Immunological and genetic studies of RHD (rabbit haemorrhagic disease) virus strains

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Abstract

The aim of this paper is to describe the immunological response in rabbits infected with 4 strains of the RHD virus (BS89, Rainham, Asturias, Frankfurt) in respect of indicators of non-specific cellular immunity and their genetic analysis on the basis of the N-terminal fragment of the gene encoding VP60 structural protein. The study was performed on strains of RHD virus that have never been analysed before in the scope of those parameters. The results of the study showed, that 4 analysed strains (BS89, Rainham, Asturias, Frankfurt) form two immunogroups: with high immunogenicity and with low immunogenicity, whereas genetic analysis of those strains also 'divided' them into two genetic groups, nevertheless the immunogroups and genogroups were of a different content.

Key words: *RHD virus, immunological, genetic analysis, immunogroups, genogroups.*

(Centr Eur J Immunol 2009; 34 (2): 61-67)

Introduction

The RHD virus is an etiological agent responsible for rabbit plague (RHD - rabbit haemorrhagic disease), which was classified into the *Caliciviridae* family in 1992. The virus was first described in 1984 in China, while in Europe first records of the disease come from 1986 [1-5]. Presently, the virus is present in Asia, Europe, Central and South America, Africa, Australia and New Zealand, and recently also in the USA, causing great damage to breeding of the animals [1-5].

Previous immunological research in this field referred to 2 Chinese strains (with no name) of the RHD virus, where in the dynamic system the ability of MN cells absorption was assessed, as well as the quantity of interpheron in rabbits infected with such viral strains. In turn, in other studies, also Chinese, made in the static system on rabbits administered with 3 strains of the RHD virus, a significant role of T and B lymphocytes and MN cells was found in this infection. In turn, domestic research performed in the dynamic system referred to many indicators of specific and non-specific immunity, both cellular and humoral, in rabbits infected with 2 French strains (Fr-1, Fr-2), 4 Czech strains (V-351, V-561, V-562, V-558) and 10 Polish strains (SGM, KGM, MAŁ, Kr-1, K-1, PD, GSK, Ż, ŻD, BLA) and showed varied immunological response of such RHD virus strains, principally in the area of non-specific cellular immunity, which may point to immunotypes among this virus [4].

However, genetic analysis based on phylogenesis of 56 French strains of the RHD virus from the years 1988-95 [6] and 104 French strains from the years 1993-2000 [7], based on the fragment of the gene encoding VP60 protein, showed that they create 6 genogroups. Also, comparative studies of 47 British strains of the RHD virus, obtained in the years 1955-2000, and 12 European strains of the virus allowed to select 8 genetic groups [8]. In turn, the analysis of 44 strains of the RHD virus obtained from dead rabbits in the years 1981-1995 in 17 countries (Austria, Belgium, China, France, Czech Republic, Germany, Hungary, Ireland, Israel, Italy, Korea, Mexico, Slovakia, Spain, Sweden, Switzerland, England), suggested the existence of 3 genetic groups [9]. The last study was confirmed by the analysis performed by the Hungarian team in 2006 [10] which on the basis of the fragment of the gene encoding VP60 assessed 17 strains of the RHD virus obtained in the years 1988-2003, and showed that they create three genogroups. However, our own studies [3] regarding three Czech strains of the RHD virus obtained

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from dead rabbits in 1988 (CAPM V-558), 1992 (CAPM V-562) and 1996 (CAPM V-561), based on the analysis of three genome fragments: two coming from N- and C-terminal regions of VP60 protein and p30 protein, showed that these form two genetic groups (G1 - V-558 and V-562; G2-V-561) [3]. Other studies on the sequences encoding VP60 structural protein in 7 Polish strains (SGM, KGM, PD, BLA, GSK, ŻD, LUB) [2] and 5 European strains (German, Czech, French, Italian, Spanish) of the RHD virus showed very close relationship, which the authors link to the fact that they were identified during the several first years of the RHD virus occurrence in Europe. However, the comparison based on the analysis of the full genome of two strains of the RHD virus coming from the Saudi Arabia and the Kingdom of Bahrain, where rabbit plague took place respectively in 1996 and 2000 [11], to 59 strains of the RHD virus coming from various countries of the world obtained in the years 1955-2000, included them to two separate genetic groups despite the origination from a single region, which would prove that they appeared in the area at different times. Facts in the area of genetic analysis of various strains of the RHD virus have proved that, as it is presently adopted, genogroup creation is principally conditioned by the year [6, 8, 12], and to a lesser extent by their identification site [6, 9].

