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Inhibition of hemagglutination activity of influenza A viruses by SP-A1 and SP-A2 variants expressed in CHO cells

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Abstract

Surfactant protein A (SP-A) inhibits hemagglutination (HA) activity and infectivity of Influenza A viruses (IAV). As we have showed before in different assays, SP-A2 gene products are more active than SP-A1. Here, we hypothesized that SP-A1 and SP-A2 mammalian CHO-cell-expressed proteins also differentially modulate HA inhibition of IAV. We found that both SP-A1 and SP-A2 equally displayed $\alpha(2,3)$ -linked sialic acids, and had similar activity against a strain (PR-8) that preferentially binds to $\alpha(2,3)$ -linked sialic acids. Based on these Findings, we speculate that in human lung SP-A1 and SP-A2 will not be different in their activity against IAV that preferably bind to $\alpha(2,3)$ -linked sialic acids (like avian strains).

Keywords

Surfactant protein A variants; Collectins; IAV

Introduction

Influenza A viruses (IAV) initiate infection by attachment to host cells via multivalent interactions of its haemagglutinins with sialyloligosaccharide moieties of cellular glyco-

conjugates [1]. Soluble macromolecules containing sialic acids (SA) from animal sera and mucosal fluids can act as decoy receptors and competitively inhibit virus-mediated hemagglutination and infection [1]. Most commonly, IAV receptors contain $\alpha(2,3)$ -linked SA and $\alpha(2,6)$ -linked SA. Of interest, avian IAV strains have a binding preference for $\alpha(2,3)$ -linked sialic acids [2,3]. IAV have two envelope glycoproteins, haemagglutinin (HA) and neuraminidase that are involved in viral interactions with cells and/or other viral particles. HA is heavily glycosylated in most IAV strains and is responsible for viral binding to cell-surface sialoglycoproteins, subsequently leading to viral adsorption [4,5]. SP-A has been shown to inhibit haemagglutination activity and infectivity of IAV [6]. This activity may involve Ca^{2+} -independent binding of IAV to sialylated carbohydrates associated with the carbohydrate recognition domain of SP-A [7]. Mice lacking SP-A exhibit more severe infection with IAV [8]. In contrast to mice that have one SP-A gene, humans have two functional genes, *SP-A1* and *SP-A2*. SP-A1 and SP-A2 gene products have sequence differences that may affect SP-A conformation and, in turn, SP-A function. Indeed, SP-A1 and SP-A2 were found to differ in their ability to stimulate phagocytosis [9,10], bind carbohydrates [11], inhibit surfactant secretion [12], and stimulate production of $\text{TNF-}\alpha$ by macrophage-like THP-1 cells [13–15], as well as in their aggregation properties and oligomerization patterns [12,16,17]. In addition, SP-A1 and SP-A2 mRNAs are differentially present in the respiratory tract sites [18,19]. In all functional assays SP-A2 was found to be more active than SP-A1. Because of the above functional and structural differences and the fact that different individuals may have different amounts of SP-A1 and SP-A2 in their lungs, as shown in a recent study [20], it became important to establish if SP-A1 and SP-A2 also differ in their activity against IAV.

Materials and methods

SP-A1 and SP-A2 variants were expressed from stably transfected Chinese hamster ovary (CHO)-K1 mammalian cells and purified as described previously [15]. For this study, SP-A variants expressed in T-REx-CHO (Tre) inducible mammalian cell system (Invitrogen, Carlsband, CA) were used as well. As a negative control, SP-A variants were produced using the baculovirus-mediated insect expression system, which does not express sialylated proteins, as described previously [14]. As a positive control, human SP-A that was purified [21] from the bronchoalveolar lavage fluid obtained from a patient with alveolar proteinosis (AP BAL hSP-A), as described previously [22], was used.

