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Recombinant Human PLD2(rhPLD2) May Significantly Inhibit Expression of GPI-PLD of Guinea Pigs of Chronic Asthma *in vivo*

ZHU Ling[1)*], YU Chuan-Xing[2)], ZOU Wei-Bin[1)], HE Xiao-Li[1)], LIN Jun-Jin[1)]

([1)] *Deparment of Immunology and Center of Neuroscience, Fujian Medical University, Fuzhou* 350004, *China*;
[2)] *Internal Medicine of Second People's Hospital of Fujian Province, Fuzhou* 350003, *China*)

Abstract The effect of recombinant human phospholipase D2 (rhPLD2) *in vivo* was investigated on the secretion of serum glycosyl phosphatidylinositol-specific phospholipase D (GPI-PLD) in guinea pigs of chronic asthma. After treating the guinea pigs attacked by chronic asthma with rhPLD2, the GPI-PLD activity detection was carried out by phase separation of human placental alkaline phosphatase in Triton X-114. Compared with the healthy guinea pigs(NS group), the serum GPI-PLD in the guinea pigs of chronic asthma are much higher than that of control groups, $P \leq 0.01$. Our results showed that rhPLD2 could significantly reduce the secretion of GPI-PLD when the guinea pigs were attacked by chronic asthma.

Key words rhPLD2; GPI-PLD; chronic asthma; guinea pig

重组人磷脂酶 D2 在体内能明显抑制慢性哮喘豚鼠血清中 GPI-PLD 的表达

朱 玲[1)*], 余传星[2)], 邹伟斌[1)], 何小丽[1)], 林俊锦[1)]

([1)] 福建医科大学基础免疫系及神经生物学研究中心,福州 350004;
[2)] 福建省第二人民医院内科,福州 350003)

摘要 通过建立慢性豚鼠哮喘模型,研究重组人磷脂酶 D2(rhPLD2)干预慢性哮喘豚鼠血清中糖基化磷脂酰肌醇特异性磷脂酶 D(GPI-PLD)含量的变化,探寻可能的机制. 以 rhPLD2 干预慢性哮喘豚鼠,用 TX-114 分相法检测豚鼠血清中 GPI-PLD 酶活性. 与正常状态豚鼠相比较,慢性哮喘状态豚鼠其血清中 GPI-PLD 酶活性显著升高. 但以 rhPLD2 及地塞米松干预慢性哮喘豚鼠后,该指标显著下降. rhPLD2 可通过抑制其血清中 GPI-PLD 酶活性表达,来抑制哮喘发生过程中的炎症反应.

关键词 重组人磷脂酶 D2; 糖基化磷脂酰肌醇特异性磷脂酶 D; 慢性哮喘; 豚鼠
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As a pulmonary disorder, asthma was characterized by the generalized reversible obstruction of airflow, allergenic inflammation and airway hyperresponsiveness. Structural alterations including airway wall thickening, fibrosis in the lamina reticularis and adventitia of the airway, mucus metaplasia, myocyte hypertrophy and hyperplasia, and neovascularization are all readily appreciated in the asthmatic airway[1,2].

More recently, greater studies have demonstrated that a variety of inflammatory factors belong to the glycoprotein family, some kinds of which are anchored to plasma membrane by glycosyl phosphatidyl-inositol (GPI), which is well exemplified by some enzymes, receptors, differentiation antigens (CDs) and other biologically active proteins; with the covalently attached GPI moiety as a membrane anchor, they widely participate in the cell interaction. So, the release of GPI moiety may access to regulate these proteins' function[3]. It is well

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* Corresponding author: Tel: 0591-83569048
E-mail: zhling62@pub6.fz.fj.cn
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福建省科技攻关重点项目(No. 2004Y010)及福建省产业技术研究开发项目(No. 200504)
*联系人 Tel: 0591-83569048, 13675005699
E-mail: zhling62@pub6.fz.fj.cn

known that glycosyl phosphatidylinositol-specific phospholipase D (GPI-PLD) can hydrolyzes the covalently attached GPI moiety, bearing some effect on these GPI-anchored proteins and further involving in the function of relational cell and some disease. There are ever increasing reports of the relationship between GPI-PLD and some disease such as acute or chronic hepatitis, bronchial pneumonia, the syndrome of systemic inflammation and so on[4]. But the relationship of GPI-PLD with guinea pig model of chronic asthma is still a virgin soil. We show a great interest for it.