The purpose of the present study is to assess the immunological response in rabbits infected with 4 not previously analysed strains of the RHD virus (BS89, Rainham, Asturias, Frankfurt) in respect of indicators of non-specific cellular immunity and their genetic analysis on the basis of the N-terminal fragment of the gene encoding VP60 structural protein.

Material and Methods

The study was performed on 60 mixed-race rabbits of various 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-zoo-technical supervision [13]. 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 [14]. After transporting to the Department's vivarium, the animals were subject to a two-week adaptation period and tested for the presence of anti-RHDV antibodies with ELISA test with ready kits (Institutio Zooprofilattico Sperimentale, Italy). The animals were fed with full-portion rabbit feed (16% Królik, Motycz Poland) in the quantity of 0.15-0.20 kg/day and had unlimited access to water. The rabbits were divided into 4 groups of infected animals (10 in each) and 4 groups of control animals (5 in each). Animals in study groups were infected intramuscularly with one of 4 strains (Italian-BS89, English-Rainham, Spanish-Asturias, German-Frankfurt) of the RHD virus suspended in glycerol, and in the same manner glycerol was administered to control animals. Each of the administered viral strains came from a naturally dead animal from which liver was sampled and prepared as a 20% homogenisate cleared by centrifugation and chloroforming, so that each antigen is characterised with a uniform number of viral particles defined with density as 1.34 g/dm^3. Titre in the HA test for the BS89 strain amounted to 10240, while for the Asturias, Frankfurt and Rainham strains it was negative, as these are non-haemagglutinogenic strains.

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. In blood, the ratios of non-specific cellular immunity were marked, namely the adherence capacity according to Lorente [15], absorption according to Brzuchowska and Ładosz method, modified by Deptuła et al. [quote 1] and cidality of PMN cells in the nitrotetrazolium blue (NTB) reduction test with cytochemical method – spontaneous test and stimulated test according to Park et al. [16], as well as in the spectrophotometric test according to Raman and Poland [17]. Furthermore, coefficient of metabolic activity in neutrophilic granulocytes (WAMG) was calculated for the spontaneous test and stimulated test according to Grządzielska [18] and stimulation index according to Lechowski [19].

Virus was obtained for genetic studies from livers sampled from rabbits experimentally infected with 4 strains of the RHD virus originating from naturally dead rabbits on the territory of Germany – Frankfurt (1996), United Kingdom – Rainham (1993), Spain – Asturias (2000) and Italy – Bs89 (1989). The genetic analysis was performed based on the RT-PCR and PCR reaction, performed according to standard procedures using starters allowing for amplification of the fragment of the gene encoding VP60 protein from the N-terminal part of the length of 510 nucleotides: P1 starters (sense) 5'gagctcgagcgacaacaggc and P2 (antisense) 5'caaacacctgacccggcaac, constructed on the basis of the genome sequence of FRG strain [12, 20]. The obtained nucleotide sequences of the genome fragment of 4 strains of the RHD virus were compared with one another, creating a phylogenetic tree using the method of observed differentiation in DNAMAN software (Lynnon BioSoft, Canada).

The results of immunological studies have been presented in Tables 1-4, while results of genetic studies – in Fig. 1.

Results

When analysing the indicators of non-specific cellular immunity (Tables 1-4), it must be stated that as regards adherence capacity on the presently studied strains of the RHD virus, growth of this parameter has been recorded for

Table 1. Indices of non-specific cellular immunity in rabbits experimentally infected with BS89 strain of RHD virus

