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Research Article

Antipoteinase Vaccines in Modeling Influenza Virus Infection

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Abstract

Isoforms of trypsin-like protease isolated from the lungs of healthy mice demonstrated high protease activity. Percentage of their protein purification ranged from 59% to 99.94%. The increase of protease activity in blood serum, especially to the 3rd isoform was observed following 4-fold immunization of albino rats with trypsin-like protease isoforms. 60% of the animals of the group 4 previously infected with a lethal dose of influenza virus and then treated with anti-protease immune sera were still alive for the observation period of 14 days. 100 % of mice of the control group who did not get treatment but were infected with a lethal dose of influenza A virus died in 4 -5 days after the infection.

Keywords: Influenza virus, Proteases, Purification of virus

Introduction

The proteolytic activation is an important event in the cycle of viral infection [1].

In cases of interfering into any stage of this cycle the parental viruses, virions, can give rise to numerous progeny that will possess no infecting properties as its structure does not contain active fusion proteins to ensure virus penetration into a healthy cell. Therefore, proteolytic activation determines the virulence of viruses and their ability to produce infection. Apparently, the viral ability to infect certain tissues of the body is predetermined by the presence in organs and tissues of enzymes required to provide proteolytic activation for viral progeny [2].

Nowadays photolytic enzymes are provoking interest in almost all the fields of medicine. Moreover, there have been a number of diseases which pathogenesis involves proteinases.

The purpose of this study was to secure trypsin-like protease from the lungs of healthy mice and to obtain hyper immune serum derived from their plasma for treating simulated influenza.

Materials and Methods

Lungs taken from 100 white mice were used to isolate trypsin-like protease. Virus A / PR / 8/34 was used to induce influenza. White rats were used to prepare hyper immune anti-proteinase sera in order to study their protective function on white mice infected with a lethal dose of influenza A.

To isolate and purify trypsin-like proteases obtained from the lungs of healthy mice we used the method of ion exchange chromatography on DEAE-cellulose-32 that included lung homogenization, extraction, ultrasonic disintegration, centrifugation, dialysis and gradient elution.

To obtain hyper immune sera we used Wistar rats weighing 170-200 g. Immunizations of the rats was carried out 4 times once a week with each isoform of trypsin-like proteases, isolated from normal mouse lung with Freud's complete adjuvant. Each rat received 560 units of protein and 890 units of protease. Total hyper immune serum blood sampling was performed on the 7th day after the last immunization. The activity of trypsin-like proteases was determined by K. N. Veremeennko [3], and the protein content was evaluated by Lowry O. [4].

Results

Having purified proteinases obtained from the lungs of healthy mice we got 6 isoforms possessing proteinase activity. The isoforms were cleansed of protein ballast by 60 - 99.98% (Table 1).

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The isoform 6 demonstrated the highest specific proteolytic activity (33.54 IU), and the isoform 1 possessed the lowest proteolytic activity.

For 6 isoforms of trypsin-like protease we obtained 7 groups of immune sera. We immunized 2 groups of animals (10 White rats in each) with the 1st form serum simultaneously. The first group had been previously used in the work with Staphylococcus number 209. Protease content was evaluated in the hyper immune sera obtained. As shown in Table 2, there was a statistically significant increase in the activity of endogenous proteases in the animals in response to all trypsin-like protease isoforms derived from lungs of healthy mice. The highest registered activity of endogenous protease serum was in response to the introduction of protease-3 ei-isoform obtained from the lungs of healthy mice. Compared to the controls (30.646 mg / Arg / ml), it increased as much as twice (43.911 mg / Arg / 0.1 ml). This protease increase in the body of albino rats appeared as nonspecific because after 8 month storage at +4° protease was not detected in these sera, while its blocking effect persisted.

Table 1: Purification of the DEAE- cellulose -32 trypsin-like proteinase obtained from the lungs of uninfected white mice.

№ Fraction	Molarity	№ proteinase isoforms	Specific proteolytic activity	% protein purification
78-88	0,01 NaCl	I	4,391	99,84
124-150	0,03 NaCl	II	11,926	99,94
204-224	0,06 NaCl	III	5,934	99,89
280-288	0,08 NaCl	IV	5,656	99,85
296-302	0,09NaCl	V	5,116	58,92
315-334	0,1 NaCl	VI	33,543	89,90

Studying the protective properties of anti-protease sera and normal rat serum on control white mice infected with lethal dose of influenza virus A / PR / 8/34 (IV passage; control group for the virus) intranasally demonstrated the control mice died on 4th -5th days. The animals, which were instilled the normal rat serum 6 times, died on the 7th day. It should be noted that during the treatment with antisera the mice injected with the serum group 2 began to die faster than other mice (Table 3). Probably, the serum group 2 contained a factor that increased the hemagglutinin dissociation and thus promoted accelerated accumulation of infectious virus. The mice treated with immune serum of group IV died the fewest. The lethality in this group of mice reduced by 60% and on the 14th day after infection there was neither hemagglutinin, nor infectious virus detected in the in the blood serum and lungs of the animals. The groups of immune sera to I and II isoforms also demonstrated protective properties, though they were weak as 30% of the test animals recovered, but not died. The control group (virus without serum) we observed total (100%) death of the mice on the 5th day after the inoculation.

