Specific immunity in rabbits infected with RHD (rabbit haemorrhagic disease) virus strains with various capacity of erythrocytes haemagglutination

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Abstract

The paper compares the immunological response by defining chosen parameters of specific immunity in rabbits infected with two strains of the RHD virus differentiating in haemagglutination capacity – the haemagglutinogenic French Fr-1 strain, and non-haemagglutinogenic English Rainham strain, which have not been studied in this aspect.

Key words: *rabbit, immune parameters.*

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Introduction

The mechanism of pathogenic action of the RHD (rabbit haemorrhagic disease) virus belonging to the *Caliciviridae* family, is not fully known. However, it is pointed that its important element is the affinity to minor blood vessels and causing the so-called DIC syndrome (disseminated intravascular coagulation) [1, 2]. Also, the capacity of RHD virus to clot erythrocytes seems to be an element impacting on the pathogenic action of the RHD virus, which may condition its malignity, including animal morbidity and mortality. Despite the homogeneity recorded in the binding systematic of the RHD virus [3] pointing to haemagglutinogenicity of this pathogen is its typical feature, presently five strains of the RHD virus have been registered which do not have that property, namely: English Rainham strain obtained in 1993 [4], Polish Blaszki strain (BLA) obtained in 1994 [5], Spanish Asturias strain obtained in 1996 [6], German Frankfurt (Fra) strain obtained in 1996 [7] and Chinese whn-1 strain, for which no year of identification has been specified [8]. Moreover, there have been reports [7, 9] of strains within the RHD virus with intermediate features, which react variably in the haemagglutination (HA) test. Such haemagglutinogenic activity at the border of negative result is shown by the Polish ŻD strain obtained in 2000, which after single passage on rabbits shows full haemagglutinogenic activity, and was called ŻD1 [9]. Another strain indicating haemagglutination capacity at the borderline is the German Hagenow strain, obtained in 1990, characterised with very low or negative result in the HA test [7]. Using the molecular biology methods [8], it has been shown that haemagglutination capacity of the RHD virus is related to the construction of its VP60 protein, and in particular placement of the following in this protein: in the P2 region at the 3' end – phenylalanine amino acids (304), alanine (305), serine (309), while at the 5' end – glycine (359), asparagine (365), alanine (365) and asparagine (386).

The purpose of this study was to compare the immunological response by defining the percent of T and B lymphocytes and their subpopulations (Th, Tc/Ts) in rabbits infected with two strains of the RHD virus differentiating in haemagglutination capacity, namely the haemagglutinogenic French Fr-1 strain, and non-haemagglutinogenic English Rainham strain, which had not been studied in this aspect. Also, the mortality analysis was performed in rabbits infected with these strains of the RHD virus.

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Material and Methods

The study involved 30 mixed rabbits of various sex, weighing between 2.5 and 3.5 kg, qualified as conventional animals from a farm under continuous veterinary and zootechnical supervision. Groups of infected animals (10 units for each strain of the RHD virus) were administered the RHD virus via intramuscular route – respectively the haemagglutinogenic Fr-1 strain and non-haemagglutinogenic Rainham strain (with density of 1.31 g/cm^3), cleared by chloroforming and centrifugation, suspended in 1 ml of glycerol. Titre in the (HA) haemagglutication inhibition test for the haemagglutinogenic French Fr-1 strain of the RHD virus amounts to 10240, while for the non-haemagglutinogenic English Rainham strain of the RHD – titre amounts to 0. For each group of infected animals, there was a corresponding group of control animals in the amount of 5 units, who received intramuscularly 1 ml of a substance in which the virus was suspended (glycerol). Blood for the tests was drawn from peripheral vein of rabbit ear at hour '0', namely before administration of the viral antigen or glycerol, and at 4, 8, 12, 24, 36 h after infection. After this time, the tests were discontinued due to mortality of animals. The number of T and B lymphocytes and their subpopulations in the form of percentage were marked at the flow cytometer – Fascscan Calibur Company by Becton Dickinson, using monoclonal antibodies (mouse anti-rabbit) by Serotec to identify CD5$^+$ (T lymphocyte), CD4$^+$ (Th lymphocyte), CD8$^+$ (Tc/Ts lymphocyte), CD19$^+$ (B lymphocyte) and CD25$^+$ [10].

Results

When analysing the obtained test results (Tables 1 and 2), it must be stated that as regards the parameters tested, double the amount of changes were caused by the haemagglutinogenic French Fr-1 strain of the RHD, where the changes were principally manifested as growth, which fell at 8-36 h after infection and referred to the following in the presented order: percentage of B lymphocytes (with CD19$^+$ receptor), percentage of T lymphocytes (with CD5$^+$ receptor), lymphocytes with CD25$^+$ receptor, Th lymphocytes (with CD4$^+$ receptor) and Tc/Ts lymphocytes (with CD4$^+$ receptor). In turn, in the case of non-haemagglutinogenic English Rainham strain of the RHD, changes were recorded in equal proportions in the form of growth and drop, falling at 12-36 h of the experiment, and principally regarded Th lymphocytes (with CD4$^+$ receptor) and Tc/Ts (with CD8$^+$ receptor).