PLD2 essentially requires phosphatidylinositol bisphosphate for its enzymatic activity. Through the hydrolyzing phosphatidylcholine creating choline and phosphatidic acid (PA), this bio-active protein has a host of function such as signaling transduction, exocytosis in most of cells, which may involve in the release of inflammatory factors. The recombinant human phospholipase D2 (rhPLD2) has been made by ourself through engineering protein production modified from the wild-type PLD2.

We further examined the effect of rhPLD2 on the activity of GPI-PLD. In the present study, we used guinea pig as a chronic asthmatic model to determine the possible role of rhPLD2 in the treatment with asthma. Our interest is to probe the relationship between GPI-anchored protein, GPI-PLD and PLD2 in a guinea pig asthmatic model in which chronic airway inflammation is maintained by repeated allergen ovalbumin (OVA) inhalation as well as the mechanism involved.

1 Materials and Methods

1.1 Animals

Male guinea pigs with weight about 250 ±50 g were purchased from Shanghai Shenwang animal breed corporation. The animals were housed in specific pathogen-free environments and were allowed access to food and water ad libitum.

1.2 OVA exposure

A 1 % solution of OVA in double-distilled water was used for antigen exposures. Filtered air was passed through an Pari BOY nebulizer (Bairui corp, German) to generate an aerosol. The size distribution of the aerosol was determined using a particle counter (Aerodynamic Particle Sizer, TSI). The aerosol sizes were distributed log-normally with a count median aerodynamic diameter of 0.82 μm and geometric SD of 1.46 μm. A mean OVA concentration of 3.8 ng/ml was measured in the chamber during the exposures.

1.3 Guinea pig model of chronic asthma

All guinea pigs were initially sensitized and then challenged with OVA before the onset of rhPLD2 treatment: guinea pigs were given an intraperitoneal injection of OVA (250 mg OVA versus to 1 kg weight of the animal, suspended in 1 ml NS.) on days 1, followed by inhalation of OVA (1 % solution,) on days 14. After that, every animal was performed by an administration of nebulized 1 % OVA for about 5 min every other day. It would take another 20 days till the guinea pig model of chronic asthma was built up. Nonsensitized control animals was received only the PBS.

1.4 Treatment and grouping of animals

52 guinea pigs attacked by chronic asthma were stochsticly divided into 5 groups, followed by consecutive treatment with an intraperitoneal injection of NS, dexamethasone (DXM) and a series of concentration of rhPLD2 respectively, before earlier 45 min of the onset of asthma 3 times. The following Table 1 has described for it. After each time induces asthma to end, the second day guinea pig has the phenomena such as dyspnea, discontinuity sneezes and so on.

Table 1 Treatment and group of animals

Group	Number of animal	Dosage of treatment
NS	10	1 ml
DXM	10	5 mg/kg
rhPLD2	11	1.5 mg/kg
rhPLD2	11	3.0 mg/kg
rhPLD2	10	6.0 mg/kg

1.5 Collection of speciment

Intraperitoneal injection of pentobarbitol sodium (30 mg/kg) with strict aseptic performance followed by puncturing into experimental animal heart for 1 ml of blood sample. A series of guinea pig blood samples were collected at different time: 4 days preceding asthma attack, 9 hours after guinea pig challenged by allergen (1 % OVA), and 6, 12, 18 hours after treating asthmatic animals with NS, DXM and rhPLD2, respectively. Add 1 ml of blood sample into a 1.5 ml EP for 30 s at room temperature without agitation, and transfer them into 4 ℃ refrigeratory for 12 hours. The sample was then centrifuged at 4 000 r/min for 10 min at 4 ℃ ensued by collection of supernatant into 0.5 ml EP at - 80 ℃.

