Int J Clin Exp Pathol 2016;9(7):7365-7369
www.ijcep.com /ISSN:1936-2625/IJCEP0023321

Original Article

Adipocyte fatty acid binding protein in OVA-induced asthmatic mice

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Received January 5, 2016; Accepted March 20, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: Objective: To investigate the expression of adipocyte fatty acid binding protein (A-FABP) in OVA-induced asthmatic mice. Methods: Healthy female BALB/c mice were randomly divided into control group and asthma group. A-FABP in lung tissues was located using immunohistochemical staining and quantified by Western blot. A-FABP in serum was quantified by ELISA. Results: A-FABP mainly expressed in the cytoplasm of airway epithelial cells (AECs), macrophages and vessel endothelial cells (VECs). The expression of A-FABP was significantly higher in asthma group than control group ($P<0.05$). Conclusion: A-FABP might be involved in the pathophysiological process of asthma.

Keywords: Asthma, A-FABP, mice

Introduction

Asthma is a common chronic airway disease characterized by periods of reversible airflow obstruction known as asthma attacks [1]. The epidemiological surveys have shown that the incidence, mortality, and morbidity of asthma are increasing [2, 3]. However, the pathogenesis of asthma has been still unclear.

Studies indicated that asthma has an association with characteristic airway immunopathology involving mast cells [4, 5], Th2 lymphocytes [6-9], eosinophils [10, 11] and goblet cell [12].

The bronchial epithelium is an important barrier to the external environment, providing defense against inhaled particles such as allergens and playing a vital role in asthma pathophysiology by regulating such diverse processes as airway remodeling and mucus production [13]. However, the contribution of airway epithelial cells (AECs) to allergic airway inflammation is not fully understood.

Adipose tissue's products regulate the metabolic functions of various tissues, including brain, pancreas and liver, to maintain systemic homeostasis. Adipocytes respond to metabolic and immune signals through lipolysis and secreting a variety of hormones and cytokines [14, 15]. The intracellular functions of fatty acid binding protein (FABP) in lipid metabolism and inflammation have been studied [16, 17]. FABP is important for the pathogenesis of several diseases, including diabetes, atherosclerosis, and fatty liver [18-21]. A-FABP was mainly expressed in adipocytes and macrophages.

To investigate the correlation of A-FABP and asthma, a mice model of asthma induced by OVA was established. In this study, we detected the expression of A-FABP in serum and lung tissues respectively.

Materials and methods

Animals

The experimental study was conducted on 35 female BALB/c mice aged 5 or 6 weeks old and weighing between 15.5 to 18.5 g, provided by Hebei Provincial Laboratory Animal Center. Room temperature was maintained at 20-24°C with relative humidity of 53-57%. Animals were kept in a 12 hours light/12 hours dark cycle, fed without ovalbumin and free to drink water.

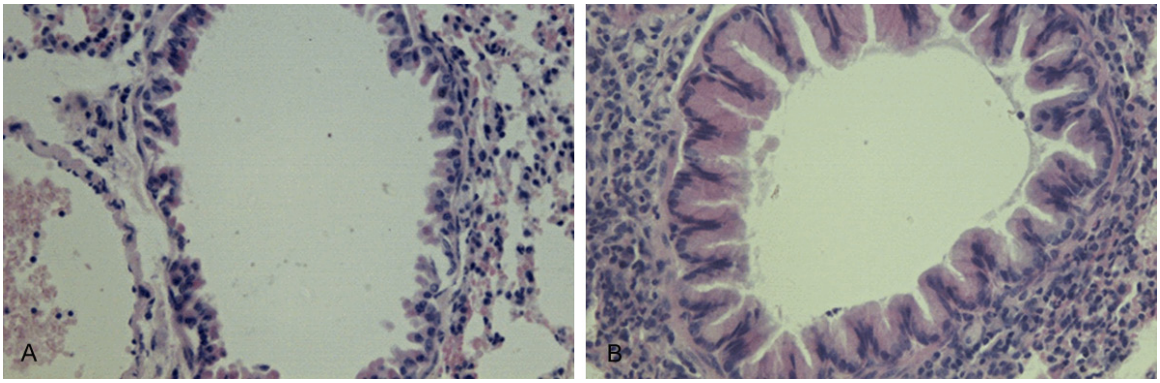

Figure 1. Pathological changes of the expression in lung tissues. A: AECs of mice in control group was complete, HE staining, ×400; B: AECs of mice in asthma group.

