Peripheral blood lymphocytes in rabbits infected with Czech strains, CAMPV-562 and CAMPV-558 of RHD virus

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Abstract

The studies pertained determination of lymphocyte T and B levels and levels of their subpopulations (Th, Tc/Ts, CD25+ cells) in peripheral blood of rabbits infected with two Czech strains, CAMPV-562 and CAMPV-558 of RHD virus, in hours 0, 4, 8, 12, 24, 36 following the infection. The studied lymphocytes were identified using flow cytometry and monoclonal antibodies reactive with CD5+ (for lymphocytes T), CD4+ (for lymphocytes Th), CD8+ (for lymphocytes Tc/Ts), CD25+ and with IgM receptor on lymphocytes B. In individual hours disease signs and mortality of rabbits were also recorded. Analysis of the results proved that the two Czech strains of RHD virus induced a similar immune response in the range of the studied lymphocytes although the response was more intense in rabbits infected with CAMPV-562 and it involved CD25+ cells and lymphocytes B. Clinical signs induced by the two strains were similar but the mortality was different: it amounted to 65% in rabbits infected with the CAMPV-562 strain and just 5% in rabbits infected with the CAMPV-558 strain.

Key words: *RHD virus, lymphocytes T, Th, Tc/Ts, B, rabbit.*

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Introduction

The RHD (*rabbit haemorrhagic disease*) virus belongs to *Caliciviridae* family [1], it is pathogenic and highly lethal for farm rabbits and wild rabbits. The disease induced by the virus, termed now the rabbit plague, was described for the first time in China in 1984 [2], in Poland in appeared at the verge of 1987/88 [3], but at present it is manifested on all continents [quoted after 4]. The disease exhibits an acute or superacute course with mortality indices ranging from 80% to 100% [quoted after 4].

The biological traits of the virus includes the almost identical ability to agglutinate human erythrocytes of any blood group by various strains of the virus [5] (even if till now four strains have been described, including Rainham, BLA, Asturias, Frankfurt strains [6-9], which do not manifest the trait); a relatively low genetic variability of RHD virus strains [4, 10, 11], and presence of ten antigenic variants (PV97, Vt97, Triptis, Hartsmanndorf, FR99-05, FR03-23, FR00-REU, RH29/03, CUB-04, Iowa) which have been distinguished in recent years [4, 10, 11]. The strains were also shown to induce distinct immune responses [4, 12-31]. Within the latter range the studies included just one Spanish strain [32], two Chinese strains [33, 34], two French strains [13, 16, 23, 24, 27, 28, 30], two Czech strains [4, 12, 13, 17- 20] and nine Polish strains [14, 15, 19-23, 25-31] of RHD virus. In the case of the two Chinese (unnamed) strains their administration to rabbits in the form of inactivated vaccine was demonstrated to augment ingesting capacity of peritoneal macrophages between hours 24 and 120 post immunisation [33] and an increase in serum interferon level in 18th hour post immunisation [34]. In the studies [33] the strains were found to increase peripheral blood lymphocyte T and B levels, examined using rosette tests. On the other hand, infection with the Spanish strain [32], was followed by an increase in numbers of monocytes and macrophages mainly in the liver, spleen and lungs of of infected rabbits, which was detected by histopathology [32]. In the case of French

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strains, Fr-1 [30, 27, 23] and Fr-2 [16, 23, 24, 27, 30]; Czech strains: CAMPV-351 [17], CAMPV-561 [17- 19] and Polish strains: Kr-1 [14, 15, 22, 29, 30], KGM [14, 30], SGM [15, 23, 27, 30], MAŁ [23, 30], BLA [19, 21, 29], PD [31], GSK [31], Ż [31], ŻD [31] of RHD virus, the studies were conducted in a dynamic set-up and they involved evaluation of non-specific cell-mediated immunity [14-17, 22-24, 27, 29, 30] and humoral immunity [14, 15, 18, 19, 21-23, 27, 31]. The studies proved that the strains induce distinct patterns of lesions, mainly within the scope of indices of non-specific cell-mediated immunity. On the other hand, the appraisal conducted also in a dynamic set-up of specific cell-mediated and humoral immunities of a single Italian strain (BS89), a single English strain (Rainham), two French strains (Fr-1, Fr-2), two Czech strains (CAMPV-351, CAMPV-561) and five Polish strains (Kr-1, BLA, SGM, KGM, MAŁ) [12, 13, 20, 25, 26, 27, 35] showed that the strains induced more pronounced alterations in indices of specific cell-mediated immunity and the extent of the alterations was related to the applied strain of RHD virus.