1.6 Butanol extraction of placental alkaline phosphatase (PLAP)

An individual human term placenta (frozen immediately after delivery) was thawed, its membranous and fibrous tissue discarded, the remainder chopped with scissors and then homogenized in an equal volume of 0.25 mol/L sucrose, 5 mmol/L $MgCl_2$, 24 mmol/L KCl, 50 mmol/L Tris-HCl, pH 7.5 (1 ml/g of tissue) for twice. The homogenate was centrifuged at 6 000 g for 10 min, the supernatant was then re-centrifuged at 150 000 g for 40 min. The resulting pellet was washed with the homogenizing buffer (0.3 mg/g of tissue) and finally resuspended with the same buffer to give a protein concentration of approximately 25—35 mg/ml. Then the crude placental extraction in volume of 0.65 ml were mixed with 0.35 ml of ice-cold butanol and incubated at

25 ℃ for 15 min in order to acquire the original PLAP with GPI moiety anchor. The mixture then cooled on ice and centrifuged at 12 000 g for 30 min at 4 ℃. The sample of the lower, aqueous phase were dialyzed against the dialysis buffer (0.15 mol/L NaCl, 0.1 mmol/L $MgCl_2$, 0.01 mmol/L zinc acetate, 10 mmol/L Hepes-NaOH, pH 7.0) for 24 hours. The dialysate was further ultra-filtrate using ultra-filtrate tube at 6 500 g for 1 hour at 4 ℃. Aliquote and store at - 80 ℃.

1.7 Preparation of partition phase of sample

The extraction of placental alkaline phosphatase and buffer C (50 mmol/L Tris-HCl, 10 mmol/L NaCl, 2.5 mmol/L $CaCl_2$, 0.1 % Triton X-100, pH 7.4) in a total volume 30 μl were mixed with 20 μl serum GPI-PLD, and incubated at 37 ℃ for 60 min in the presence or absence of the inhibitor of GPI-PLD (1,10-phenantroline).

1.8 Triton X-114 phase separation

250 μl portion of alkaline phosphatase were incubated on ice with 250 μl buffer C including 1.25 % (w/V) Triton X-114 for 15 min. A 20 μl sample was removed for determination of total alkaline phosphatase activity (T). The tube were incubated at 37 ℃ for 15 min to promote phase separation, centrifuged for 3min at 12 000 r/min and a 20 μl sample of upper phase was removed for alkaline phosphatase assay (A).

1.9 Alkaline phosphatase assay

Alkaline phosphatase activity was determined by incubating enzyme sample with 5 mmol/L *p*-nitrophenyl phosphate (pNPP) in 1 mmol/L $MgCl_2$, 1 mmol/L diethanolamine, pH 10, at 37 ℃ in a total volume 0.5 ml. The reaction was stopped with 1.5 ml of 1 mol/L NaOH, 5 mmol/L EDTA and the amount of *p*-nitrophenol produced was determined by measurement of A_{405}.

The following equation was used to determined the activity of GPI-PLD:

Percent of enzyme transform substrate = [A/T (sample) - A/T(contrast)] ×100 %

1.10 Data analysis

Statistical significance was evaluated with the program SPSS 11.5 for Windows. Student's *t*-test and ANOVA were employed for comparing groups of samples, as appropriate. A P value of < 0.05 was considered significant.

2 Results

2.1 Determination of the integrality of PLAP

After phase separation in Triton X-114, the PLAP activity amounted to 96 % were found to partition exclusively into the relatively hydrophobic detergent phase, while in aqueous phase the activity of PLAP was too low to be detected. It indicated that the butanol extraction of PLAP was intact and suitable for detection of GPI-PLD activity.

2.2 Detection of PLAP activity

PLAP activity was determined by incubating enzyme sample with appropriate p-nitrophenyl phosphate as described in the methods section. The result showed that the activity of PLAP may amount to 2 000 U/L, as well as, the extraction of PLAP with butanol at the pH 8.4 was free of other impure proteins, so that it can reduce side-effect on both PLAP activity and GPI-PLD activity (see Table 2).