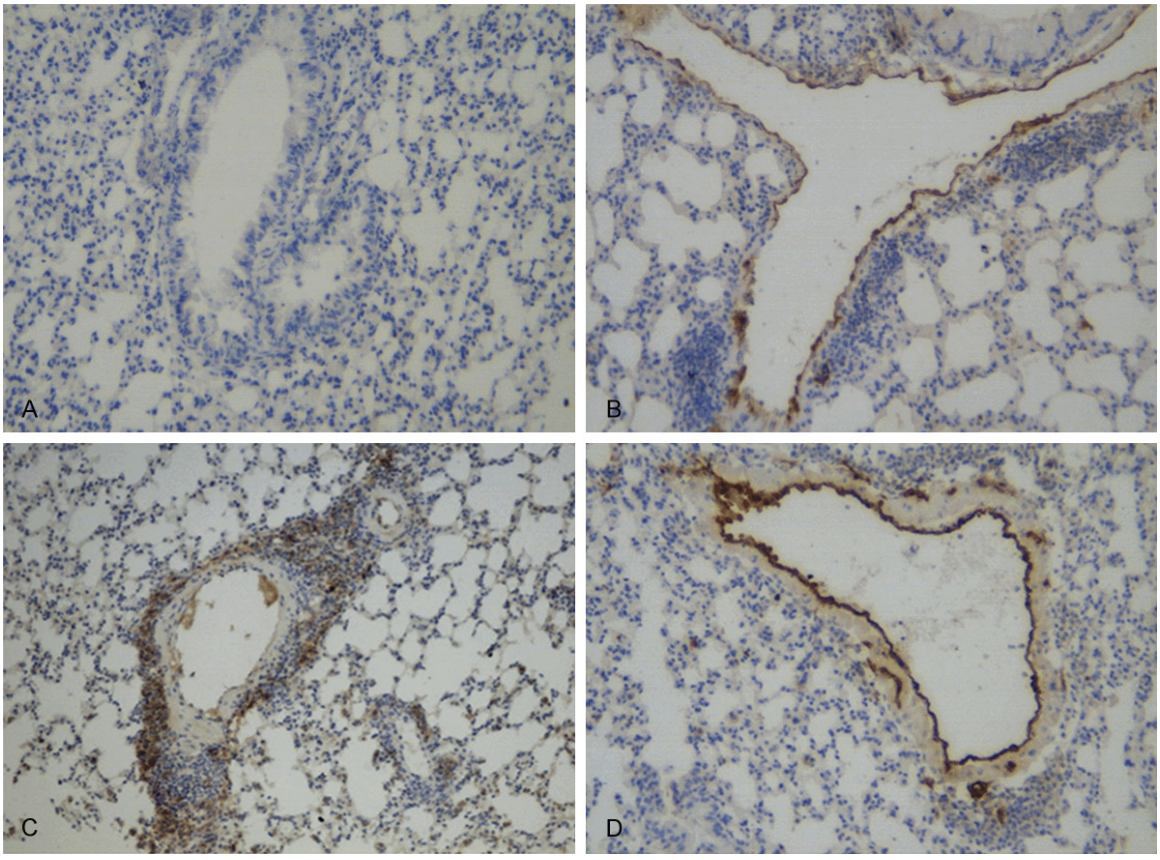

Figure 2. Immunohistochemical staining of A-FABP in lung tissues. A: Immunohistochemical staining was negative in control group, ×200; B: Immunohistochemical staining was positive in AECs of asthma group, ×200; C: Immunohistochemical staining was positive in macrophages of asthma group, ×200; D: Immunohistochemical staining was positive in VECs of asthma group, ×200.

Experimental procedures were performed in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of the People's Republic of China in 1998, and was approved by the Animal Ethics Committee of Children's Hospital of Hebei Province.

Asthmatic mice model [22]

Animals were divided into control group (n=15) and asthma group (n=20). Mice in asthma group were immunized by intraperitoneal injection of 80 μg OVA (Sigma-Aldrich, St. Louis, USA) emulsified in 10 mg of alum (Pierce,

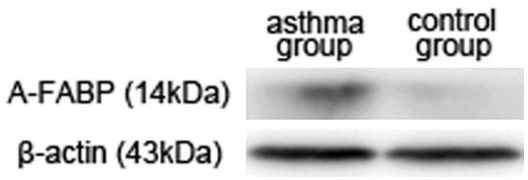

Figure 3. Western blot of A-FABP in lung tissues.

