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Phospholipases as a factor of pathogenicity in microorganisms

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Abstract

Many microorganisms secrete enzymes which ensure their penetration into the host cells. Phospholipases belong to this type of molecules capable to derange or destroy cell surface membranes. Recent data from in vitro and in vivo studies establish the role of phospholipases as virulence factors. Except direct cytolytic effect the enzymes express effect on some immune responses. The increased incidence of antibiotic resistance needs new therapeutic approaches for treatment of infections. Phospolipases represent a promising target for development of a novel class of therapeutics with divergent mechanism of action.

Keywords: Phospholipases; Infections; *Candida albicans*

1. Introduction

During the evolution microbes have developed different mechanisms to penetrate into the host cells. One of them is the production of hydrolytic enzymes which destroy cell membranes. Lipids and proteins are the targets of constitutive and inducible lipases and proteases. The term “phospholipases” is referred to a heterogeneous group of enzymes capable to hydrolyze one or more ester linkage in glycerophospholipds. According to the specific bond cleaved in the phospholipid molecule they are indicated as A, B, C and D [1]. Many studies have addressed the role of phospholipases as a virulence factor in protozoa, bacteria and fungi. Since the discovery that *Clostridium perfringes* alpha-toxin (phospholipase C, PLC) possesses enzymatic activity, there arises a considerable interest in this type of microbial products [2]. A great impetus for investigation the correlation between PLC synthesis and *Clostridium* virulence was the cloning of PLC gene [3]. By the use of PLC-deficient strains it have been directly proved the essential role of PLC in gas gangrene pathogenesis [4]. However, the expectations that all PLC would have virulence properties have not been confirmed. Many data in animal models of infections support that phospholipase B (PLB) acts as pathogenic factor. This minireview briefly summarizes the recent results on phospholipases as an integral part of microbial virulence properties.

2. Phospholipases as a virulence factor in protozoa and bacteria

Logically, the first investigations have been focused on the importance of phospholipases in host cell penetration and lysis by pathogenes. Such data have been accumulated for protozoans *Toxoplasma gondii* [5] and *Entomaeba histolytica* [6,7]. Further, phospholipase A (PLA) was isolated from *Rickettsia rickettsii* [8,9] and some other bacteria [4,10]. *Lysteria monocytogenes* secretes two different phospholipases C, named PLC-A and PLC-B [11,12]. The in vivo experiments showed that the mutant *Lysteria* strains

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lacking genes for one or both lipases were 500-fold less virulent than the parent strain [13]. PLC-B depletion decreased the virulence in a greater extent compared to PLC-A deficiency. These data indicate that different phospholipases could play distinct role as pathogenic factors. *Pseudomonas aeruginosa* is a major pathogen in chronic lung infection, causing inflammation and leading to cystic fibrosis. The following lung destruction correlates with the *Pseudomonas* colonization [14]. The pathogen produces nonhemolytic (PLC-N) and hemolytic (PLC-H) phospholipases, both with MW about 77 kDa. They act synergistically to destroy host cell membrane and cause lysis [15]. Purified PLC causes vascular permeability, hepatonecrosis, renal tubular necrosis, splenomegaly and even death after injection at high doses into mice [16,17]. *Pseudomonas* isolates from patients with traheal, urinary and wound infections express high levels of PLC secretion [18]. Mutants lacking the gene encoding PLC-H were with decreased virulence in a mouse model of *Pseudomonas* infection [10] and in rabbit model of pneumonia [19]. Investigations on *Pseudomonas* PLC also point that extracellular phospholipases can destroy phagolysosome membranes and thus allow liberation of cryptococci into cytoplasm. Two PLC-producing strains have been compared with a PLC-deficient mutant in their intracellular survival in human endothelial cells. While all three strains were equally uptaken by the cells, lysis of vacuoles and higher intracellular concentration was detected for PLC-producing strains. The mutant deficient strain was observed in vacuoles with intact membranes [20]. *P. aeruginosa* also secretes phospholipase D, commonly expressed in eukaryotes, but rarely synthesized by prokaryotes. PLD-deficient mutant was less virulent in pulmonary infection in rats than the parent strain [21].