Table 2 The relationship of the content of pNPP and A_{405} values

pNPP (μg)	0	1.4	2.8	4.2	5.6	7.0	8.4	9.8
A_{405}	0.048	0.389	0.920	1.349	1.867	2.236	2.718	3.042

The enzyme activity of PLAP was examined by pNPP, which the standard curve is $y = 0.026\,6 + 0.315\,2x$, $r = 0.998$. After calculation, its activity is 2 000 U/L approximately

2.3 Effect of intervening factor on serum GPI-PLD of guinea pig attacked by chronical asthma

Detection of GPI-PLD activity was performed by phase separation in Triton X-114. We have compared the activity of serum GPI-PLD of asthmatic animals with the healthy animals (NS group). Furthermore, we also watched the effect of NS, DXM and different dosage of rhPLD2 on the activity of GPI-PLD at different time (6 hours, 12 hours, 18 hours after treatment asthmatic animals with NS, DXM and rhPLD2 respectively). The results were described in Fig. 1 and Fig. 2.

3 Discussion

Asthma is a complex chronic inflammatory disease of the airways that involves the activation of many inflammatory and structural cells, all of which release inflammatory mediators that result in the typical pathophysiological changes of asthma. Among of these inflammatory mediators, many are glycoproteins, which either immediately excrete out of cytoplasm or anchored to membrane by specific glycosyl anchorage after synthesized in cell[5]. Investigators have discovered that GPI-anchored proteins characterized by GPI just belong to this kind of glycoproteins. All of which may be involved in cell recognition, cell development, as well as, broadly participation in inflammatory reaction[6]. We want to know what will change for GPI-anchored proteins when asthma attacks. Because of the complexity of GPI-anchored proteins, and until recently, still have scientists undefined which of these GPI-anchored proteins involve in asthma, detection of GPI-anchored proteins in asthma will be a hard nut to crack. Hence, we made a detection of

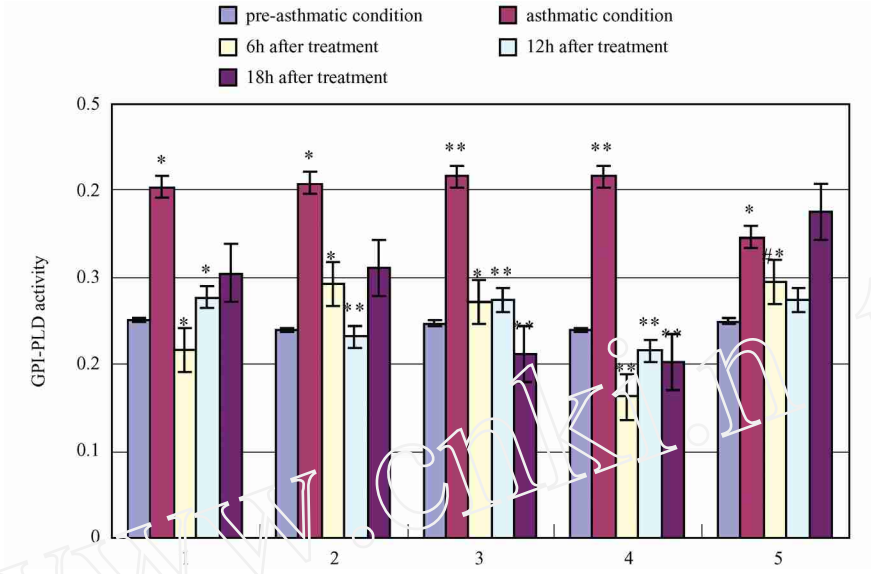

Fig. 1 Comparation of serum GPI-PLD activity for different groups at different time

In vitro, when the detergent existence, this enzyme can degrade the GPI- anchor protein specially. The application in GPI- anchor protein or the enzyme as the substrate which including hydrophobic bases to determine its conversion rate of hydrophobic substrate to water affinity substrate after GPI-PLD function, to express the GPI-PLD enzyme activity level by this method

1: 1.5 mg/kg rhPLD2; 2: 3.0 mg/kg rhPLD2; 3: 6.0 mg/kg rhPLD2; 4: DXM; 5: NS.