Present studies aimed at determining percentages of T and B lymphocytes and of their subpopulations (Th, Tc/Ts, CD25+ lymphocytes) in peripheral blood of rabbits infected with two Czech strains of RHD virus, CAMPV-562 and CAMPV-558, which have escaped till now attention of investigators.

Material and Methods

The studies were performed on 60 rabbits, weighing 2.0 kg to 3.0 kg, classified as conventional animals [36]. The animals were housed individually in metal cages in vivarium of the Chair of Microbiology and Immunology, the zoohygienic conditions of which corresponded to national standards [4]. Antigens used in the experiment involved two Czech strains, CAMPV-562 and CAMPV-558, originating Microbial Bank, Veterinary Institute in Brno (Czech Republic) [4]. The original material for preparation of the antigens was the liver of naturally RHD affected rabbits. The antigens, in the form of 20% homogenisates of liver were subjected to purification, centrifugation and freeze-drying, according to the earlier described procedure [23]. Titres of the used in the experiment two Czech strains of RHD virus in the haemagglutination test amounted to 5120-10240. The animals devoted to infection formed two groups. The first group of 20 animals included rabbits administered with intramuscular injection of CAMPV-562 strain RHD virus suspended in 1 ml sterile physiological saline, while 20 animals of the second group received analogously Czech strain CAMPV-558 of the virus. Each group of infected animals was accompanied by the group of 10 control rabbits which received intramuscular injection of 1 ml sterile physiological saline. Blood for tests was isolated from the marginal ear vein just before the infection (hour 0), and then in hours 4, 8, 12, 24, 36 after infection, i.e. to the time of appearance of the first clinical signs of rabbit plague.

Determination of lymphocytes T and B and their subpopulations

In rabbit blood percentages of lymphocytes T, Th, Tc/Ts, lymphocytes with CD25+ receptor and lymphocytes B were determined using the technique described by Deptuła et al. [37]. The lymphocytes were identified using monoclonal mouse anti-rabbit antibodies (Serotec, USA) to CD5+ receptor (on lymphocytes T), CD4+ receptor (on lymphocytes Th), CD8+ receptor (on lymphocytes Tc/Ts), CD25+ receptor (on lymphocytes T) and IgM receptor on lymphocytes B in a flow cytometer (Becton Dickinson, FACScan, USA).

Registration of clinical signs and mortality

In individual rabbits morbid signs and mortality (%) were recorded in individual hours of the experiment till the 36th hour of the experiment.

Statistical analysis

Results of the studies were subjected to statistical analysis using Student's *t*-test at $p \leq 0.05$ and presented in Tables 1 and 2 in the form of means ($\bar{x}$) and standard deviation (SD±).

Results

Analysis of the demonstrated levels of peripheral blood lymphocytes in rabbits infected with one or the other strain (CAMPV-562, CAMPV-558) of RHD virus (Tables 1 and 2) justifies the conclusion that significant alterations in proportions of lymphocytes T and B and in their subpopulations (Th, Tc/Ts, CD25+) were registered only following infection with the CAMPV-562 strain and, in addition, in only some of the subpopulations. The increased percentage involved lymphocytes with the CD25+ receptor and lymphocytes B in the 36th hour of the experiment.

The clinical signs noted in the infected animals were similar: they developed between 24th and 36th hour following infection and involved dejection, disturbed respiration, lack of appetite and augmented coagulability of blood. Till the 36th hour of the experiment mortality in rabbits infected with the CAMPV-562 strain, which induced alterations in lymphocyte subpopulations, amounted to 65%, while the mortality following infection with the CAMPV-558 strain, which induced no alterations in percentage of lymphocytes, amounted to 5% only.

Discussion

Upon analysis of the immune pattern which developed following infection with CAMPV-562 and CAMPV-558

Table 1. Lymphocytes and their subpopulations in peripheral blood of rabbits experimentally infected with Czech strains CAMPV-562 of RHD virus

