·综 述·

肺表面活性物质相关蛋白质D与哮喘的研究进展

张丽丽,鲍永霞*
(哈尔滨医科大学附属第二医院呼吸科,哈尔滨 150086)

【摘 要】 哮喘是呼吸系统常见的慢性炎症性疾病。关于哮喘的生物学标记物种类较多,但目前尚未发现具有特异性的标记物。肺表面活性物质相关蛋白质D(SP-D)主要存在于肺内,但在肺以外的器官系统中也有少量表达。SP-D在机体防御及免疫监视中发挥重要作用,与多种肺部疾病密切相关。其中SP-D是哮喘发生、发展的重要参与者,可作为哮喘的生物学标记物。现就SP-D的功能及其在哮喘中的生物学标记物作用作一综述。

【关键词】 肺表面活性物质相关蛋白质D;哮喘;生物学标记物
【中图分类号】 R562.2+5 【文献标志码】 A 【DOI】 10.11915/j.issn.1671-5403.2017.09.168

Research advances in correlation between pulmonary surfactant-associated protein D and asthma

ZHANG Li-Li, BAO Yong-Xia*
(Department of Respiratory Diseases, the Second Affiliated Hospital of Harbin Medical University, Harbin 150086, China)

【Abstract】 Asthma is a common chronic inflammatory disease of the respiratory system. There are many kinds of biomarkers for asthma, but no specific ones have been found yet. Pulmonary surfactant-associated protein D (SP-D) is mainly located in the lung, but also mildly expressed in the other organ systems. SP-D plays important roles in body defense and immune surveillance, and is closely related to many pulmonary diseases. The protein is essentially involved in the development and progression of asthma, and can be used as a biomarker of bronchial asthma. In this we article reviewed the function of SP-D and its role as biomarker in asthma.

【Key words】 pulmonary surfactant-associated protein D; asthma; biomarkers

Corresponding author: *BAO Yong-Xia*, *E-mail*:*baoyongxia@126.com*

肺表面活性物质相关蛋白质D(surfactant-associated protein D,SP-D)主要由肺泡Ⅱ型细胞、Clara细胞和黏膜下细胞合成和分泌,并且在传导性气道上皮细胞中也有少量存在。SP-D属于亲水性蛋白,可通过多种机制参与肺内免疫[1]。SP-D还可针对病原体和沉积在肺部的异物颗粒发挥调理素作用,然后与肺泡巨噬细胞通过特定受体相互作用,使它们被吸收并引起下游的免疫反应。SP-D参与多种肺部疾病的发生、发展过程。哮喘患者外周血中SP-D水平升高及结构完整性改变,表明SP-D与哮喘疾病反应相关联[2]。SP-D在肺内的限制性表达,使肺部疾病的特异性标志物分析成为可能。迄今为止,SP-D及其在哮喘中的生物学标志物作用的研究越来越受到人们的关注。本文就SP-D的功能及SP-D与哮喘的关系做一综述。

1 SP-D的功能

1.1 免疫监视与免疫保护

免疫系统是由固有免疫系统和特异性免疫系统组成,其中参与固有免疫应答的细胞有巨噬细胞、树突状细胞和自然杀伤细胞,而表达在这些细胞表面的模式识别受体(pattern-recognition receptor, PRR)可在免疫应答的识别过程中发挥一定作用。作为PRR的重要家族之一,C型凝集素受体蛋白可识别构成各种病原菌细胞壁的一系列糖基,因此也被称为病原体识别受体蛋白[3]。其中一些凝集素分子可与脂多糖(lipopolysaccharide, LPS)竞争,结合于细胞表面受体[4],引起细胞因子转录增加;另外一些凝集素通过活化T淋巴细胞参与特异性免疫系统的调节[5],再通过树突状细胞抗原提呈作用、免