* $P < 0.05$, * * $P < 0.01$, * # $P < 0.01$. Results indicate that after 6 hours treatment with NS, serum GPI-PLD activity was even markedly higher than asthmatic condition

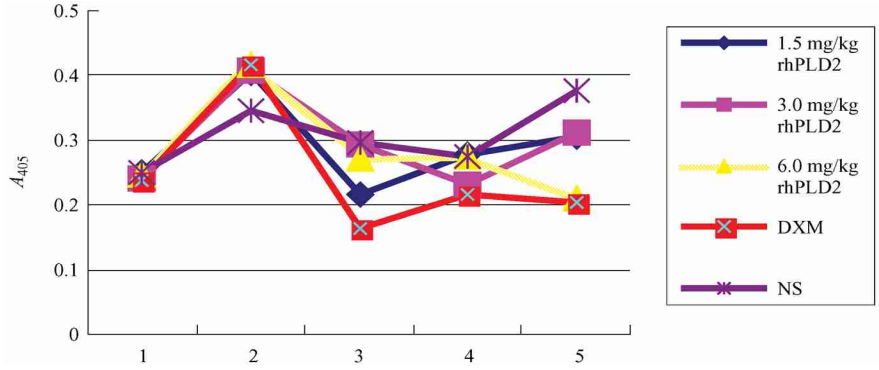

Fig. 2 Detection of GPI-PLD of different groups at different times

1: Pre-asthmatic condition; 2: Asthmatic condition; 3: 6 hours after treatment 4: 12 hours after treatment; 5: 18 hours after treatment

Placenta alkalinity phosphatase (PLAP) was used as GPI-PLD substrate, but PLAP also had its substrate, namely paranitrophenyl phosphate (pNPP) which produced the nitrophenol by PLAP hydrolysis. There is a positive correlation between the content and the A_{405} value. So through this determination of the enzyme activity, PLAP reflects the enzyme activity of GPI-PLD

GPI-PLD, which can specificly hydrolyze GPI moiety to release GPI-anchored proteins[7]. It has demonstrated that GPI-PLD is abundant in mammal serum and associates with high density lipoproteins (HDL), and GPI-PLD activity has been identified in a number of tissues including most of cells, pancreatic islets, and liver. Although GPI-PLD appears to cleave GPI-anchored proteins from the cell surface, it is catalytically inactive in serum. However, because regulation of GPI-anchored proteins interaction, at least, is via GPI-PLD or GPI-PLC, we figure out the level of GPI-PLD in serum may reflect the role of GPI-anchored proteins on the disease pathogenesis (asthma)[8,9].

Our results showed that serum GPI-PLD activity had no statistical difference for either the healthy or asthmatic guinea pigs in different groups before treatment with rhPLD2, DXM and NS, respectively. This indicates the experimental animals had satisfactory homogeneity. There were marked difference of GPI-PLD activity between pre-asthmatic animals and asthmatic animals in different groups. The results show that P value was intervenient between 0.001 and 0.027 in the pre-asthmatic and

asthmatic guinea pigs. It means that GPI-PLD may be involved in pathogenesis of asthma. Group datum are reported as means ± SE. Statistical significance of differences in sample means was determined by paired Student's t-test. In all cases, statistical significance was accepted at the $P < 0.05$ level.

After treatment of asthmatic animals, we watched the markedly descendant GPI-PLD activity for both groups of rhPLD2 and DXM. The extent can be played down to 39.4% of asthmatic GPI-PLD activity for DXM treatment, while only 50.7% for rhPLD2 treatment ($P < 0.01$ for both of them). There was no significant difference for NS treatment ($P = 0.249$). Therefore we deduced that both rhPLD2 and DXM might play a role in oppression of GPI-PLD activity.

As can be seen in Fig. 2, the injection of NS had no effect on GPI-PLD activity, rhPLD2 showed dose dependently inhibition of the activity of GPI-PLD. 6.0 mg/kg rhPLD2 had long-term effect (may last for more than 18 hours) on GPI-PLD activity, while the effective time of 3.0 mg/kg, as well as, 1.5 mg/kg of rhPLD2 was comparatively shorter; after 18 hours, the GPI-PLD activity re-increased to the level of asthmatic animals (P is 0.113 and 0.055 respectively). Our results also showed that DXM had comparative effect on the suppression of GPI-PLD activity with 6.0 mg/kg of rhPLD2. And it seemed that the extent of suppression of GPI-PLD was higher for DXM at the dot time of 6 hours, 12 hours and 18 hours.