Rockford, USA) in a total volume of 200 μl on Days 0 and 14. Mice were challenged via the airways with 1% OVA in saline for 40 min on Days 24, 25, 26, 27, and 28 by ultrasonic nebulization.

ELISA

About 0.8 ml blood from a mouse was obtained by decapitation 48 hours after the last OVA challenge. The serum was separated by centrifugation at 2000 rpm for 10 minutes after 1 hour's standing. Serum A-FABP was determined by an A-FABP ELISA kit (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

Immunohistochemical staining

The right upper lobes of mice were harvested and fixed in 4% paraformaldehyde. The expression of A-FABP in lung tissue was detected using immunohistochemical staining. HE staining was also performed to evaluate the pathological changes of lung tissues.

Western blot

About 100 mg lung tissue was cut into pieces, and total protein was extracted with RIPA lysate containing PMSF (1% NP-40, 1% SDS, 150 mmol/L NaCl, 50 mmol/L Tris-Cl, pH 7.5, 10% glycerol, 1 mmol/L sodium vanadate, 1 mmol/L PMSF). The protein concentration was determined by the CBB (Coomassie brilliant blue) method.

50 μg sample protein per lane was separated by 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene-difluoride (PVDF) membrane. The membranes were incubated in 5% skimmed milk for 2 h at 37°C, and overnight at 4°C with primary antibodies (goat anti-A-FABP, 1:1000 dilution, Santa Cruz). β-actin was used as a loading control. The membranes were incubated in rabbit-anti-goat second antibody for 1.5 h at 37°C. The membranes were exposed to the negative films to develop target bands after incubated with enhanced chemiluminescence (Santa Cruz, USA). The intensities of bands were quantitated by Quantity One (Bio-Rad, USA).

Results

Changes in behaviors of mice

Mice in asthma group presented with anxiety, shortness of breath, nares flaring, abdominal muscle twitching and gatism after OVA challenge. Some of them were severe with limbs collapse and hair losing luster. Mice in control group did not present with those symptoms.

Pathological changes of lung tissues in mice

AECs of mice in asthma group proliferated and desquamated. A large number of inflammatory cells, especially eosinophil granulocyte (EOS), infiltrated surrounding the airways and vessels. EOS existed in pulmonary interstitial and alveolar spaces as well. AECs of mice in control group were complete. Only few inflammatory cells were observed surrounding the airways and vessels (**Figure 1**).

Expression of A-FABP in serum and lung tissues

Serum A-FABP of mice in asthma group was significantly higher than control group (2.12±0.13 ng/ml vs. 2.01±0.10 ng/ml, t=2.477, P=0.019).

As the results of immunohistochemical staining, A-FABP mainly expressed in the cytoplasm of AECs, macrophages and vessel endothelial cells (VECs) (**Figure 2**).

The expression of A-FABP lung tissues of mice in asthma group was significantly higher than control group (P<0.05) according to the Western blot analysis (**Figure 3**).

Discussion

Asthma is chronic airway inflammatory disease involving various inflammatory cells and cytokines. Its pathogenesis is too complex to be fully understood for now. Furuhashi *et al.* [23] found that asthmatic model mice who took a potent A-FABP inhibitor BMS309403 orally would not have diabetes, atherosclerosis and

asthma. It indicated that A-FABP was a novel cytokine involved in the pathogenesis of asthma. The effective inhibitor of A-FABP would be a novel drug for the prevention and treatment of asthma.

A-FABP widely distributed in a variety of normal tissues and cells, such as adipocytes, macrophages, AECs [24] and dendritic cells [25], mainly in adipocytes and macrophages. In our study, A-FABP expression was significantly higher in mice of asthma group than control group both in serum and lung tissues ($P<0.05$). Results of immunohistochemical staining suggested that A-FABP expressed in macrophages, AECs and VECs. As a peripheral protein, A-FABP can bind saturated and unsaturated fatty acids to stimulate the transport and metabolism of fatty acids. A-FABP also binds tretinoin and eicosanoid and is involved in the transport and metabolism of them. A-FABP might promote the occurrence and development of asthma through the regulation of metabolism of fatty acids, tretinoin and eicosanoid.

In our study, the higher level of mice in asthma group might have two reasons. One was the excitation of Th2 cells induced by OVA promoted the release of IL-3 and IL-4. IL-3 and IL-4 could up-regulated the expression of A-FABP had been proved in previous study [24]. Another is the increasing levels of free fatty acid (FFA) and peroxisome proliferators activated receptor γ (PPARγ) induced the expression of A-FABP in macrophages [16].

