Non-specific humoral immunity in rabbits infected with the selected German strains of the RHD (rabbit haemorrhagic disease) virus

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Abstract

The study aimed to analyse selected parameters of non-specific humoral immunity (myeloperoxidase – MPO activity, as well as lysozyme – LZM concentration and activity) in rabbits infected with three German (Hagenow, Frankfurt, Triptis) strains of the RHD virus with different biological properties. The results of the study showed that biological properties (i.e. different reactivity in the HA test and formation of antigen variant), have impact on the analysed non-specific humoral immunity factors, as antigen variant Triptis causing high mortality at a very short time was the most immunogenic strain, while the strain with variable haemagglutination capacity Hagenow proved to be the least immunogenic strain.

Key words: *RHD virus, haemagglutination capacity, antigenic variant.*

(Centr Eur J Immunol 2009; 34 (4): 218-221)

Introduction

Biological diversity recorded among the strains of the RHD (rabbit haemorrhagic disease) virus, causing death among rabbits, became the reason for thorough analysis of the group of non-haemagglutinogenic strains of the RHD virus (there are only 5 of them) [1] and the continuously growing group of strains referred to as antigen variants, which at present include as many as 28 strains, and which show high pathogenicity accompanied with the unusually acute course of the disease [1].

Therefore, the objective of the study was to analyse the formation of the selected factors of non-specific humoral immunity (myeloperoxidase – MPO activity, as well as lysozyme – LZM concentration and activity) in rabbits infected with three (Hagenow, Frankfurt, Triptis) strains of the RHD virus originating from Germany, and characterised with differentiation among biological properties.

Material and Methods

The study was performed on 60 mixed-race rabbits of both sexes, weighing in the range of 3.20-4.20 kg, marked as conventional animals, coming from a licensed breeding farm under continuous veterinary-and-zootechnical supervision [2]. During the experiment, the animals stayed at the vivarium of the Department of Microbiology and Immunology, Faculty of Natural Science, University of Szczecin, where zoo-technical parameters were conformant to the standards recommended in Poland [3]. After transporting to the Department's vivarium, the animals were subject to a two-week adaptation period. The animals were fed with full-portion rabbit feed (16% Królik z Motycza) and had unlimited access to water. The rabbits were divided into infected groups (10 groups for each studied strain of the RHD virus), and each group of infected animals corresponded to the group of control animals (10 animals in each group). Animals in infected groups were administered intramuscularly a dose of the RHD virus suspended in 1 ml of glycerol with the number of viral particles defined with density within the range of 1.34 g/dm^3, while rabbits in control groups received 1 ml of glycerol as placebo. Rabbits in particular infected groups were administered Hagenow strain – showing haemagglutination capacity at the borderline of positive result, non-haemagglutinogenic Frankfurt strain, and haemagglutinogenic antigen variant Triptis of the RHD virus.

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Each of the viral strains originated from a naturally deceased animal and was prepared as 20% homogenisate cleared by centrifugation and chloroforming, and then by suspension in glycerol in the 1 : 1 proportion [4].

Blood for the tests was drawn both in infected and control groups from peripheral vein of rabbit ear at hour '0', namely before administration of the RHD virus or glycerol, and at 4, 8, 12, 24, 36 h of the experiment. According to the recommendations of the Local Ethical Committee in Szczecin (permit no. 11/06), the experiment was terminated upon the occurrence of the first symptoms of the disease or in the event of animal death, which was recorded and expressed as mortality percentage. In blood, myeloperoxidase (MPO) activity was assessed acc. to Graham method described by Zawistowski [5], comprising the marking of the activity of this enzyme in PMN cells by determining the colour intensity in histochemical reaction, where MPO activity index was expressed as a factor calculated according to the Afanasyev formula [6]. In turn, the lysozyme (LZM) concentration in serum was determined with the method of platelet diffusion acc. to Hankiewicz [7], as compared to model *Micrococcus lysodeikticus* strain. LZM activity index was calculated with the formula presented by Szmigielski [8]. The results were subject to the statistical analysis with t-Student test, and have been presented in Tables 1-3.

Results

When analysing the results obtained for the selected non-specific humoral immunity factors, it must be stated that in the case of MPO activity, non-haemagglutinogenic Frankfurt strain of the RHD virus did not cause changes, strain with variable haemagglutination capacity Hagenow indicated increase at 36 h, while haemagglutinogenic variant Triptis caused a decrease in this factor at 4, 8, 12, 24 h from virus administration.