3. Phospholipases as a virulence factor in fungi

The ability of phospholipases to participate in pathogenic processes during fungal infections is postulated for *Cryptococcus neoformans* [22]. *Cryptococcus* PLB expresses lysophospholipase hydrolase and lysophospholipase transcyclase activities. Mutants lacking PLB gene were less infective in mouse inhalational and rabbit meningitis models [23]. Phospholipases are important pathogenicity determinants in *Candida albicans* infection. The yeast is a common commensal microorganism in humans and animals, and it is a major opportunistic fungal pathogen. Candidosis have become a great medical and economic problem for hospitals, as the mortality rate for candidemia ranges from 40 to 60%. The yeast produces over 75 known toxic substances which contaminate the tissues where it weakens the immune system in the glands, kidneys, bladder, lungs, liver and especially the brain and nervous system. Invasive *Candida* mycoses develop from saprophytic colonization with the yeast in the digestive tract, which is registered in about every second individual. Under certain conditions such as reduction of the commensal intestinal flora by antibiotics, immunosuppressive therapy, HIV infection, colonization with *Candida* may get out of control and cause systemic infections. Until recent years much attention have been paid to reveal the immunologic mechanisms that underlay *Candida* infection. The virulence factors responsible for the initiation of the pathologic processes are less exploited. The yeast secretes all four types (A, B, C and D) of phospholipases [24]. By disruption of cell membranes phospholipases ensure yeast penetration into the host cells and further organ colonization which may last as organ failure [25,26]. The in vitro studies with HT-29 epithelial cells give direct evidence that *Candida* phospholipase B is a virulence factor. Parent *Candida* strain penetrated and damaged epithelial monolayer more efficiently than PLB-deficient strain [27]. High phospholipase production is correlated with an increased ability of adherence and a higher mortality rate in animal models. It is established that the blood isolates of *C. albicans* possessed greater phospholipase activity than commensal isolates. High lipase producers were invasive in infant mouse model, while low producers were not [28,29]. Using in vitro model Klotz et al. [30] proved that *Candida* cells first adhere to the endothelium, disrupt cell continuity and then penetrate into the substance of the vascular tissue due to its phospholipase activity. In vivo data demonstrate that PLB-producing *Candida* strain penetrates deep into the gastric mucosal and submucosal tissues, while PLB-deficient mutant is not invasive [31,32]. Thus, PLB most likely appears to be a pathogenic factor through damaging of host cell membranes after the adherence is realized. The investigations

of phospholipase D1 suppose that it is less important in *Candida* pathogenesis [33]. PLD1-producing and PLD1-deficient strains showed unchanged yeast growth in a model of oral infection. At the same time, deficient mutants were less virulent in two different mouse models [33]. Recent data show that yeast lipases are encoded by a gene family from at least ten members [34,35]. Having in view the ability of the pathogen to colonize a broad spectrum of tissues and to persist as a commensal or as an aggressive invader, it might be expected that through gene variety *Candida* ensures its own penetration and survival into the host cells. The investigations on phospholipases as a virulence factor in *Candida* infections just now begin to develop. The fact that these enzymes are detected in other fungi like *C. neoformans* and *Aspergillus niger* and *A. flavus* points that they may represent common pathogenic factor.

The efficacy of the limited number of antifungals that are available to treat the *Candida* infection is additionally reduced owing to the increased incidence of drug resistance. Amphotericin B has remained the drug of choice for severe invasive fungal infections for nearly 40 years. The side effects due to its renal toxicity lead to dose reduction or discontinuation of the treatment. As a result full eradication of the pathogen is not achieved and organ contamination is not escaped. Hence, there is a need to identify new antifungal targets and compounds that inactivate them. Data implicating phospholipases as virulence factors in *Candida* infection suggest that this enzyme system may represent an important antifungal target [36–38]. First steps in this direction have been made already [39]. By the application of synthetic lipase inhibitors in combination with fluconazole a beneficial effect in *Candida* infection was achieved. A combination of inhibitors and classical antibiotics seems to be very perspective, because ensures pathogen killing and suppression of its penetration into the host cells.

4. Noncytolitic properties of phospholipases

Recently, new aspects of phospholipase activity have been revealed in addition to their lytic effect. Subcytolytic concentrations of PLC cause cell destruction through activation of arachidonic acid cascade and proteinkinase C [4]. Extracellular lipase from *P. aeruginosa* inhibits the monocyte chemotaxis and chemiluminescence [40]. One of the newly described phospholipase activities concern their ability to modulate host immune responses. PLC from *C. perfringens* induces synthesis of interleukine-8 (IL-8) by endothelial cells [41]. Indirect effect on IL-6 and IL-8 secretion by epithelial cells have been proven for *E. histolytica* and *Rickettsia conorii* [42,43].