收稿日期:2017-06-07;修回日期:2017-06-30
通信作者:鲍永霞,E-mail:baoyongxia@126.com

疫球蛋白E(immunoglobulin E, IgE)或组胺依赖的过敏性反应发挥免疫作用[6]。SP-D是C型凝集素超家族中胶凝素家族成员,可覆盖在多种病原菌表面,通过多种不同机制,如聚集、细胞凋亡[7]及调理作用等抑制病原体生长并中和病原体。Djiadeu等[8]证明SP-D可结合Jurkat T淋巴细胞并且推迟Fas(CD95)-Fas配体和TNF相关凋亡诱导配体(TNF-related apoptosis-inducing ligand, TRAIL)-TRAIL受体引起的T淋巴细胞凋亡,而Pandit等[9]研究发现SP-D可通过调节受体和细胞因子的表达诱导活化淋巴细胞凋亡。以上研究表明SP-D对免疫系统具有监视作用,维持局部免疫稳态。另一项研究通过聚合酶链反应(polymerase chain reaction, PCR)检测SP-D的染色体组DNA,逆转录聚合酶链反应(reverse transcriptase polymerase chain reaction, RT-PCR)检测SP-D的mRNA翻译,Western blotting测定蛋白质表达,酶联免疫吸附试验(enzyme linked immunosorbent assay, ELISA)定量分析SP-D,结果发现,在厌氧培养条件下,金黄色葡萄球菌及绿脓杆菌SP-D的表达均明显增加[10],提示细菌可能具有产生SP-D的能力或SP-D参与细菌的特异性免疫反应过程。

1.2 抗炎效应

首先,肺内SP-D不仅通过球状头部与信号调节蛋白α(signal regulatory protein α, SIRPα)结合来抑制p38丝裂原活化蛋白激酶(p38 mitogen-activated protein kinase, p38MAPK)激活,从而抑制炎症介质反应[11],还可诱导辅助性T淋巴细胞2(helper T lymphocytes 2, Th2)向Th1转变,减弱气道过敏反应[12],抑制嗜酸性粒细胞趋化和趋化因子触发的脱颗粒[13]。另外SP-D可与CD32嗜酸性粒细胞受体CD32直接相互作用抑制嗜酸性粒细胞阳离子蛋白产生[14]。其次,SP-D可调节Ⅰ型过敏反应,抑制变应原诱导的CD27-CD4+CRTh2+(chemoattractant receptor-homologs molecule expressed on Th2 cell)(Th2)细胞增殖,避免组织过度的炎性损伤,因此可作为季节性过敏性鼻炎的潜在治疗靶点[15]。Liu等[7]通过急性胰腺损伤小鼠模型发现,相比于SP-D基因敲除的小鼠,野生型小鼠细胞凋亡水平降低,提示SP-D具有抗炎作用。类似的是,研究发现在Toll样受体4(Toll-like receptor 4, TLR-4)过表达的早产儿坏死性小肠结肠细胞中,SP-D可减弱炎性因子IL-8水平[4],而在特发性肺纤维化患者体内,SP-D可通过信号转导通路SIRPα/Rho相关卷曲螺旋蛋白激酶(Rho associated coiled-coil forming protein kinase, ROCK)/细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK)抑制巨噬细胞产生IL-12p40[16]。这些研究均表明SP-D具有抗炎效应,可延缓疾病进程。

1.3 环境暴露与SP-D异源性表达

SP-D易受环境因素的影响,随之发生功能改变。研究通过检测慢性阻塞性肺疾病(chronic obstructive pulmonary diseases, COPD)患者支气管肺泡灌洗(bronchoalveolar lavage, BAL)液样本中的SP-D发现,其浓度与血清SP-D浓度增加相关[17]。这可能和肺泡气-血屏障被破坏有关,该结论得到了Sin等[18]的吸烟影响研究支持。此外,LPS诱导急性肺损伤的动物模型体内产生气道炎症,导致SP-D分子溢出进入循环系统增加,造成血清SP-D浓度升高[19],与上述文献结果一致。Flayer等[20]通过延长SP-D基因缺失小鼠暴露于臭氧条件的时间后,气道中性粒细胞数及BAL液中2型固有样淋巴细胞(type-2 innate lymphiod cells, ILC2s)数增加。ILC2s在白细胞介素-33(interleukin-33, IL-33)的刺激下产生IL-5、IL-13等Th2型细胞因子,在哮喘等变态反应的发病机制中发挥着重要作用。另外,有研究发现野生型小鼠暴露于酒精和香烟烟雾的代谢产物即丙二醛-乙醛加合物蛋白(malondialdehyde-acetaldehyde adducts, MAA)时,小鼠肺内SP-D可与MAA结合,促使肺巨噬细胞肿瘤坏死因子α(tumor necrosis factor-α, TNF-α)和IL-6等亲炎症细胞因子表达上调,导致肺巨噬细胞在肺内的趋化和聚集[21],提示SP-D可能是肺损伤的启动因子。总之,上述研究均证明SP-D可依赖特定个体环境暴露导致肺损伤。