	Parameters		Values of parameters in hours									
			0		4		8		12		24	
			Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (7)	K (5)
	Adherence capacity (%)	$\bar{x}$	41.24	39.19	49.26*	40.26	46.20	46.03	40.56	39.01	34.95	43.10*
		SD ±	5.59	2.55	6.53	4.25	4.79	4.17	3.63	2.74	4.31	2.98
Absorption capacity	absorption index (l.b.)	$\bar{x}$	3.75	4.63	2.66	4.55*	3.11	4.98*	3.08	4.67*	3.33	4.41*
		SD ±	0.21	0.45	0.19	0.43	0.36	0.48	0.19	0.43	0.39	0.43
	% of absorbing cells	$\bar{x}$	57.00	67.89	53.00	70.20*	54.00	66.26	56.00	68.70	65.00	68.09
		SD ±	0.96	4.86	3.92	3.89	4.04	3.91	4.57	4.00	4.58	3.09
NBT reduction test	spectrophotometeric (10^9/l)	$\bar{x}$	3.00	4.13	1.42	4.42*	1.57	4.99*	1.91	5.60*	5.22	4.95
		SD ±	0.60	0.88	0.48	0.77	0.39	1.31	0.39	1.16	2.36	0.75
	spontaneous (l.b.)	$\bar{x}$	13.20	10.11	10.00	10.81	9.60	9.93	8.60	9.81	7.50	8.81
		SD ±	0.84	0.96	0.71	1.18	0.89	1.00	0.51	0.89	0.71	0.86
	stimulated (l.b.)	$\bar{x}$	27.00	21.11	22.60	20.20	21.60	19.87	19.60	18.85	19.50	16.38
		SD ±	2.00	1.78	1.95	1.72	1.14	1.69	0.40	1.97	0.32	0.61
	stimulation index (l.b.)	$\bar{x}$	2.04	2.07	2.26	2.05	2.26	2.09	2.28	1.88	2.02*	2.11
		SD ±	0.14	0.30	0.13	0.47	0.22	0.33	0.27	0.20	0.25	0.18
WAMG	spontaneous (l.b.)	$\bar{x}$	0.33	0.33	0.25	0.29	0.22	0.27	0.17	0.28*	0.25	0.34
		SD ±	0.07	0.06	0.03	0.07	0.07	0.06	0.05	0.08	0.06	0.03
	stimulated (l.b.)	$\bar{x}$	0.68	0.63	0.56	0.52	0.50	0.53	0.39	0.53*	0.53	0.50
		SD ±	0.11	0.06	0.05	0.04	0.19	0.05	0.08	0.05	0.29	0.06

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

Asturias strain (12, 24 h), Frankfurt (36 h) and BS89 (4 h), with the drop in the case of Rainham strain (8, 24, 36 h) and BS89 (24 h). In turn, as regards the absorption index, growth of this index was only recorded for Asturias strain (4, 8, 12, 24 h) and drop in the case of BS89 strain (4, 8, 12, 24 h) of the RHD virus. The values of the percentage of absorbing cells showed increase for Frankfurt strain (8, 12 h), with drop for Asturias strain (8, 12, 24 h) and BS89 (4 h) of the RHD virus. In the case of spectrophotometric NBT test, all strains studied recorded a drop in this factor – Asturias at 4, 8, 24 h, Frankfurt at 12, 24 h, Rainham at 12 h and BS89 at 4, 8, 12 h, while as regards spontaneous NBT test. Three non-haemagglutinogenic strains studied indicated a drop in this parameter – Asturias at 4, 8, 12, 24 h, Frankfurt at 4, 8, 12, 24 and 36 h, while Rainham at 24, 36 h. In the stimulated NBT test, only Frankfurt strain of the RHD virus showed changes in the form of a drop at 4, 8, 12, 24, 36 h, while as regards the stimulation index, changes in the form of growth at 24 h after infection were recorded for 3 strains: Asturias, Rainham and BS89 of the RHD virus. WAMG coefficient in the spontaneous NBT test was characterised with a drop for Asturias strain at 4 h, for Frankfurt strain at 4, 8, 12, 24, 36 h, while for BS89 strain at 12 h. In turn, WAMG values of the stimulated NBT test were recorded as growth for Rainham strain (36 h) and drop for Asturias (4, 8, 24 h), Frankfurt (4, 8, 24, 36 h) and BS89 (12 h) strains.

Phylogenetic analysis based on the comparison of the sequence of N-terminal region of the gene encoding VP60 structural protein showed that the 4 studied strains of the RHD virus (Asturias, Bs89, Frankfurt, Rainham) formed two genetic groups. The first group comprised English Rainham strain (1993) and German Frankfurt strain (1996), while the other – Spanish Asturias (2000) and Italian Bs89 (1989) (Fig. 1).

