表达上调，与轻中度组、对照组比较差异有统计学意义（P＜0.05）；3 组 MUC5A、AQP5 表达量比较差异有统计学意义（P＜0.05），轻中度组、重度组 MUC5A 表达量上调，而 AQP5 表达量下调见表 3。

表 3 3 组吞噬受体表达比较（$\bar{x}\pm s$）

Table 3 Comparison of related receptor expression between three groups（$\bar{x}\pm s$）

Groups	Cases	TLR4	MARCO	SR-A1	MUC5AC	AQP5
Control group	40	0.92± 0.65	1660.73± 344.16	859.45± 229.07	1.65± 0.45	6.89± 1.36
mild - moderate group	44	1.14± 0.73	1705.31± 326.27	778.79± 210.79	5.38± 2.04*	3.86± 0.75*
Severe group	40	2.74± 1.02$^{\#\triangle}$	1642.19± 405.16	748.57± 309.31	7.64± 2.15$^{\#\triangle}$	1.79± 0.59$^{\#\triangle}$
F	-	9.52	0.22	1.82	17.25	21.76
P	-	＜0.05	＞0.05	＞0.05	＜0.05	＜0.05

Note: comparison between the control group and mild-moderate group, *P＜0.05; comparison between the control group and severe group, $^{\#}$P＜0.05; comparison between the mild -moderate group and severe group, $^{\triangle}$ P＜0.05.

2.3 巨噬细胞 PI 与受体的相关性

Spearman 相关分析显示，巨噬细胞 PI 与 TLR4、MUC5A 表达量呈负相关（r＝－0.52、－0.36，P＜0.05），与 AQP5 表达量呈正相关（r＝0.41，P＜0.05）。

3 讨论

巨噬细胞广泛分布于 COPD 肺泡及气道表面，通过吞噬炎性细胞并分泌细胞因子调节和始动局部免疫炎症反省，是患者肺部防御系统最丰富的免疫细胞。研究显示，COPD 患者受多方面影响，巨噬细胞对气道凋亡上皮、颗粒物质及病原微生物的吞噬能力下降，使得肺部固有免疫受损，从而促使 COPD 急性加重发作[10]。巨噬细胞作为肺部对外来病原菌及异物的第一道防线，其活性、功能对肺组织极为重要。有研究显示，COPD 患者巨噬细胞在支气管黏膜层及管腔内数量增加，且其功能也发生了改变。Belli 等[11]发现 COPD 患者因香烟烟雾的影响，气道巨噬细胞凋亡加速，并与其吞噬功能的抑制程度相关。曾华东等[12]采用单一烟熏的方法制备 COPD 模型大鼠，发现大鼠巨噬细胞所占比例显著升高，但其吞噬功能却发生改变，受到显著抑制。Barnawi 等[13]研究报道，COPD 患者气道巨噬细胞在体外的杀菌活性显著下降。本研究中，COPD 组患者细胞总数均多于对照组，而巨噬细胞比例却显著下降，差异比较有统计学意义（P＜0.05），这与上述文献报道不符，其原因有待进一步研究。但与上述文献报道相符的是 COPD 组患者巨噬细胞吞噬功能均受到抑制，重度组受抑制作用较明显，3 组 PI 比较差异有统计学意义（P＜0.05），证实 COPD 患者气道巨噬细胞的吞噬功能受到抑制，且随着病情加重，其抑制作用加强。

巨噬细胞通过表面模式识别受体对病原菌的识别及受体介导的内吞、消化、呈递等方式发挥吞噬作用。目前临床研究发现，介导巨噬细胞吞噬作用的受体包括清道夫受体（SRs）、Toll 样受体（TLRs）、补体受体等[14]。其中起代表作用的受体包括 TLR4、MARCO、SR-A1 等。TLRs 在激活信号传导途径引起巨噬细胞杀伤和清除病菌体方面起重要作用。陈恩竹等[15]研究发

现，TLR4 可诱导其他受体基因的表达，并通过 IRAK4、MyD88、p38 途径上调 SRs，促使巨噬细胞对革兰氏菌的吞噬作用提高。van de Garde 等[16]研究报道，COPD 与哮喘患者肺泡及气道巨噬细胞 TLR4、TLR2 mRNA 表达均较健康对照组上调。郭彩霞等[17]研究发现，TLR4 配基 LPS 激活巨噬细胞后，可释放金属基质蛋白而影响 COPD 病情发展。本研究中，轻中度组与对照组 TLR4 表达量比较差异无统计学意义，但重度组 TLR4 表达上调，与轻中度组、对照组比较差异有统计学意义（P＜0.05），与上述文献报道基本相符。进一步研究发现，巨噬细胞 PI 与 TLR4mRNA 表达量呈负相关（r＝ -0.52，P＜0.05），由此提示重度 COPD 患者通过激活 TLR4 而介导免疫反应来保护机体，但 TLR4 的过度表达可引起持续性炎症反应，使得巨噬细胞的吞噬功能减退，进而对机体产生损害。值得注意的是，3 组 MARCO、SR-A1 表达量比较差异无统计学意义（P＞0.05），且巨噬细胞 PI 与 MARCO、SR-A1 的表达无明显相关性。由此提示，清道夫受体并不是 COPD 患者中介导巨噬细胞吞噬作用的重要受体。

COPD 除了炎症细胞介导的慢性炎症外，黏膜下腺增生及黏膜杯状细胞增多所致的气道黏液高分泌状态也是其重要病理特征。呼吸道黏液中的黏蛋白与水比例失衡，导致痰液黏稠而加重感染。国外研究显示，气道黏液高分泌状态与巨噬细胞的吞噬功能减退具有相关性[18]。MUC5A 是气道黏液栓中重要的大分子蛋白。MUC5A 的大量分泌是气道炎症反应的结果，而其大量分泌又是细菌繁殖的良好培养基[3]。AQP5 则是肺组织、气道中重要的水转运通道，AQP5 的表达下调可导致气道水分减少而使得黏蛋白浓度增加[4]。董玉等[19]研究报道，COPD 患者 MUC5A 与 AQP5mRNA 表达呈负相关，二者的表达变化与 COPD 的病情有关。临床对于 MUC5A、AQP5 与巨噬细胞吞噬功能的报道较少。Metcalfe 等[20]研究发现，COPD 患者 TLR4 mRNA 的表达与 MUC5A、AQP5 蛋白表达量及 mRNA 表达量均具有相关性，其猜测 MUC5A、AQP5 的表达变化是气道炎症介导的结果，可反过来影响炎症反应而影响 TLR4 的表达，进而影响巨噬细胞吞噬功能。本研究中，巨噬细胞 PI 与 MUC5A 表达量呈负相关（r＝－0.36，P＜0.05），与 AQP5 表达量呈正相关（r＝0.41，P＜0.05），其具体机制有待进一步研究。

综上所述，COPD 患者巨噬细胞占细胞总数的比例下降，其吞噬功能也受到抑制，且随着病情的加重，抑制更明显，其机制可能与 TLR4、MUC5A 表达上调及 AQP5 表达下调等有关。

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临床症状的快速恢复。

综上所述，在支气管哮喘患者中应用乌司他丁效果显著，可有效缓解临床症状，改善血清 IL-2、IL-4 及 T 细胞亚群的表达，调节机体免疫功能，临床应用价值高。

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