

OVER SOME SPECIFIC ASPECTS OF ACTIVE AND PASSIVE IMMUNITY IN ROTA- AND CORONAVIRUS INFECTIONS IN MAMMALS

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Summary

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Rota- and coronaviruses cause intestinal infections in mammals. Severe damages of the whole epithelium and especially the intestinal villi with fatal consequences for the absorptive processes in the duodenum are observed. A crucial significance for the prevention of clinical manifestations of both agents has the presence of antibodies in the intestinal lumen as a protective film over the epithelial cells. Therefore the oral vaccination is applied for simultaneously reception of secretory and humoral immunity. The aim of the study was to find an effective immune protection in nonvaccinated animals. Goats have been vaccinated by the intramammary route with "Ro-Co" vaccine. An "immune milk" with a high quantity of IgA antibodies was received. The reception of such milk in 3–4 hours interval provides permanent presence of antibodies in the intestine. An effective way for passive immunoprophylaxis and metaphylaxis was found.

Key words: "immune milk", intramammary vaccination, protection, rota- and coronaviruses

INTRODUCTION

Rota- and coronaviruses cause in mammals intestinal infections which are accompanied with severe damage of the whole epithelium and the villi with fatal consequences for the absorption processes of the duodenum. The presence of specific antibodies in the gut lumen as a protective film over the epithelial cells is of crucial significance for the prevention of the clinical manifestations of both viruses. Therefore oral vaccinations with live attenuated vaccinal strains for simultaneous achievement of humoral and especially secretory immunity are applied.

The secretory immunity is the most important for the vaccinated mammals because it protects them from a clinical manifestation in contact with the field rota- and coronaviruses.

The presence of antibodies in the colostrum and the milk of their mothers plays a crucial role for the protection of sucking children and animals, because their regularly intake provide neutralization of rota- and coronaviruses which enter in the digestive tract. The basic problems arise when the rota- and coronaviruses attack unvaccinated populations.

This fact required finding an effective immune protection against them. This led to the manufacture and the implementation of the so-called "immune milk", which is consistent with the fact that primarily rotavirus infections dominate in children (Yonkova, 1986), but in animals, rotavirus infections occur more often in calves (Haralambiev *et al.*, 1983) and the coronavirus infections affect mostly the swine (Motovski, 1988) and cause transmissible gastroenteritis (TGE) in breeding farms with high mortality in piglets if the mothers are not immune.

Cases of TGE caused by coronavirus were described for first time in the world in dogs in Germany (Albrecht & Lüpcke, 1976) and then in other countries including in Bulgaria (Lüpcke & Filipov, 1982).

For obtaining "immune milk", a "Ro-Co" vaccine developed by Haralambiev and co-workers in 1983 (Lüpcke & Yonkova, 1991), designed for simultaneous immunization against rota- and coronavirus infections in calves, was used initially experimentally by Yonkova & Lüpcke (1989).

MATERIAL AND METHODS

Three lactating goats, vaccinated in different ways, were used: the first goat was vaccinated orally, the second goat – intramammary and the third goat – intracis-ternally.

Two revaccinations of all goats at three-day intervals were applied via the same route and 10 days after the last revaccination milk samples were collected and milk sera were prepared.

The samples were tested for antibodies against rotaviruses by agar gel immunodiffusion (AGID).

We tested lyophilised milk sera from 9 "immune milk" samples in AGID using commercial agar for serological diagnos-

tics (Sanofi S.A., France). The agar was prepared for each sample in the following configuration: 6 wells with 5 mm diameter at 4 mm distance from the central 5 mm well (variant 1) and 6 wells with 8 mm diameter at 5 mm distance from the central 8 mm well (variant 2).

Re-suspended lyophilized rotavirus antigen was added to the central wells and milk sera in dilutions from 1:2 to 1:64 for titration in the surrounding wells. Then the samples were placed in a moist chamber at room temperature. The detection of the precipitation lines was performed at 48th, 72th and 96th hour.

RESULTS

The first precipitation lines appeared at the 48th hour after betting at the lower dilutions and they were observed especially clearly in samples from variant 1. After 72 h we recorded the detected titres, so we could compare them with those reported after 96 h. The results are presented in Table 1.

The titres of antibodies in samples from variant 1 were generally lower than the titres in the samples set in variant 2.

We found that the titres recorded after 72 h were also lower than those found at hour 96. Both variants (1 and 2) showed the same results at these time intervals.

The results from the test carried out by the 120th hour showed that after the 96th hour no longer changes occurred regarding to titres, but the lines appeared to be a little more clear.

DISCUSSION

The results of our studies indicate the AGID can be used in the routine practice for determination of antibody titres against rotaviruses in the "immune milk".

Table 1. Titre of antibodies in samples from the variant 1 and variant 2

Milk serum	Variant	Hour of recording	Titre					
			1:2	1:4	1:8	1:16	1:32	1:64
No 1	1	72				X		
		96					X	
	2	72					X	
		96						X
No 2	1	72			X			
		96				X		
	2	72				X		
		96					X	
No 3	1	72			X			
		96				X		
	2	72				X		
		96					X	
No 4	1	72			X			
		96				X		
	2	72				X		
		96					X	
No 5	1	72		X				
		96			X			
	2	72			X			
		96				X		
No 6	1	72			X			
		96				X		
	2	72				X		
		96					X	
No 7	1	72				X		
		96					X	
	2	72					X	
		96						X
No 8	1	72		X				
		96			X			
	2	72			X			
		96				X		
No 9	1	72		X				
		96			X			
	2	72			X			
		96				X		

The tested samples from lyophilised “immune milk” were stored for more than 10 years without losing their immune activity.

These observations encouraged us to carry out further in-depth studies of the composition of the immunoglobulins:

classes IgA, IgM and IgG in the obtained “immune milk”, because it is known that especially dimeric IgA antibodies are more stable against proteolytic degradation and thus they remain longer capable for a neutralisation of the rota- and coronaviruses into the lumen and on the intes-

tinal mucosa (Taskov & Nikolova, 1997).

It means that the most suitable way for the preparation of "immune milk" are the vaccinations which induce mainly the formation of antibodies such as class IgA. It is possible in the contact between live but well attenuated vaccinal strains and the immunocompetent cells found in the mucosa (Taskov & Nikolova, 1997; Bojkov *et al.*, 1997).

The combined intramammary and intracisternal application of vaccinal rota- and coronaviruses strains would be the base of our future research for improvement the technology of an "immune milk" production. This is due to the fact the oral administration of milk with high titers of specific secretory antibodies in threatened and not vaccinated children and young animals, is the only way for an effective passive immunization. It can be applied as metaphylaxis for limitation of viral replication and reduction of the damages from the viruses on the intestinal mucosa.

CONCLUSIONS

The "immune milk" is produced by induction of mucosal immunity and intramammary and intracisternal application of live attenuated strains in mammary gland. It may be divided into small portions and frozen at -20°C in plastic containers or lyophilised in vials. The titres have to be controlled by agar gel immunodiffusion reaction (AGID) which is easy and widely applicable in practice.

The "immune milk" can be administered at intervals which ensure the continuing presence of antibodies, particularly the more resistant to proteolytic degradation secretory antibodies of the class IgA, located both in the gut lumen and on the intestinal epithelium.

The "immune milk" may be a widely applicable tool for an effective immunization against the rota- and coronavirus infections in mammals.

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