IAV was grown and purified as described previously [23]. The A/Puerto Rico/8/34(H1N1) (PR-8) strain was provided by Dr J. Abramson (Department of Pediatrics, Bowman Gray School of Medicine, Wake Forest University, Winston Salem, NC). The A/Phillippines/82(H3N2) strain, called “Phil82” later in the paper, was provided by Dr E. M. Anders (Department of Microbiology, University of Melbourne, Melbourne, Australia). HA titers of IAV were determined by titration of virus samples in PBS with thoroughly PBS-washed human type O, Rh (–) red blood cells, as described [23]. HA inhibition was measured by serially diluting SP-A preparations in 96-well round-bottom plates (Serocluster U-Vinyl plates; Costar, Cambridge, MA) using PBS as diluent. After adding IAV solution, giving a final concentration of 40 HA Units per ml, the IAV/SP-A mixture was pre-incubated for 15 min, followed by addition of human erythrocyte suspension in PBS. The entire procedure was performed at room temperature. The minimal concentration of SP-A required to fully inhibit HA caused by the virus was determined by reading the plates after 2 h. HA inhibition was detected as the formation of a pellet of red blood cells.

Presence and linkage patterns of terminally linked SAs on SP-A preparations were analyzed by reducing SDS-PAGE and Western blotting, followed by detection with digoxigenin-labeled lectins according to manufacturer’s instruction (DIG Glycan Differentiation Kit,

Roche Diagnostics GmbH). A control gel was included as reference (Coomassie stain). The MAA and SNA lectins were used to detect $\alpha(2,3)$ -linked and $\alpha(2,6)$ -linked sialic acids, respectively. Control glycoproteins were included to assess lectin binding efficiency and specificity. Quantitative comparisons were obtained by densitometric analysis of blots.

Results

To study, if SP-A1 and SP-A2 gene products differentially inhibit the HA activity of IAV, we tested SP-A variants expressed in two systems—mammalian (CHO) cells (non-inducible and inducible), and insect cells. The density of sialic acids on SP-As was tested by densitometry of glycan blots. A representative glycan blot is shown in Fig. 1. The density of specific sialic acids linkages found on SP-A preparations correlated with SP-A activity. There were no $\alpha(2,6)$ -linked sialic acids found in CHO-cell-expressed SPA variants, and no activity against the Phil82 strain (used as a negative control) that binds to $\alpha(2,6)$ -linked sialic acids was found for recombinant proteins (Table 1), respectively. CHO cell-expressed SP-A1 and SP-A2 had similar HA inhibitory activity to hSP-A against PR-8 (Table 1). Within each set of preparations (I or II, Table 1) there was no significant difference between SP-A1 and SP-A2 in either $\alpha(2,3)$ -linked sialic acids content or in their ability to inhibit HA of IAV. Recombinant preparations II (Tre-system) had lower concentrations of $\alpha(2,3)$ -linked sialic acids and greater SP-A concentrations were required to inhibit PR-8 activity. Neither natural hSP-A nor CHO-cell-produced SP-A variants had any detectable high mannose oligosaccharides based on glycan blotting (not shown). Glycan blots were also performed with insect cell-derived SP-A1 and SP-A2 (used as a negative control), and neither of them showed any sialic acid linkages, or any measurable hemagglutinin inhibiting activity against either IAV (Table 1).

Discussion

The activities of SP-A1 and SP-A2 have been shown previously to differ in a variety of assays. Here, we showed for the first time that CHO-cell-produced-SP-A variants had similar activity to that of natural hSP-A with regards to their ability to inhibit HA of IAV specific to $\alpha(2,3)$ -linked sialic acids. However, no significant difference between SP-A1 and SP-A2 activities was observed.