Discussion

When analysing the results in the area of non-specific cellular immunity for the presently studied four strains of the RHD virus (BS89, Rainham, Asturias, Frankfurt), it must be stated that the results can be compared to the previous results obtained in this area on 13 strains of the RHD virus, including

Table 2. Indices of non-specific cellular immunity in rabbits experimentally infected with Rainham strain of RHD virus

	Parameters		Values of parameters in 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)
	Adherence capacity (%)	$\bar{x}$	38.12	39.19	40.58	40.26	28.88	46.03*	30.46	39.01	29.96	43.10*	13.40	34.45*
		SD ±	6.65	2.55	6.33	4.25	4.93	4.17	4.58	2.74	4.04	2.98	0.00	3.21
Absorption capacity	absorption index (l.b.)	$\bar{x}$	4.50	4.63	3.77	4.55	3.90	4.98	4.38	4.67	4.13	4.41	5.32	4.62
		SD ±	0.46	0.45	0.49	0.43	0.58	0.48	0.55	0.43	0.72	0.43	0.00	0.42
	% of absorbing cells	$\bar{x}$	59.20	67.89	64.00	70.20	69.20	66.26	68.80	68.70	70.00	68.09	64.00	62.25
		SD ±	3.03	4.86	3.46	3.89	3.63	3.91	6.87	4.00	6.78	3.09	0.00	4.22
NBT reduction test	spectrophotometeric (10^9/l)	$\bar{x}$	5.34	4.13	2.76	4.42	3.91	4.99	2.64	5.60*	4.82	4.95	5.83	4.91
		SD ±	0.62	0.88	0.67	0.77	0.57	1.31	0.48	1.16	0.69	0.75	0.00	0.51
	spontaneous (l.b.)	$\bar{x}$	9.40	10.11	11.00	10.81	10.00	9.93	8.40	9.81	6.20	8.81*	6.00	8.22*
		SD ±	0.55	0.96	0.71	1.18	0.71	100	0.55	0.89	0.45	0.86	0.00	0.65
	stimulated (l.b.)	$\bar{x}$	28.20	21.11	23.60	20.20	21.00	19.87	20.80	18.85	20.80	18.09	20.00	16.38
		SD ±	1.30	1.78	1.67	1.72	0.71	1.69	0.84	1.97	0.84	2.16	0.00	0.61
	stimulation index (l.b.)	$\bar{x}$	3.00	2.07	2.14	2.05	2.10	2.09	2.46	1.88	3.34*	2.02	2.30	2.11
		SD ±	0.14	0.30	0.25	0.47	0.19	0.33	0.21	0.20	0.23	0.25	0.00	0.18
WAMG	spontaneous (l.b.)	$\bar{x}$	0.50	0.33	0.26	0.29	0.30	0.27	0.30	0.28	0.17	0.25	0.40	0.34
		SD ±	0.06	0.06	0.06	0.07	0.10	0.06	0.02	0.08	0.09	0.06	0.00	0.05
	stimulated (l.b.)	$\bar{x}$	0.50	0.63	0.55	0.52	0.63	0.53	0.74	0.53	0.59	0.50	1.33*	0.69
		SD ±	0.16	0.06	0.08	0.04	0.19	0.05	0.10	0.05	0.32	0.06	0.00	0.05

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

2 French (Fr-1, Fr-2) [4], 4 Czech (CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-559) [3] and 7 Polish (SGM, MAŁ, Kr-1, K-1, KGM, BLA, PD) strains [1, 5, 22, 23]. When discussing the results in the area of adherence capacity, it can be concluded that growth in this coefficient at 12, 24 h after rabbit infection with Asturias strain of the RHD virus is partly comparable with growth falling at 36, 52 h after rabbit infection with MAŁ strain [1]. In turn, the recorded growth falling at single hours of the study for Frankfurt – 36 h and BS89 strains – 4 h, is not comparable to previous results recorded on French (Fr-1, Fr-2) [1], Czech CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-559) [3] and Polish (SGM, MAŁ, Kr-1, K-1, KGM, BLA, PD) strains [1, 5, 22, 23]. However, the recorded drop in this parameter for Rainham strain of the RHD virus at 8, 24, 36 h is almost analogical to results obtained for CAMP V-351 strain of the RHD virus at 12, 48, 56, 72 h from infection [3], while the obtained drop at 24 h for BS89 strain of the RHD is comparable to the result observed for CAMP V-562 strain [3]. In the case of absorption index, it must be stated that the present study is not comparable to previous results, as they are longer lasting than in the case of previously tested strains of the RHD virus – CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558 [3], SGM, MAŁ [1], K-1 [5], KGM [23]. As regards the percentage of absorbing cells, it can be concluded that the growth in this factor recorded at 8, 12 h for Frankfurt strains of the RHD virus is partly identical as growth obtained for MAŁ strain of the RHD [1] – growth at 4, 60 h, although the lesions fall onto different times during the infection. The drop in percentage value of absorbing cells falling at 8, 12, 24 h from infection for Asturias strain of the RHD can be considered analogical to the one obtained for KGM strain, which took place at 8, 56, 60 h of the study [22]. In turn, a single lesion in the form of drop at 4 h from infection with BS89 strain of the RHD is not reflected in previous studies for Fr-1, Fr-2 [1], CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-559 [3], SGM, MAŁ, Kr-1, K-1, KGM, BLA, PD [1, 5, 22, 23]. The results in the area of spectrophotometric NBT test, which in the present study manifested in all four analysed strains (Asturias, Frankfurt, Rainham, BS89) of the RHD virus exclusively with the drop in value, are close to the results obtained previously for strains