The virulence of *P. aeruginosa* is to some extent explained in regard to PLC activity. The pathogen provokes extensive influx of neutrophils during the development of lung infection. This effect is due to an enhancement of IL-8 level. Low concentration of PLC-H increased IL-8 secretion, while higher concentrations led to a decrease of IL-8 synthesis and release [44]. In addition, PLC-N was not active. Despite the recruitment of neutrophils, which are the major effector cells taking care for *Pseudomonas* elimination, the pathogen is able to survive and persist in lungs. The activated neutrophils react with respiratory burst and generation of oxygen metabolites to kill the pathogen. PLC-producing strains suppress the respiratory burst of human neutrophils in contrast to PLC-deficient mutants [45]. *Pseudomonas* lipase and PLC are defined as virulence factors because of their ability to generate excessive inflammatory response in vivo [46]. The effector cells of inflammation appear to be neutrophils, basophils, monocytes, platelets and mast cells through release of mediators, like leukotriens (LT), 12-hydroxyeicosatetraenoic acid (HETE), histamine, etc. PLC-H activated polymorphonuclear cells to generate LTB_4, beta-glucoronidase and histamine [47] and also, powerfully increases the formation of 12-HETE by human platelets [48,49]. The enzyme enhances the release of histamine and LTs from rat peritoneal mast cells and human granulocytes [50,51]. Lipase although inactive supports PLC-induced proinflammatory effect of human platelets and leukocytes [52].

Phospholipases C and A2 can remove processed antigens from the surface of antigen presenting cells [53]. Regulation of IL-12 production by human blood monocytes have been supposed for *Candida* lipase [54]. It have also been proven that phospholipases possess antigenic properties. Sera from patients with systemic candidiasis contain antibodies against PLB and their increase correlates with the disease progression [55]. High titers of anti-PLC antibodies have been established in *P. aeruginosa* infections [56,57]. Being

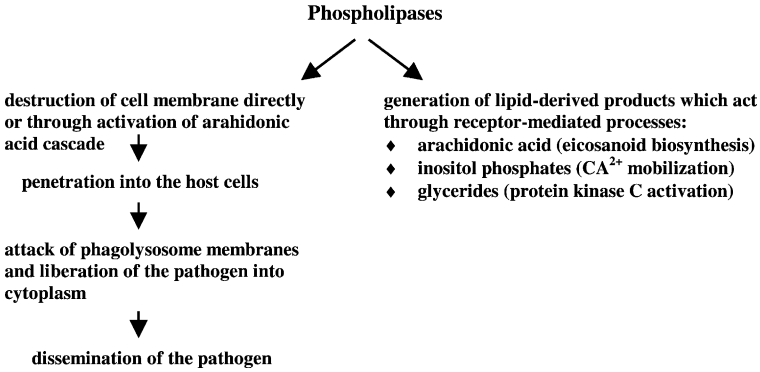

Fig. 1. Mechanism of phospholipase action based on comparison of parent and phospholipase-deficient mutants.

a naturally secreted proteins phospholipases might be used as a diagnostic and therapeutic tool in *Candida* and *Pseudomonas* infections [58].

5. Concluding remarks

All these data implicating phospholipases as virulence factors for many pathogenic species suppose that this enzyme system is an appropriate target for prevention of infectious processes. Phospholipases affect not only the initial processes of penetration and damage of host cells but also provoke inflammation and change cytokine production. The elucidation of mechanisms of their action is an area only partially elaborated until now. One direction which is in initial state concerns the question, whether the lipid-derived products generated by phospholipases express activity. Limited data suppose that such products are implicated as mediators and second messengers in signal transduction [59]. The nomenclature of phospholipases is based on the specificity of the ester link that is cleaved. Therefore, a single enzyme can possess phospholipase B activity (release of fatty acids) and also can express lysophospholipase and transcyclase activity. In addition, all three activities are controlled by a single gene product, which makes difficult the biological action of PLB to be connected with only one of them. The use of different experimental models is another confusing point in data interpretation. Cloning and disruption of phospholipase genes is the most promising approach. Data received by comparison of phospholipase-deficient mutants with parent strains suppose a mechanism of their action, although not rigorously proven (Fig. 1). Strategies for screening substances that can inhibit synthesis or inactivate secreted molecules are needed to develop novel drugs for clinical application.

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