Our results showed that the activity of GPI-PLD hit to a top level when the guinea pigs were attacked by asthma; after treatment with rhPLD2 or DXM, the serum GPI-PLD activity re-receded to the level of pre-asthmatic conditions. We found that NS was of no effect on GPI-PLD activity. This indicates that GPI-PLD may associate with asthma. Then what mechanism appreciates the effect of GPI-PLD on asthma. The reports about the relationship between GPI-PLD and asthma mostly concentrate on the role of GPI-anchored proteins. It has been reported that dipeptidase is a sort of GPI-anchored protein, which can regulate the concentration of LTD4, one of the most important inflammatory mediators of the acute phase of chronic asthma[10]. Studies have showed that the membrane-linked dipeptidase has K_m value 11 times as much as that of the hydrolyzed. So the depeptidase released from the cell surface may have larger catalytic activity to lower the content of LTD4. Some investigators thereby believed that GPI-PLD might restraint the acute symptoms of chronic asthma. However, other researchs show that the releasing of some GPI-anchored protein may promote the development of asthma. CD14, the receptor of LPS, is expressed on peripheral blood neutrophils and monocytes as a GPI-anchored protein[11]. There is evidence that CD14 may be a marker for asthma. Some studies showed that $CD14^+$ cell are necessary for increased survival of eosinophils in response to lipopolysaccharide[12]. Therefore, GPI-PLD may abate the effect of EOS on asthma via releasing of GPI moiety. This seems not to agree with our results. But other studies showed that soluble CD14 binding LPS may transport LPS to target site to activate the EOS, however, there are no studies to date examining whether soluble CD14 can interact directly with EOS to mediate EOS response. Further studies are needed to address these questions.

According to our results, we preferred to GPI-PLD promoting the development of asthma in that the content of serum GPI-PLD can be markedly repressed by DXM. The effective of anti-inflammation of DXM has been broadly applied for asthma in clinic medicine.

Because GPI-PLD appears catalytically inactive in serum, we postulate that the improvement of serum GPI-PLD is due to the promoting expression of GPI-PLD, therefore causing to enhance the secretion of GPI-PLD. DXM and rhPLD2 might repress the expression of GPI-PLD, but we still couldn't explicitly define the mechanism involved. DXM's effect are mediated primarily by glucocorticoid receptor (GR) lied in the cytoplasm, DXM binds to GR, resulting in the receptor activation. After processing cascades of signaling transduction, ligand-activated receptor translocates to the nucleus, where it functions as a modulator of gene transcription, activating the transcription of specific sets of genes while repressing the expression of others, the whole effect mights result in the deduced expression of GPI-PLD[13].

The repression of GPI-PLD activity by extrinsic rhPLD2 is an intriguing discover. rhPLD2, one of recombinant isoforms of PLD2 expressed in yeast, has been modified in some functional domains. It has been reported that the wild-type PLD2 may hydrolyze phosphatidylcholine into phosphatidic acid and choline, which, as second signal molecules, play a vital role in signal transduction, membrane trafficking, reconstitution of cytoskeleton, cell profiling and cell differentiation[14,15]. Also studies showed that PLD2 may contribute to some disease pathogenesis such as tumor and asthma. Therefore, we presumed that rhPLD2 might depress asthma via modification of its functional domains to change its corresponding function. This is an interesting issue deserved to be further probe. Our results showed that rhPLD2 might block asthma attack by reducing the expression of GPI-PLD, but how does rhPLD2 repress the expression of GPI-PLD, which itself is a valuable question. In the biological world, there often exists such a phenomenon: the homologue proteins may compete with the same wild-type proteins or the proteins shared with the concensus conformation. Both PLD2 and GPI-PLD belong to PLD superfamily[16]. It has been known that GPI-PLD, like all other excretive proteins, has a process of exocytosis through ER, Golgi

apparatus and budding vesicles[17]. In the whole process, every step owns recognizing mechanism to permit the protein further transport. The signal peptide at the N terminus of protein, and the protein conformation itself, as a recognizing signal, play a vital role that determines the partition of the excretive protein[18]. Therefore, after extrinsic rhPLD2 went into cytoplasm, owing to its homologue conformation with GPI-PLD, it may block the transport of GPI-PLD so as to reduce the expression of GPI-PLD by puzzling the cell recognizing system. Besides of homologue theory, our previous datum indicate that rhPLD2 can inhibit the secretion of PAF, which is a potently inflammory mediator. After binding its receptor, PAF can promote to express and exocyte a series of inflammory proteins. So the deduction of PAF perhaps contributed to the depression of GPI-PLD.