Table 3. Indices of non-specific cellular immunity in rabbits experimentally infected with Asturias strain of RHD virus

	Parameters		Values of parameters in hours									
			0		4		8		12		24	
			Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (10)	K (5)	Z (9)	K (5)
	Adherence capacity (%)	$\bar{x}$	35.01	39.19	37.58	40.26	40.07	46.03	62.49*	39.01	57.62*	43.10
		SD ±	6.92	2.55	5.20	4.25	9.05	4.17	11.17	2.74	8.07	2.98
Absorption capacity	absorption index (l.b.)	$\bar{x}$	4.73	4.63	2.31*	4.55	1.39*	4.98	1.20*	4.67	1.39*	4.41
		SD ±	0.32	0.45	0.44	0.43	0.21	0.48	0.16	0.43	0.16	0.43
	% of absorbing cells	$\bar{x}$	74.80	67.89	62.40	70.20	52.80	66.26*	55.60	68.70*	40.00	68.09*
		SD ±	5.59	4.86	4.66	3.89	3.97	3.91	5.37	4.00	2.82	3.09
NBT reduction test	spectrophotometeric (10^9/l)	$\bar{x}$	2.68	4.13	1.40	4.42*	2.50	4.99*	4.22	5.60*	3.50	4.95
		SD ±	0.46	0.88	0.58	0.77	0.87	1.31	1.73	1.16	1.21	0.75
	spontaneous (l.b.)	$\bar{x}$	5.80	10.11	5.00	10.81*	3.80	9.93*	5.60	9.81*	2.75	8.81*
		SD ±	0.86	0.96	0.70	1.18	0.66	1.00	0.51	0.89	0.43	0.86
	stimulated (l.b.)	$\bar{x}$	12.40	21.11	9.60	20.20	6.60	19.87	8.40	18.85	7.50	18.09
		SD ±	1.82	1.78	1.67	1.72	1.03	1.69	0.51	1.97	0.93	2.16
	stimulation index (l.b.)	$\bar{x}$	1.70	2.07	1.88	2.05	1.77	2.09	1.88	1.83	3.10*	2.02
		SD ±	0.33	0.30	0.12	0.47	0.37	0.33	0.20	0.09	0.70	0.25
WAMG	spontaneous (l.b.)	$\bar{x}$	0.33	0.33	0.14	0.29*	0.15	0.27	0.26	0.28	0.11	0.25
		SD ±	0.10	0.06	0.09	0.07	0.05	0.06	0.05	0.08	0.01	0.06
	stimulated (l.b.)	$\bar{x}$	0.57	0.63	0.29	0.52*	0.23	0.53*	0.33	0.53	0.31	0.50*
		SD ±	0.08	0.06	0.08	0.04	0.08	0.05	0.13	0.05	0.15	0.06