In turn, as regards LZM concentration, it was recorded that the strain with variable haemagglutination capacity Hagenow caused increase at 4, 8, 12 h from infection, whereas non-haemagglutinogenic Frankfurt strain of the RHD virus

Table 1. Selected factors of non-specific humoral immunity in rabbits infected with RHD virus – strain Hagenow

Parameters			Values of parameters in hours in hours											
			0		4		8		12		24		36	
			Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (8)	K (10)	Z (8)	K (10)
Myeloperoxidase (MPO) activity (l.b.)		$\bar{x}$	2.58	2.53	2.60	2.49	2.73	2.47	2.72	2.46	2.54	2.41	2.54*	2.09
		SD±	0.09	0.28	0.09	0.21	0.05	0.20	0.06	0.19	0.15	0.20	0.11	0.18
Lysozyme (LZM)	concentration (mg/l)	$\bar{x}$	1.76	1.53	2.30*	1.41	2.14*	1.28	2.72*	1.13	1.52	1.14	2.16	1.40
		SD±	0.91	0.33	0.44	0.27	0.94	0.27	0.60	0.13	0.86	0.28	0.28	0.12
	activity index (l.b.)	$\bar{x}$	0.0010	0.0010	0.0015	0.0007	0.0009	0.0012	0.0020*	0.0007	0.0013	0.0011	0.0027	0.0013
		SD±	0.0003	0.0001	0.0004	0.0001	0.0018	0.0002	0.0013	0.0002	0.0008	0.0002	0.0006	0.0004

$\bar{x}$ – mean value, SD± – standard deviation; Z – infected animals, K – control animals; ( ) – number of animals.

Table 2. Selected factors of non-specific humoral immunity in rabbits infected with RHD virus – strain Frankfurt

Parameters			Values of parameters in hours in hours											
			0		4		8		12		24		36	
			Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (1)	K (10)
Myeloperoxidase (MPO) activity (l.b.)		$\bar{x}$	2.64	2.53	2.59	2.49	2.42	2.47	2.29	2.46	2.15	2.41	2.02	2.09
		SD±	0.11	0.28	0.10	0.21	0.08	0.20	0.17	0.19	0.12	0.20	0.07	0.18
Lysozyme (LZM)	concentration (mg/l)	$\bar{x}$	1.92	1.53	5.93*	1.41	5.26*	1.28	4.25*	1.13	2.46*	1.14	0.78	1.40*
		SD±	1.45	0.33	1.09	0.27	3.31	0.27	3.59	0.13	0.77	0.28	0.24	0.12
	activity index (l.b.)	$\bar{x}$	0.0038	0.0010	0.0052*	0.0007	0.0024	0.0012	0.0200*	0.0007	0.0022	0.0011	0.0006	0.0063*
		SD±	0.0010	0.0001	0.0020	0.0001	0.0010	0.0002	0.0020	0.0002	0.0040	0.0002	0.0002	0.0004

$\bar{x}$ – mean value, SD± – standard deviation; Z – infected animals, K – control animals; ( ) – number of animals.

Table 3. Selected factors of non-specific humoral immunity in rabbits infected with RHD virus – strain Triptis

Parameters			Values of parameters in hours in hours											
			0		4		8		12		24		36	
			Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (1)	K (10)
Myeloperoxidase (MPO) activity (l.b.)		$\bar{x}$	2.06	2.53	1.91	2.49*	1.75	2.47*	1.69	2.46*	1.65	2.41*	1.65	2.09*
		SD±	0.34	0.28	0.02	0.21	0.10	0.20	0.07	0.19	0.06	0.20	0.00	0.18
Lysozyme (LZM)	concentration (mg/l)	$\bar{x}$	1.18	1.53	2.75*	1.41	1.63	1.28	0.77	1.13*	1.50	1.14	1.50	1.40
		SD±	0.83	0.33	0.96	0.27	0.70	0.27	0.09	0.13	0.10	0.28	0.00	0.12
	activity index (l.b.)	$\bar{x}$	0.0010	0.0010	0.0020*	0.0007	0.0009	0.0012*	0.0007	0.0007	0.0020*	0.0011	0.0011	0.0063*
		SD±	0.0001	0.0001	0.0000	0.0001	0.0005	0.0002	0.0001	0.0002	0.0010	0.0002	0.0000	0.0004

$\bar{x}$ – mean value, SD± – standard deviation; Z – infected animals, K – control animals; () – number of animals.

caused increase at 4, 8, 12, 24 h and decrease at 36 h, while haemagglutinogenic Triptis variant of the RHD virus caused increase at 4 h and decrease at 12 h from infection.

As regards changes in LZM activity, the strain with variable haemagglutination capacity Hagenow caused increase at 12 h from infection, non-haemagglutinogenic Frankfurt strain of the RHD virus caused increase at 4, 12 h and decrease at 36 h, whereas haemagglutinogenic Triptis variant of the RHD virus caused increase at 4, 24 h and decrease at 8, 36 h from virus administration.

Furthermore, the recorded mortality at 36/48 h from infection for the strain with variable haemagglutination capacity Hagenow amounted to 90%, while for non-haemagglutinogenic Frankfurt strain of the RHD virus and haemagglutinogenic Triptis variant of the RHD virus – 100%.