Lymphocytes with a specific CD receptor (%)		Parameters in particular hours											
		0		4		8		12		24		36	
		Z	K	Z	K	Z	K	Z	K	Z	K	Z	K
		n=20	n=10	n=20	n=10	n=20	n=10	n=20	n=10	n=19	n=10	n=7	n=10
Lymphocytes T	x̄	69.2	64.9	67.3	60.9	69.9	62.7	67.4	56.7	65.4	67.0	67.7	61.7
(CD5+)	SD±	7.1	7.9	9.6	5.3	6.7	3.0	4.9	7.6	4.3	5.0	7.5	4.0
Lymphocytes Th	x̄	48.4	48.5	55.1	47.5	46.2	41.5	49.8	40.3	45.4	51.0	55.1	40.5
(CD4+)	SD±	5.0	6.1	3.1	5.0	4.7	5.6	3.1	4.3	1.2	6.2	7.7	5.4
Lymphocytes	x̄	21.7	20.2	19.9	21.2	19.2	24.6	21.7	18.9	20.5	24.1	21.9	20.7
Tc/Ts (CD8+)	SD±	3.5	0.5	2.7	0.8	3.4	3.0	2.9	3.6	3.4	2.0	3.3	2.7
Lymphocytes B	x̄	23.2	22.7	31.4	28.6	21.5	29.2	25.1	25.4	20.8	20.2	52.3*	23.3
(IgM)	SD±	3.0	3.0	4.0	3.3	3.9	3.5	2.6	2.5	3.5	3.9	6.0	2.6
Lymphocytes	x̄	17.6	15.2	20.5	15.1	18.7	17.4	15.2	26.5	18.9	22.3	67.7*	26.4
CD25+	SD±	2.0	0.8	1.5	2.0	2.3	2.2	3.0	4.0	2.0	4.1	7.0	3.0

*Z – infected animals; K – control animals; * – difference significant at p≤0.05*

Table 2. Lymphocytes and their subpopulations in peripheral blood of rabbits experimentally infected with Czech strains CAMPV-558 of RHD virus

Lymphocytes with a specific CD receptor (%)		Parameters in particular hours											
		0		4		8		12		24		36	
		Z	K	Z	K	Z	K	Z	K	Z	K	Z	K
		n=20	n=10	n=20	n=10	n=20	n=10	n=20	n=10	n=19	n=10	n=19	n=10
Lymphocytes T	x̄	60.9	57.3	57.2	52.0	58.0	53.5	49.9	52.0	54.8	52.6	52.6	59.4
(CD5+)	SD±	7.9	7.8	7.0	7.3	8.4	5.8	5.0	6.0	7.0	7.5	6.7	6.0
Lymphocytes Th	x̄	39.8	39.3	37.0	38.4	34.8	38.3	35.7	35.5	35.4	34.9	39.1	40.4
(CD4+)	SD±	3.2	1.8	4.5	5.2	3.6	5.1	3.9	3.0	4.1	4.5	3.8	4.7
Lymphocytes	x̄	23.2	19.5	19.9	18.9	20.5	20.4	17.9	19.9	19.3	21.0	18.6	21.8
Tc/Ts (CD8+)	SD±	3.3	3.9	2.9	3.2	4.1	4.9	3.7	3.0	2.3	2.2	3.2	2.5
Lymphocytes B	x̄	22.6	24.3	26.0	26.5	28.3	29.3	26.5	28.0	31.5	29.1	40.4	34.9
(IgM)	SD±	2.6	2.0	1.4	4.9	4.3	2.4	2.6	4.0	3.5	4.0	5.7	4.9
Lymphocytes	x̄	32.1	36.8	35.1	30.2	30.5	31.3	34.0	37.8	39.8	33.8	51.0	31.3
CD25+	SD±	3.4	2.4	4.0	5.4	2.0	2.0	2.6	2.2	4.0	5.0	3.4	4.0

Z – infected animals; K – control animals

strains of RHD virus allows to note that the absence of changes in percentage of lymphocytes T is consistent with the earlier obtained results of infection with the Czech strain of CAMPV-351 [12], Italian strain of BS89 and English strain of Rainham [35], although it differs from results which were obtained following infection with the Czech strain of CAMPV- 561 [12], French strains of Fr-1 [27] and Fr-2 [13, 26] and Polish strains of Kr-1 [25] and BLA [20]. Infection with the CAMPV-561, Fr-1, Fr-2, Kr-1, BLA strains of RHD virus was followed also by an increase in

the percentage of lymphocytes T. However, the increase was persisted longer and was noted in hours 4, 8, 12, 24 and 36 following the infection.

Results of our own studies on percentage of lymphocytes Th, Ts/Ts following infection with CAMPV-562 and CAMPV-558 strains were consistent with the results obtained following infection with the Czech strain of CAMPV-351 [12], which also was followed by no significant alterations in the percentage of the lymphocytes. Present studies have not confirmed the observations obtained following infection with the Czech strain of CAMPV-561 [12]; French strains of Fr-1 [27] and Fr-2 [13, 26]; Polish strains of Kr-1 [25], BLA [20], Italian strain of BS89 or the English Rainham strain [35]. Strain CAMPV-561, Fr-1, Fr-2, Kr-1 and BLA have increased of lymphocytes Th, Tc/Ts in hours 4, 8, 12, 24, 36 of the experiment [12, 13, 20, 25-27], while the strains of BS89 and Rainham resulted in both increase (hours 4, 8, 12, 24, 36) and a decrease (hours 4, 12, 24) in percentage of lymphocytes Th and Tc/Ts [35].