In turn, rabbit mortality at 36 h after administration of the analysed two strains of the RHD virus varied, as mortality recorded just for non-haemagglutinogenic English Rainham strain of the RHD amounted to 90%, while for haemagglutinogenic Fr-1 strain – amounted to 0%.

Discussion

The presently obtained test results regarding the percentage of T and B lymphocytes and their subpopulations (Th, Tc/Ts) may be compared to the results obtained earlier for haemagglutinogenic French Fr-2 strain [11], Czech CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558)

Table 1. Specific immunity factors in rabbits experimentally infected with RHD virus – non-haemagglutinogenic Rainham strain

PARAMETER TESTED		PARAMETER VALUES AT SPECIFIC HOURS											
		0		4		8		12		24		36	
		Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (1)	K (5)
T Lymphocytes (CD5$^+$) (%)	$\bar{x}$	54.00	57.24	56.28	56.85	59.80	57.85	55.62	58.11	55.43	61.14	53.90	57.82
	SD±	3.16	1.49	4.74	1.54	3.35	1.50	3.03	1.57	3.89	2.04	0.00	1.98
Th Lymphocytes (CD4$^+$) (%)	$\bar{x}$	42.50	43.29	43.56	42.81	42.36	45.71	36.78	43.56*	38.00	47.19*	36.20	41.98*
	SD±	3.98	1.54	4.73	1.32	4.20	1.97	2.94	2.06	4.19	2.59	0.00	1.16
Tc/Ts Lymphocytes (CD8$^+$) (%)	$\bar{x}$	17.26	17.10	16.1	16.86	18.96	15.03	17.36	14.92	20.90*	15.68	24.70*	18.21
	SD±	2.31	1.05	2.42	0.97	2.33	0.72	1.49	1.03	2.16	0.95	0.00	0.33
Lymphocytes with receptor CD25$^+$ (%)	$\bar{x}$	28.16	23.77	23.58	20.89	24.08	23.94	24.30	24.35	23.35	24.64	32.70	22.65
	SD±	2.41	1.21	3.93	1.09	1.46	2.05	3.55	1.94	2.13	1.22	0.00	1.43
B Lymphocytes (with receptor CD19$^+$) (%)	$\bar{x}$	20.82	21.96	19.56	20.57	22.12	21.56	21.66	20.56	21.60	21.93	31.90*	23.48
	SD±	2.00	1.03	1.22	1.71	2.57	1.38	2.00	1.56	1.49	1.32	0.00	1.84

Legend: $\bar{x}$– mean value; SD – standard deviation at p=0.05; Z – infected animals, K – control animals, ( ) – number of animals.

Table 2. Specific immunity factors in rabbits experimentally infected with RHD virus – haemagglutinogenic Fr-1 strain

PARAMETER TESTED		PARAMETER VALUES AT SPECIFIC HOURS											
		0		4		8		12		24		36	
		Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)
T Lymphocytes ($CD5^+$) (%)	$\bar{x}$	46.42	48.87	45.28	48.00	55.28*	47.70	52.90*	41.20	52.32	52.32	55.10*	43.92
	SD±	2.33	10.26	11.12	3.12	9.58	5.71	7.35	17.47	8.74	11.54	12.58	4.98
Th Lymphocytes ($CD4^+$) (%)	$\bar{x}$	38.00	40.40	36.33	36.43	42.12*	35.83	44.56*	36.20	41.26	42.02	35.10	34.35
	SD±	1.97	11.32	6.36	7.80	7.15	6.65	4.67	6.29	6.58	10.82	0.57	7.40
Tc/Ts Lymphocytes ($CD8^+$) (%)	$\bar{x}$	29.65	21.16	24.83	29.45	30.50	29.86	32.49	30.06	30.12	25.21	19.16	28.36*
	SD±	6.36	4.35	6.47	3.98	9.01	5.54	10.02	3.29	8.67	5.23	4.14	5.02
Lymphocytes with receptor $CD25^+$ (%)	$\bar{x}$	7.92	9.40	13.46*	9.97	7.80	8.85	9.60*	5.58	14.68	12.80	12.80*	5.68
	SD±	1.11	1.58	2.8	1.28	1.29	2.33	1.51	1.47	1.25	1.31	1.24	1.93
B Lymphocytes (with receptor $CD19^+$) (%)	$\bar{x}$	17.14	18.24	11.50*	6.67	10.50*	4.57	9.66*	4.45	19.56*	14.82*	12.15	5.98
	SD±	5.39	4.90	4.70	2.02	3.23	1.58	1.77	1.47	2.44	2.43	12.13	1.05

Legend: $\bar{x}$ – mean value; SD – standard deviation at p=0.05; Z – infected animals, K – control animals, ( ) – number of animals.

[1, 12, 13], and Polish Kr-1 [14] strains, and for one non-haemagglutinogenic Polish BLA strain [15].