Discussion

The analysis of the results for 3 parameters of non-specific humoral immunity for the presently studied German strains of the RHD virus with different biological properties (Hagenow, Frankfurt, Triptis) was performed in comparison to previous studies comprising 15 strains of the RHD virus – 2 haemagglutinogenic French Fr-1 and Fr-2 strains [9], 4 haemagglutinogenic Czech – CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558 strains [10], and 9 Polish – SGM, MAŁ [9], Kr-1, KGM [11], BLA [12], PD, GSK, Ż, ŻD strains [13], among which only the BLA strain of the RHD virus is a non-haemagglutinogenic strain, while ŻD is a strain at the edge of negative result in the haemagglutination test.

And so, when comparing the results presently obtained in the area of MPO activity for the three studied strains of the RHD virus (Hagenow, Frankfurt, Triptis), it must be stated that the lack of changes recorded for non-haemagglutinogenic Frankfurt strain of the RHD virus is identical as the results previously obtained for haemagglutinogenic French strains Fr-1 and Fr-2 [9], Czech CAMP V-561 and CAMP V-562 strains [10] and Polish MAŁ strain [9], while the decrease in this factor recorded for haemagglutinogenic antigen variant Triptis of the RHD virus falling at 8, 12, 24 h from infection differs from the results obtained previously, as decreases were recorded in the previously studied strains, yet these were changes falling later and lasting shorter, namely at 52 h for haemagglutinogenic Czech CAMP V-558 strain [10] and Polish SGM strain [9], and at 60 h for haemagglutinogenic Polish KGM strain of the RHD virus [11]. In turn, the increase in this factor falling at 36 h for the strain with variable haemagglutination capacity Hagenow of the RHD virus is similar to the increase obtained at 24 h for haemagglutinogenic Czech CAMP V-558 strain [10].

As regards the changes obtained for the three studied German strains (Hagenow, Frankfurt, Triptis) of the RHD virus in the area of LZM concentration, it can be stated that increase recorded at 4, 8, 12 h for the strain with variable haemagglutination capacity Hagenow and at 4, 8, 12, 24 h for non-haemagglutinogenic Frankfurt strain of the RHD virus can be compared in the aspect of intensity with haemagglutinogenic Czech CAMP V-351 strain [10] and Polish Ż strain [13], although in the case of these strains changes fell much later after administration of antigen, namely at 24, 36, 72 h (CAMP V-351) and 52, 56, 60 h (Ż strain). However, the short increase (just at 4 h from infection) in this factor, recorded for haemagglutinogenic antigen variant Triptis of the RHD virus, cannot be compared with previous studies in this area. In turn, the presently recorded decrease for haemagglutinogenic antigen variant Triptis of the RHD virus, falling at 12 h from infection, and for non-haemagglutinogenic Frankfurt strain of the RHD virus at 36 h, can be compared with the results obtained for haemagglutinogenic Czech CAMP V-561 (decrease at 12 h) and CAMP V-562 (decrease at 52 h) strains [10].

When analysing the results in the area of LZM activity obtained for the three studied German strains (Hagenow,

Frankfurt, Triptis) of the RHD virus, it can be stated that increase falling at 12 h from infection of animals with the strain with variable haemagglutination capacity Hagenow of the RHD virus is not similar to previous results, whereas increase recorded at 4, 12 h for non-haemagglutinogenic Frankfurt strain of the RHD virus and at 4, 24 h for haemagglutinogenic antigen variant Triptis of the RHD virus to some extent resembles the results obtained for Polish haemagglutinogenic Kr-1 strain [11], where increase was recorded at 4, 12, 24 h from virus administration. Also, the decrease in values obtained at 36 h in the case of rabbit infection with non-haemagglutinogenic Frankfurt strain of the RHD virus, and at 8, 36 h for haemagglutinogenic Triptis variant, confirms the results obtained for Polish haemagglutinogenic Kr-1 strain [11] of the RHD virus, where the decrease in this factor fell at 48 h.

Considering high mortality caused already at 36/48 h from administration of the three strains of the RHD virus (Hagenow, Frankfurt, Triptis), it must be observed that a similar mortality ratio was rarely recorded in such a short time from administration of the RHD virus, although 100% mortality at 60 h was obtained for haemagglutinogenic French Fr-2 strain [9], Czech CAMP V-561 strain [10], and Polish Kr-1 [11] and ŻD [13] strains of the RHD virus.

Conclusion

In conclusion, it can be observed that most changes within the studied non-specific humoral immunity factors were recorded for LZM concentration, while the most intensive changes to the factors studied were caused by haemagglutinogenic antigen variant Triptis of the RHD virus, whereas less intensive changes were recorded for non-haemagglutinogenic Frankfurt strain, while strain with variable haemagglutination capacity Hagenow of the RHD virus caused the least number of changes. To conclude, it can be stated that biological properties, which in the case of the three studied German strains (Hagenow, Frankfurt, Triptis) comprised different reactivity in the HA test and formation of antigen variant, impacted on the immunological image formed by the analysed non-specific humoral immunity factors, as antigen variant Triptis causing high mortality at a very short time was the most immunogenic strain, while the strain with variable haemagglutination capacity Hagenow proved to be the least immunogenic strain.

Acknowledgements

The paper has been sponsored from the grant no. N308 03832/3662.

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