1.4 调节吞噬作用

SP-D在肺真菌感染时的功能和机制尚不清楚。只有少数研究评估了SP-D在新生隐球菌感染反应中的作用[22]。在体内,SP-D糖识别域可结合新生隐球菌的糖基产生凝聚反应,并且对免疫细胞(如单核巨噬细胞、中性粒细胞)具有趋化作用,如以钙离子依赖方式增强中性粒细胞对病原体的吞噬。研究发现,通过上述机制,肺泡巨噬细胞被吸引到病原体周围并参与新生隐球菌感染的初始控制。同时有研究证明SP-D的黏附作用可进一步增强巨噬细胞对新生隐球菌CAP59荚膜缺失突变株的吞噬和杀伤作用[23],促进机体对新生隐球菌快速有效的清除,这与Abdel-Razek等[24]的研究结果一致。

2 SP-D与哮喘

2.1 SP-D在哮喘中的作用

哮喘是以气道炎症和组织结构重构为特征的慢性气道疾病。首先,气道炎症的典型表现是嗜酸性粒细胞和Th2型细胞因子如IL-4、IL-5、IL-13水平升高[25],而重症哮喘属于中性粒细胞和嗜酸性粒细胞性的气道炎症[26]。中性粒细胞丝氨酸蛋白酶能裂解SP-D糖识别域,使其功能失效[27]。作为远端气道非特异性免疫的一个重要组成部分,SP-D失调是哮喘的一个重要特征。通过蛋白免疫印迹法检测SP-D发现,重症哮喘患者BAL液中除了有50 kD的条带,还存在28 kD和17 kD两种微弱条带,而血清中除了有50 kD条带外,还存在38 kD、28 kD和17 kD三种强烈条带[2],提示重症哮喘血清中SP-D可能发生降解和功能损害。其次,有研究表明痰上清液中的SP-D与哮喘气道组织重塑过程有关[28]。气道重塑是哮喘的主要病理学特征之一。气道重塑是气道受到慢性炎症反复刺激的结果,其特点是气道结构发生变化,细胞因子、炎性介质等在其中发挥重要作用。近年来有研究表明哮喘气道结构的改变在轻型哮喘和重型哮喘中均存在不同程度的气道壁平滑肌增厚、基底膜增厚等。SP-D主要由肺泡Ⅱ型细胞和Clara细胞分泌,并作为免疫活性物质增强呼吸道的抵抗能力,通过识别和加速清除坏死和凋亡细胞以保持免疫稳态,但在超常增生的肺泡Ⅱ型细胞表达可明显增加。在博莱霉素诱导的肝纤维化小鼠模型中,肺泡SP-D可调节巨噬细胞和成纤维细胞的数量、纤维化细胞因子的表达以及肺纤维化组织重塑[29],提示SP-D可能参与气道组织重塑过程。该研究还发现重症哮喘患者BAL液中SP-D水平增加与平滑肌面积和基底膜厚度呈正相关,进一步证明SP-D在哮喘气道重塑过程中发挥重要作用。

2.2 SP-D作为哮喘的生物学标志物

Benfante等[30]选取了29例哮喘患者,其中包括19例轻度哮喘患者,10例重度哮喘患者,同时选取9名健康人作为对照组。所有入选对象均经过临床肺功能和生物学评估,通过ELISA法检测血清中SP-D的水平。研究发现哮喘患者血清中SP-D明显高于对照组,且哮喘患者血清SP-D水平随疾病严重程度增加,与第1秒用力呼气容积占预计值的百分比(predicted percentage of forced expiratory volume in one second, FEV1%)、用力肺活量占预计值的百分比(the predicted percentage of forced vital capacity, FVC%)及用力呼出气体量为25%~75%肺活量时的平均流量(average flow rate of forced expiratory flow as 25%~75% of vital capacity, FEF25%~75%)成反比,提示血清SP-D可作为一种评估哮喘严重性的特定生物学标志物。

3 结 语

哮喘患者血液及痰液中SP-D水平明显增高,主要与哮喘患者气道细胞免疫功能缺陷、肺泡气-血屏障被破坏有关,哮喘进展可能会损害肺内非特异性免疫系统的效能。SP-D渗入血清现象,可能成为一种外周血生物学标志物,其水平升高程度可以在很大程度上反应哮喘患者的疾病严重性,但哮喘患者SP-D分泌机制及结构改变有待进一步研究。

【参考文献】

[1] Hartshorn KL, White MR, Tecle T, *et al*. Viral aggregating and opsonizing activity in collectin trimers[J]. Am J Physiol Lung Cell Mol Physiol, 2010, 298(1): L79-L88. DOI: 10.1152/ajplung.00223.2009.