In conclusion, A-FABP might be involved in the pathophysiological process of asthma. However, further studies should be performed to investigate the effects of A-FABP in asthma. And the effective inhibitor could be applied to treat asthma should be researched in future.

Disclosure of conflict of interest

None.

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References

[1] Cabieses B, Uphoff E, Pinart M, Anto JM and Wright J. A systematic review on the development of asthma and allergic diseases in relation to international immigration: the leading role of the environment confirmed. PLoS One 2014; 9: e105347.

[2] Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA and Liu X. Trends in asthma prevalence, health care use, and mortality in the United States, 2001-2010. NCHS Data Brief 2012; 1-8.

[3] Akinbami LJ, Moorman JE and Liu X. Asthma prevalence, health care use, and mortality: United States, 2005-2009. Natl Health Stat Report 2011; 1-14.

[4] Reuter S, Stassen M and Taube C. Mast cells in allergic asthma and beyond. Yonsei Med J 2010; 51: 797-807.

[5] da Silva EZ, Jamur MC and Oliver C. Mast cell function: a new vision of an old cell. J Histochem Cytochem 2014; 62: 698-738.

[6] Bochner BS, Undem BJ and Lichtenstein LM. Immunological aspects of allergic asthma. Annu Rev Immunol 1994; 12: 295-335.

[7] Brusselle G, Kips J, Joos G, Bluethmann H and Pauwels R. Allergen-induced airway inflammation and bronchial responsiveness in wild-type and interleukin-4-deficient mice. Am J Respir Cell Mol Biol 1995; 12: 254-259.

[8] Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM and Corry DB. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998; 282: 2261-2263.

[9] Shore SA. Direct effects of Th2 cytokines on airway smooth muscle. Curr Opin Pharmacol 2004; 4: 235-240.

[10] Pavord ID. Eosinophilic phenotypes of airway disease. Ann Am Thorac Soc 2013; 10 Suppl: S143-149.

[11] Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ and Pavord ID. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009; 360: 973-984.

[12] Mushaben EM, Kramer EL, Brandt EB, Khurana Hershey GK and Le Cras TD. Rapamycin attenuates airway hyperreactivity, goblet cells, and IgE in experimental allergic asthma. J Immunol 2011; 187: 5756-5763.

[13] Davies DE. The bronchial epithelium in chronic and severe asthma. Curr Allergy Asthma Rep 2001; 1: 127-133.

[14] Kershaw EE and Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89: 2548-2556.

[15] Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 2006; 55: 1537-1545.

[16] Furuhashi M and Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases

and potential as drug targets. Nat Rev Drug Discov 2008; 7: 489-503.
[17] Hertzel AV and Bernlohr DA. The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. Trends Endocrinol Metab 2000; 11: 175-180.
[18] Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE and Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 1996; 274: 1377-1379.
[19] Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S and Hotamisligil GS. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. Endocrinology 2000; 141: 3388-3396.
[20] Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF and Hotamisligil GS. Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. Circulation 2004; 110: 1492-1498.
[21] Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME, Fazio S, Wiest MM, Watkins SM, Linton MF and Hotamisligil GS. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat Med 2009; 15: 1383-1391.
[22] Itoga M, Konno Y, Moritoki Y, Saito Y, Ito W, Tamaki M, Kobayashi Y, Kayaba H, Kikuchi Y, Chihara J, Takeda M, Ueki S and Hirokawa M. G-protein-coupled estrogen receptor agonist suppresses airway inflammation in a mouse model of asthma through IL-10. PLoS One 2015; 10: e0123210.
[23] Furuhashi M, Tuncman G, Gorgun CZ, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, Sulsky R, Robl JA, Parker RA and Hotamisligil GS. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. Nature 2007; 447: 959-965.
[24] Shum BO, Mackay CR, Gorgun CZ, Frost MJ, Kumar RK, Hotamisligil GS and Rolph MS. The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. J Clin Invest 2006; 116: 2183-2192.
[25] Rolph MS, Young TR, Shum BO, Gorgun CZ, Schmitz-Peiffer C, Ramshaw IA, Hotamisligil GS and Mackay CR. Regulation of dendritic cell function and T cell priming by the fatty acid-binding protein AP2. J Immunol 2006; 177: 7794-7801.