In summary, both rhPLD2 and DXM appears to play an essential role in deduction of serumal GPI-PLD of guinea pig attacked by chronic asthma, which will provide new evidence for the role of GPI-anchored proteins in asthma, although the molecular details of how it is accomplished requires further study, it will pilot us to probe into the mechanism involved in asthma.

References

[1] Knox A J, Pang L, Johnson S, *et al*. Airway smooth muscle function in asthma[J]. Clin Exp Allergy, 2000, **30**(5): 606-614

[2] Busse WW, Lemanske R F Jr. Asthma [J]. N Engl J Med, 2001, **344**(5): 350-362

[3] Nosjean O, Briolay A, Roux B. Mammalian GPI proteins: sorting, membrane residence and functions[J]. Biochim Biophys Acta, 1997, **1331**: 153-186

[4] Deeq M A, Bowen R F, Williams M D, *et al*. Increased expression of GPI-specific phospholipase D in mouse models of type 1 diabetes [J]. Am J Physiol Endocrinol Metab, 2001, **281**: 147-154

[5] Elias J A, Lee C G, Zheng T, *et al*. New insights into the pathogenesis of asthma[J]. J Clin Invest, 2003, **111**(3): 291-297

[6] 唐建华,李文凯. 血清糖基化磷脂酰肌醇特异性磷脂酶D的检测. 湖南医科大学学报(Tang J H, Li W K. Methodological study on the assay of glycosyl-phosphatidylinositolspecific phospholipase D activity in serum[J]. Bull Hunan Med Univ), 1999, **24**(2): 119-122

[7] Malik A S, Low M G. Conversion of human placental alkaline phosphatase from a high Mr form to a low Mr form during butanol extraction. An investigation of the role of endogenous phosphoinosstide-specific phospholipases[J]. Biochem J, 1986, **240**: 519-527

[8] Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases[J]. N Engl J Med, 1990, **323**(10): 645-655

[9] Low MG, Huang KS. Factors affecting the ability of glycosyl-phosphatidylinositol-specific phospholipase D to degrade the membrane anchors of cell surface proteins. Biochem J, 1991, **279**: 483-493

[10] Chung KF, Barnes PJ. Cytokines in asthma. Thorax, 1999, **54**(9): 825-857

[11] McNamara KM, Hall SE, Wilder RS, *et al*. Periodontitis and cytokine expression in CD14 deficient patients[J]. J Int Acad Periodontol, 1999, **1**(4): 95-100

[12] Meerschaert J, Busse WW, Bertics PJ, *et al*. CD14(+) cells are necessary for increased survival of eosinophils in response to lipopolysaccharide[J]. Am J Respir Cell Mol Biol, 2000, **23**(6): 780-787

[13] Sung TC, Roper RL, Zhang Y, *et al*. Mutagenesis of phospholipase D defines a superfamily including a trans-Golgi viral protein required for poxvirus pathogenicity[J]. EMBO J, 1997, **16**(15): 4519-4530

[14] Xie Z, Ho WT, Exton J H. Association of N- and C-terminal domains of phospholipase D is required for catalytic activity[J]. J Biol Chem, 1998, **273**(52): 34679-34682

[15] Sung T C, Altshuller Y M, Morris A J, *et al*. Molecular analysis of mammalian phospholipase D2[J]. J Biol Chem, 1999, **274**(1): 494-502

[16] Miller J L. Release and extracellular transit of glycosylphosphatidylinositol protein[J]. J Lab Clin Med, 1998, **131**(2): 115-123

[17] Deckert M, Ticchioni M, Bernard A. Endocytosis of GPI-anchered proteins in human lymphocytes: role of glycolipid-based domains, actin cytoskeleton, and protein kinases[J]. J Cell Biol, 1996, **133**(4): 791-799

[18] Campbell BJ, Baker SF, Shukla SD, *et al*. Bioconversion of leukotriene D4 by lung dipeptidase[J]. Biochim Biophys Acta, 1990, **1042**(1): 107-112