Thus, by intranasal infection of mice with a lethal dose of influenza virus (2,5-2 LD50) in the presence of anti-protease immune serum to III isoform of the group 4, 60% of the animals survived for 14 days (time of observation). In the control group 100% lethality in mice was fixed on 4th -5th days.

Discussion

This research was the first attempt to study cell anti-protease immunoglobulin's blocking the progression of influenza infection. To isolate some fractions of trypsin-like protease isoforms from uninfected mice lungs we had to use larger number of animals

Table 2: The Activity of trypsin-like proteases in the blood serum of white rats after quadruple immunization to isoforms of trypsin-like protease isolated from the lungs of healthy mice.

№ Iso-Form	Control	I isoform		Iisoform	III isoform	IV isoform	V isoform	VI isoform
	17 µkg protease in 0,1 ml (dose administered)							
№ Group		1	2	3	4	5	6	7
n, animals								
1	32,0	40,0	42,0	48,6	48,5	20,0	57,0	52,2
2	29,5	28,1	38,1	46,0	44,5	32,0	38,1	44,4
3	29,2	16,2	38,1	40,0	46,0	30,0	60,0	56,4
4	24,5	38,2	36,2	33,0	38,5	32,0	52,1	50,0
5	31,6	40,5	42,0	42,6	44,0	32,0	52,1	58,0
6	21,0	-	28,1	42,4	42,5	34,5	48,9	61,0
7	29,0	-	30,1	44,0	42,5	34,5	-	-
8	43,6	-	-	-	44,3	40,5	-	-
9	21,2	-	-	-	44,2	36,8	-	-
10	26,8	-	-	-	-	30,8	-	-
11	29,0	-	-	-	-	-	-	-
12	36,8	-	-	-	-	-	-	-
13	32,4	-	-	-	-	-	-	-
14	36,6	-	-	-	-	-	-	-
15	36,5	-	-	-	-	-	-	-
Statistical manipulation								
	δ= 5,60	δ=10,45	δ=3,90	δ=4,66	δ=2,73	δ=5,34	G=6,57	G=21,63
	n =15	n =5	n=7	n=7	n=10	n=10	n=5	n=5
	M=30,65	M=32,58	M=36,37	M=42,51	M=43,91	M=32,32	M=50,20	M=38,22
	m=2,23	m =5,22	M=2,22	m=1,90	m=0,96	m=1,78	m=3,29	m=10,82
		t=1,88	t=5,20	t=7,67	t=4,31	t=9,83	t=1,43	t=5,87
	p>0,1	p<0,001	P<0,001	p0,001	p<0,001	p>0,1	p<0,001	p<0,001

Table 3: Effect of antiproteinase immune sera on the survival of mice infected with lethal dose of influenza virus A / PR / 8/34 / H0N1.

№ group	Protease isoforms	Groups of sera	Period following the infection (days)													
			6 time	1	2	3	4	5	6	7	8	9	10	14	N, survived	% survivalrate
1	I	I						2/10		2/10	2/10	2/10			2	30
2	I	II	2/10	2/10		4/10									2	30
3	II	III			2/10			2/10		2/10			2/10		2	30
4	III	IV						2/10			2/10				6	60
5	IV	V			2/10		2/10	2/14							2	30
6	V	VI			5/10	3/10	2/10								1	10
7	VI	VII				7/10	1/10	1/10							1	10
6	saline solution		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10	100
7	Influenza A virus, no serum					2/10	2/10	6/10							0	0
8	Normal rat serum						2/10	3/10		5/10					0	0
9	Immune serum of IV group without influenza virus (toxicity)		0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10	100

Note: numerator – number of mice who died; denominator – number of mice tested.

that suggests proteases in a healthy body are in very minute amount but play an important role in cases virus invading. Cellular proteases are apparently involved in the "stripping" of influenza virions. Such known anti-viral drugs as rimantadine and bonaftan act on protein M2 HA ion channels, which precede the stage of transcription of the viral genome. Inhibitors of proteolytic enzymes suppress influenza virus replication in the later stages. However, from the position of the body it would be more advantageous to prevent infectioning at earlier stage – the stage when virus particles penetrate into the cell.

According to the literature, influenza viruses A, B and C types, paramyxoviruses, rotaviruses develop infectivity only after the enhancement with trypsin or trypsin-like proteases, whereas chemotryptic proteases by inducing the cleavage of superficial proteins do not activate their function and infectivity towards embryos [5]. The simulation of influenza infection in mice demonstrated significant decrease in their death under the treatment with anti-protease immune serum, especially with that obtained to the III isoform. Whether it is due to the inhibition of a haemagglutinin molecule, or by suppressing the activity of cellular enzymes, the answer to this question requires future thorough studies.

However, our data show that it is possible to obtain an antiviral agent, which would block the influenza virus in the intercellular space and suspend further penetration of the viral RNA into healthy cells, and interrupt the development of the pathological process.

Conclusions

Isoforms of trypsin-like protease isolated from the lungs of healthy mice demonstrated high protease activity. Percentage of their protein purification ranged from 59% to 99.94%. The increase of protease activity in blood serum, especially to the 3rd isoform was observed following 4-fold immunization of albino rats with trypsin-like protease isoforms. 60% of the animals of the group 4 previously infected with a lethal dose of influenza virus and then treated with anti-protease immune sera were still alive for the observation period of 14 days. 100 % of mice of the control group who did not get treatment but were infected with a lethal dose of influenza A virus died in 4 -5 days after the infection.

Summary

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