The PR-8 strain of IAV used in this study, resembles avian IAV strains that bind specifically to sialic acids in an $\alpha(2,3)$ -linkage [2,3]. Of further interest, as we showed before, surfactant protein D and mannose binding lectin lack activity against PR-8 [24]. Hence, the activity of SPA against this strain highlights the potential importance of SP-A in innate host defense against potential pandemic strains of IAV specific to $\alpha(2,3)$ -linked sialic acids [25–27]. Because different individuals may have different proportions of SP-A1/SP-A2 in their lung, and SP-A1 and SP-A2 have differences in their sequence, oligomerization and function, as it was shown by different assays, studying the effect of SP-A1 and SP-A2 on HA inhibition of IAV was a point of interest. It has been noted that CHO cells are only capable of synthesizing $\alpha(2,3)$ -linked sialic acids due to lack of a functional copy of the gene encoding the $\alpha(2,6)$ -sialyltransferase [28]. Thus, SP-A1 and SP-A2 variants expressed in CHO-cells are an appropriate model to study the effect of SP-A on the IAV strains that specifically bind to $\alpha(2,3)$ -linked sialic acids, like avian strains. The baculovirus-derived insect cell-expressed SP-A variants lacked sialic acid and IAV inhibiting activity, and these variants were used as a negative control. Differences between SP-A1 and SP-A2 in their sequence and in oligomerization patterns, may affect, in turn, the interaction of SP-A proteins with IAV. The present data indicate that functional activity of SP-A1 and SP-A2 with respect to IAV may depend principally on the type and extent of sialylation, and not on sequence or oligomerization differences between SP-A1 and SP-A2. The latter do not play a significant

role in the anti-IAV function of SP-A. This is the first time where no differences between SP-A1 and SP-A2 have been observed. In all published reports where other than the anti-viral activity of SP-A was studied, differences were observed with SP-A2 being more active than SP-A1.

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Fig. 1.

Sialylation of SP-A1 and SP-A2 variants expressed in mammalian (CHO) cells. Sialic acid linkage analysis of SP-A variants was performed as described in "Results". Presence and linkage patterns of terminally linked sialic acids on various SP-A preparations were analyzed by SDS-PAGE and Western blotting, followed by detection with digoxigenin-labeled lectins and anti-digoxigenin-alkaline phosphatase system. To assess lectin binding efficiency and specificity control (*cont.*) glycoproteins (fetuin for $\alpha(2,3)$ -linkage and transferrin for $\alpha(2,6)$ -linkage) were included

Inhibition of hemagglutination activity of IAV by recombinant SP-A variants expressed either in mammalian or insect cells and by natural human SP-A; association with patterns of SP-A sialylation

Table 1

SP-A preparation	I (CHO, pEEs14)		II (CHO, Tre)		III (insect)		hSP-A
	1A ⁰	6A ²	1A ⁰	6A ²	1A ⁰	6A ²	
HA inhibition PR-8 strain ^a	4.1 ± 0.5	4.2 ± 1.1	14.1 ± 2.4	11.2 ± 1.0	0	0	4.4 ± 1.1
HA inhibition Phil82 strain ^b	23.4 ± 1.6	17.1 ± 4.7	>25.0	21.8 ± 3.2	0	0	6.2 ± 1.5
Density of α(2,3) sialic acids	706 ± 151	419 ± 77	198 ± 52	152 ± 61	0	0	449 ± 136
Density of α(2,6) sialic acids	0	0	0	0	0	0	381 ± 44

Values shown are mean ± SEM concentrations of SP-A (µg/ml) required for inhibition of 40 hemagglutinin units of IAV. Significantly greater concentrations of the CHO cell expressed recombinant preparations II (Tre-system) were required to inhibit HA activity of PR-8 than for CHO cell expressed recombinant preparations I (pEE14) or natural hSP-A ($P < 0.05$). Insect cell expressed recombinant SP-A variants (III) lacked sialic acids and did not inhibit HA of IAV.

^aPR-8 strain binds specifically to α(2,3)-linked sialic acids.

^bPhil82 strain binds specifically to α(2,6)-linked sialic acids. Significant differences ($P < 0.05$) between inhibitory concentrations of CHO cell expressed SP-A variants for two Influenza A viruses (PR-8 and Phil82) strains were found. Density of α(2,6) or α(2,3)-linked sialic acids on SP-A was measured by densitometric analysis of glycan blots. Recombinant SP-A had no appreciable α(2,6) sialic acids, whereas natural hSP-A did. Three experiments were performed for HA inhibition of PR-8 (except for 6A² preparation I where $n = 2$) and assessment of sialylation levels. Two experiments were performed for inhibition of Phil82