[2] Mackay RM, Grainge CL, Lau LC, *et al*. Airway surfactant protein D deficiency in adults with severe asthma[J]. Chest, 2016, 149(5): 1165-1172. DOI: 10.1016/j.chest.2015.11.012.

[3] Mayer S, Raulf MK, Lepenies B. C-type lectins: their network and roles in pathogen recognition and immunity[J]. Histochem Cell Biol, 2017, 147(2): 223-237. DOI: 10.1007/s00418-016-1523-7.

[4] Saka R, Wakimoto T, Nishiumi F, *et al*. Surfactant protein-D attenuates the lipopolysaccharide-induced inflammation in human intestinal cells overexpressing Toll-like receptor 4[J]. Pediatr Surg Int, 2016, 32(1): 59-63. DOI: 10.1007/s00383-015-3812-y.

[5] Gowdy KM, Cardona DM, Nugent JL, *et al*. Novel role for surfactant protein A in gastrointestinal graft-*versus*-host disease[J]. J Immunol, 2012, 188(10): 4897-4905. DOI: 10.4049/jimmunol.1103558.

[6] Nayak A, Dodagatta-Marri E, Tsolaki AG, *et al*. An insight into the diverse roles of surfactant proteins, SP-A and SP-D in innate and adaptive immunity[J]. Front Immunol, 2012, 3(4): 257-264. DOI: 10.3389/fimmu.2012.00131.

[7] Liu Z, Shi Q, Liu J, *et al*. Innate immune molecule surfactant protein D attenuates sepsis-induced acute pancreatic injury through modulating apoptosis and NF-κB-mediated inflammation[J]. Sci Rep, 2015, 5(4): 17798. DOI: 10.1038/srep17798.

[8] Djiadeu P, Kotra LP, Sweezey N, *et al*. Surfactant protein D delays Fas- and TRAIL-mediated extrinsic pathway of apoptosis in T cells[J]. Apoptosis, 2017, 22(5): 730-740. DOI: 10.1007/s10495-017-1348-4.

[9] Pandit H, Thakur G, Koippallil Gopalakrishnan AR, *et al*. Surfactant protein D induces immune quiescence and apoptosis of mitogen-activated peripheral blood mononuclear cells[J]. Immunobiology, 2016, 221(2): 310-322. DOI: 10.1016/j.

imbio. 2015. 10. 004.

[10] Bräuer L, Schicht M, Worlitzsch D, *et al*. *Staphylococcus* aureus and *Pseudomonas aeruginosa* express and secrete human surfactant proteins[J]. PLoS One, 2013, 8(1): e53705. DOI: 10. 1371/journal. pone. 0053705.

[11] Waters P, Vaid M, Kishore U, *et al*. Lung surfactant proteins A and D as pattern recognition proteins[J]. Adv Exp Med Biol, 2009,653(4): 74 – 97. DOI: 10. 1007/978-1-4419-0901-5_6.

[12] Madan T, Kishore U, Singh M, *et al*. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by Aspergillus fumigatus antigens and allergens[J]. J Clin Invest, 2001, 107(4): 467 – 475. DOI: 10. 1172/JCI10124.

[13] von Bredow C, Hartl D, Schmid K, *et al*. Surfactant protein D regulates chemotaxis and degranulation of human eosinophils[J]. Clin Exp Allergy, 2006, 36(12): 1566 – 1574. DOI: 10. 1111/j. 1365-2222. 2006. 02598. x.

[14] Bateman ED, Hurd SS, Barnes PJ, *et al*. Global strategy for asthma management and prevention: GINA executive summary[J]. Eur Respir J, 2008, 31(1): 143 – 178. DOI: 10. 1183/09031936. 00138707.

[15] Singh I, Qaseem A, Pathan A, *et al*. Surfactant protein-D (SP-D): a potential therapeutic target for seasonal allergic rhinitis[J]. J Allergy Clin Immunol, 2017, 139(2): AB84. DOI: 10. 1016/j. jaci. 2016. 12. 227.