The currently noted increase in percentage of lymphocytes B in the 36th hour following infection with CAMPV-562 strain has confirmed the data obtained following infection with the French strain, Fr-2 [13, 26], Polish strains, Kr-1 [25] and BLA [20] and Italian strain, BS89 [35]. Infection with the strains was followed by an increase in percentage of lymphocytes B in hours 4 (strain Fr-2), 8 (strain BS89), 12 (strains Kr-1, BLA) and 36 (strains Kr-1 and BS89) following infection. In contrast to the infection with the presently studied Czech strain of CAMPV-562, infection with another Czech strain of CAMPV-351 was followed by a decrease in the percentage of lymphocytes B in 24th hour [12], while infection with the English strain of Rainham was followed by decreased percentage of lymphocytes B in hours 4 and 8 following infection [35]. The absence of changes in our present studies in the level of lymphocytes B following infection with CAMPV-558 strain is consistent with the result obtained following infection with the Czech strain, CAMPV-561 of RHD virus [12].

In turn, the increase in CD25+ cells in the 36th hour following infection with the Czech strain of CAMPV-562 corroborates the results obtained following infection with the Czech strain of CAMPV-351, French strain of Fr-2, Italian BS89 strain and Polish strains of Kr-1 and BLA. Infection with the strains was followed by elevated percentage of CD25+ cells between 24th and 36th hour post infection [12, 13, 20, 25, 26, 35]. It should also be added that following infection with some of the above mentioned strains (Czech strain of CAMPV-351 and the English strain of Rainham) of RHD virus, the increased percentage of lymphocytes CD25+ was preceded by their decreased [12, 35] in hour 8 following infection with CAMPV-351 strain and in hours 4, 8, 12 following infection with Rainham strain [12, 35]. In turn, the absence of changes in percentage of CD25+ lymphocytes following infection with the Czech strain of CAMPV-558 is consistent with the results obtained after infection with the other Czech strain, CAMPV-561 of RHD virus [12].

As far as timing of clinical signs, their type and intensity are concerned, they did not depart from the signs recorded by other authors following infection with CAMPV-351, CAMPV-561, Fr-1, Fr-2, SGM, MAŁ, Kr-1, BS89, Rainham strains of RHD virus [4, 12, 22, 23, 35]. On the other hand, both timing and mortality among rabbits infected with the currently studied RHD viruses of Czech strains, CAMPV-562 and CAMPV-558, point to differences in their pathogenicity. The mortality of 65% recorded following infection with the Czech strain of CAMPV-562 has resembled the mortality noted in rabbits infected with the Polish Ż strain, which amounted to 60% till the 36th hour of the studies [31], although an even higher mortality till the 36th hour of studies was noted following infection with the Polish strains, GSK (80%), Kr-1 (80%) [23, 31] and Czech strain of CAMPV-561 (75%) [12]. On the other hand, the mortality of 5%, noted till the 36th hour of observation following infection with the currently studied Czech strain of CAMPV-558 is comparable to mortality indices observed following infection with the Polish strains of SGM (5%) and BLA (5%) or French strain of Fr-2 (10%) [23, 31]. It should also be added that in the cases of infection with the French strain of Fr-1 or Polish strains of Mał, ZD, PD [23, 31] mortality indices till 36th hour after the infection amounted to 0%, while those following infection with the Czech strain of CAMPV-351 amounted to 30% [12] and that with the Polish strain of KGM was 25% [31].

Summing up it can be concluded that the studied Czech strains, CAMPV-562 and CAMPV-558, induced just slight alterations in the studied lymphocyte: only infection with the strain of CAMPV-562 was followed by increased percentage of lymphocytes B and CD25+ cells. The alterations following infection with the Czech strain of CAMPV-562 most cosely resembled the changes noted following infection with the Czech strain of CAMPV-351, which was also associated with slight alterations in percentage of CD25+ and B cells [12]. Therefore, the two studied Czech strains (CAMPV-562, CAMPV-558) induced slight and similar alterations in the immune pattern and the alterations differed from those obtained following infection with Polish, French, Italian or English strains of the virus. This might indicate their distinct immunological character and might confirm the hypothesis on immunotypes which exist among strains of RHD virus. On the other hand, immunological studies on currently tested Czech strains CAMPV-562 and CAMPV-558, in the context of the induced by them mortality, suggest a relationship which may exist between virus-induced alterations in the immune pattern and pathogenicity of RHD virus. Such a relationship seems to be indicated also by studies conducted on the Czech CAMPV-561 strain, Italian BS89 strain and English Rainham strain of RHD virus [12, 35].

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