When analysing the results concerning the percentage of T lymphocytes (with $CD5^+$ receptor), it must be noted that lack of changes for non-haemagglutinogenic Rainham strain of the RHD conforms to the results obtained for Czech haemagglutinogenic strains CAMP V-351 and CAMP V-558 [1, 12, 13], yet the results do not confirm the results obtained for the non-haemagglutinogenic BLA strain, where growth of this factor was recorded at 4,8,12,36,52,56 h after infection [15]. In turn, growth recorded in this parameter (percentage of T lymphocytes with $CD5^+$ receptor) for haemagglutinogenic Fr-1 strain (at 8, 12, 36 h) of RHD partially reflects the results obtained for haemagglutinogenic Polish Kr-1 strain [16]. Furthermore, results in the area of percentage of Th lymphocytes (with $CD4^+$ receptor) for non-haemagglutinogenic Rainham strain of the RHD in the form of drop at 12, 24, 26 h after infection are not similar to the results recorded earlier both in the case of haemagglutinogenic strains (Fr-2, CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558, Kr-1) and non-haemagglutinogenic BLA strain of the RHD, similarly as growth in this factor at 8, 12 h from infection with the French haemagglutinogenic Fr-1 strain. As regards the percentage of Tc/Ts lymphocytes (with $CD8^+$ receptor), it can be noted that the results obtained for non-haemagglutinogenic Rainham strain of the RHD manifested with growth at 24, 36 h after infection, conform to the results obtained for the Czech haemagglutinogenic CAMP V-561 strain of the RHD, where growth was also recorded at 8, 12 h after infection [1, 12, 13]. It should be, therefore, noted that as regards this factor, the results obtained earlier for non-haemagglutinogenic Polish BLA strain of the RHD are also manifested in the form of growth, yet the growth occurs with greater frequency, as it falls on 12, 24, 36, 48, 52, 56 and 60 h after administration of the virus [15]. In turn, drop of the factor, recorded at 36 h after infection with the haemagglutinogenic French Fr-1 strain of the RHD, does not confirm the results obtained earlier for any of the analysed strains of this virus. Lack of changes as regards the percentage of lymphocytes with $CD25^+$ receptor for the presently analysed non-haemagglutinogenic Rainham strain of the RHD confirms previous results for haemagglutinogenic Czech CAMP V-561 and CAMP V-558 strains [1, 12, 13], and does not conform to changes in non-haemagglutinogenic BLA strain of the RHD, where growth was recorded at 36, 52, 56 and 60 h [15]. In turn, results regarding haemagglutinogenic Fr-1 strain of the RHD, manifested with growth at 4, 12, 36 h after infection, are similar to those obtained for haemagglutinogenic French Fr-2 strain [11] and for non-haemagglutinogenic Polish BLA strain [15] of the RHD, because also in these strains, changes were recorded in the first and final hours after infection (12, 52, 56 h). Growth, however, in the percentage of B lymphocytes (with $CD19^+$ receptor) at 36 h after infection in non-haemagglutinogenic Rainham strain of the RHD is similar to the one recorded in haemagglutinogenic Czech CAMP V-558 strain, where it fell on 48 h after virus administration [1, 12, 13]. It must also be noticed that the recorded growth at 12, 24, 36, 48, 52, 56 and 60 h after infection with non-haemagglutinogenic BLA strain of the RHD conforms to the present observations, although they refer to the haemagglutinogenic Fr-1 strain of the RHD, where growth was also recorded at 4, 8, 12, 24 and 36 h after infection.

When analysing the results in the area of selected specific immunity factors during rabbit infection with the RHD virus, one must also mention the study by Chinese authors [quote 2], which point to a significant role of T lymphocytes assessed in the rosette test at rabbit infection with three unspecified strains of the virus.

In turn, when analysing the percentage of mortality at the presently analysed strains, it must be stated that mortality obtained at haemagglutinogenic French Fr-1 strain of the RHD can partly be compared to previous studies [14, 17], although present study was run only to the 36th h (according to the recommendations of the Local Ethics Committee), while other studies [14, 17] regarding the same strain were performed until 60 h from infection, when 90% of mortality was registered. High, as much as 90% mortality obtained at 36 h from rabbit infection with non-haemagglutinogenic English Rainham strain of the RHD is reflected in previous studies, where haemagglutinogenic Fr-2, KGM, SGM and Kr-1 strains recorded 90-95% mortality, although this happened at 60 h after infection with these strains of the RHD virus [17].

To conclude the present results regarding specific immunity in rabbits infected with two strains of the RHD virus – haemagglutinogenic French Fr-1 strain and non-haemagglutinogenic English Rainham strain, differing with the erythrocite clotting capacity, it must be stated that the immunity image is varied for these RHD virus strains, which would confirm the existence of immunotypes among the strains of the virus. It is worth considering that the non-haemagglutinogenic Rainham strain, as regards specific immunity factors, significantly differs from the previously studied non-haemagglutinogenic BLA strain of the RHD, which would indicate that the erythrocyte clotting property is not decisive as regards changes caused in the immunity image. Furthermore, the differentiated mortality image, causing high mortality in animals infected with non-haemagglutinogenic Rainham strain of the RHD, points to a conclusion that the biological property of heamagglutination capacity does not impact on pathogenicity of strains of the RHD virus.

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