[16] Yamaguchi R, Sakamoto A, Yamamoto T, *et al*. Surfactant protein D inhibits interleukin-12p40 production by macrophages through the SIRP, Sakamoto signaling pathway[J]. Am J Med Sci, 2017, 353(6): 559 – 567. DOI: 10. 1016/j. amjms. 2017. 03. 013.

[17] Winkler C, Atochina-Vasserman EN, Holz O, *et al*. Comprehensive characterisation of pulmonary and serum surfactant protein D in COPD[J]. Respir Res, 2011, 12(1): 1 – 11. DOI: 10. 1186/1465-9921-12-29.

[18] Sin DD, Pahlavan PS, Man SF. Surfactant protein D: a lung specific biomarker in COPD? [J]. Ther Adv Respir Dis, 2008, 2(2): 65 – 74. DOI: 10. 1177/1753465808088903.

[19] Gaunsbaek MQ, Rasmussen KJ, Beers MF, *et al*. Lung surfactant protein D (SP-D) response and regulation during acute and chronic lung injury[J]. Lung, 2013, 191(3): 295 – 303. DOI: 10. 1007/s00408-013-9452-x.

[20] Flayer CH, Ge MQ, Kovacs PB, *et al*. Surfactant protein-D (SP-D) is a lung specific regulator of group 2 innate lymphoid cells (ILC2)[J]. J Allergy Clin Immunol, 2017, 139(2): AB15. DOI: 10. 1016/j. jaci. 2016. 12. 104.

[21] Sapkota M, Kharbanda KK, Wyatt TA. Malondialdehyde-acetaldehyde-adducted surfactant protein alters macrophage functions through scavenger receptor A[J]. Alcohol Clin Exp Res, 2016, 40(12): 2563 – 2572. DOI: 10. 1111/acer. 13248.

[22] Geunes-Boyer S, Beers MF, Perfect JR, *et al*. Surfactant protein D facilitates Cryptococcus neoformans infection[J]. Infect Immun, 2012, 80(7): 2444 – 2453. DOI: 10. 1128/IAI. 05613-11.

[23] Geunes-Boyer S, Oliver TN, Janbon G, *et al*. Surfactant protein D increases phagocytosis of hypocapsular *Cryptococcus* neoformans by murine macrophages and enhances fungal survival[J]. Infect Immun, 2009, 77(7): 2783 – 2794. DOI: 10. 1128/IAI. 00088-09.

[24] Abdel-Razek O, Liu X, Javidiparsijani S, *et al*. Role of surfactant protein D on *in vivo* alveolar macrophage phagocytosis of *Cryptococcus* neoformans by the regulation of p38 MAPK pathway activation[J]. Pulm Crit Care Med, 2016, 1(3): 79 – 83. DOI: 10. 15761/PCCM. 1000117.

[25] Schuijs MJ, Willart MA, Hammad H, *et al*. Cytokine targets in airway inflammation[J]. Curr Opin Pharmacol, 2013, 13(3): 351 – 361. DOI: 10. 1016/j. coph. 2013. 03. 013.

[26] Moore WC, Hastie AT, Li X, *et al*. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis[J]. J Allergy Clin Immunol, 2014, 133(6): 1557 – 1563. e5. DOI: 10. 1016/j. jaci. 2013. 10. 011.

[27] Duvoix A, Mackay RM, Henderson N, *et al*. Physiological concentration of calcium inhibits elastase-induced cleavage of a functional recombinant fragment of surfactant protein D[J]. Immunobiology, 2011, 216(1 – 2): 72 – 79. DOI: 10. 1016/j. imbio. 2010. 03. 006.

[28] Tseliou E, Bakakos P, Kostikas K, *et al*. Increased levels of angiopoietins 1 and 2 in sputum supernatant in severe refractory asthma[J]. Allergy, 2012, 67(3): 396 – 402. DOI: 10. 1111/j. 1398-9995. 2011. 02768. x.

[29] Aono Y, Ledford JG, Mukherjee S, *et al*. Surfactant protein-D regulates effector cell function and fibrotic lung remodeling in response to bleomycin injury[J]. Am J Respir Crit Care Med, 2012, 185(5): 525 – 536. DOI: 10. 1164/rccm. 2011030561-OC.

[30] Benfante A, Battaglia S, Paterno A, *et al*. Serum surfactant protein D is a potential biomarker of severity of asthma[J]. Am J Respir Crit Care Med, 2015, 191: A4311.

（编辑：张芙）