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

CAMP V-561, CAMP V-562 [3] and PD [23]. Recorded results of spontaneous NBT test only manifested with drops in the case of three out of four presently analysed strains (apart from BS89 strain) can hardly be referred to prior studies, as the results obtained for Fr-1 [1], CAMP V-351, CAMP V-561, CAMP V-558 [3], SGM, MAŁ [1], K-1 [5], KGM [22], PD [23] strains were both in the form of growth and drop of the coefficient. As regards stimulated NBT test, changes were registered in the form of drop exclusively in the case of Frankfurt strain of the RHD virus at 4, 8, 12, 24, 36 h from infection, and no changes for other three strains, so the results are similar as previously obtained for CAMP V-558 strain of the RHD virus [3]. In turn, stimulation index, manifested with growth at 24 h for Asturias, Rainham and BS89 strains, can be compared to the image of changes obtained for CAMP V-561 strain (growth at 4 h) [3], CAMP V-558 (growth at 52 h) [3] and SGM (growth at 48 h) [1]. No changes for Frankfurt strain of the RHD as regards stimulation index, which is analogical to the results for CAMP V562 [3] and PD [23] strains. However, as regards spontaneous WAMG test, drop falling at single hours of the experiment for Asturias (4 h) and BS89 (12 h) strains is analogical to the results obtained for CAMP V-562 (52 h) [3] strain, while the drop obtained at 4, 8, 12, 24, 36 h from infection for Frankfurt strain of the RHD is similar to changes recorded for Fr-2 [1], CAMP V-351 [3] and CAMP V-561 [3] strains. When analysing the values obtained for stimulated WAMG in the form of growth at 36 h for Rainham strain of the RHD, this can be compared to growth at 56 h for MAŁ strain of the RHD [1], while the drop falling at 4, 8, 24 h for Asturias strain and at 4, 8, 24, 36 h for Frankfurt strain of the RHD is similar to the results obtained for CAMP V-561 strain of the RHD virus [3]. The obtained single drop at 12 h for BS89 strain is confirmed by results for CAMP V-558 strain of the RHD [3].

When analysing the results of genetic studies, it must be concluded that two genetic groups created by 4 analysed strains (Asturias, Bs89, Frankfurt, Rainham) may be a result of the isolation time of such strains, although this can only be referred to group I comprising Rainham strain from 1993 and Frankfurt strain from 1996. This explanation is conformant to the present trend that genogroup creation is a result of the time of strain identification, and not the place of origin

Table 4. Indices of non-specific cellular immunity in rabbits experimentally infected with Frankfurt strain of RHD virus

	Parameters		Values of parameters in 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 (8)	K (5)
	Adherence capacity (%)	$\bar{x}$	36.57	39.19	37.66	40.26	41.43	46.03	35.04	39.01	49.34	43.10	47.61*	34.45
		SD ±	3.26	2.55	5.16	4.25	2.77	4.17	3.75	2.74	3.80	2.98	4.48	3.21
Absorption capacity	absorption index (l.b)	$\bar{x}$	7.33	4.63	8.61	4.55	7.22	4.98	5.95	4.67	4.52	4.41	4.44	4.62
		SD ±	0.62	0.45	0.49	0.43	0.59	0.48	0.74	0.43	0.77	0.43	0.51	0.42
	% of absorbing cells	$\bar{x}$	89.50	67.89	87.00	70.20	80.50*	66.26	80.50*	68.70	70.00	68.09	78.00	62.25
		SD ±	1.00	4.86	6.22	3.89	2.52	3.91	6.81	4.00	8.62	3.09	0.00	4.22
NBT reduction test	spectrophotometeric (10^9/l)	$\bar{x}$	7.79	4.13	6.74	4.42	8.62	4.99	2.23	5.60*	2.62	4.95*	3.53	4.91
		SD ±	2.25	0.88	3.08	0.77	3.76	1.31	0.51	1.16	0.71	0.75	0.36	0.51
	spontaneous (l.b)	$\bar{x}$	10.00	10.11	6.75	10.81*	6.00	9.93*	5.00	9.81*	4.50	8.81*	3.50	8.22*
		SD ±	1.63	0.96	0.63	1.18	0.82	1.00	0.71	1.89	0.58	0.86	0.71	0.65
	stimulated (l.b)	$\bar{x}$	16.50	21.11	13.75	20.20*	13.75	19.87*	13.00	18.85*	11.00	18.09*	10.00	16.38*
		SD ±	1.29	1.78	1.71	1.72	1.26	1.69	2.16	1.97	0.82	2.16	0.00	0.61
	stimulation index (l.b)	$\bar{x}$	1.67	2.07	2.07	2.05	2.31	2.09	2.66	1.88	2.49	2.02	2.92	2.11
		SD ±	0.16	0.30	0.23	0.47	0.22	0.33	0.30	0.20	0.47	0.25	0.59	0.18
WAMG	spontaneous (l.b)	$\bar{x}$	0.20	0.33	0.12	0.29*	0.13	0.27*	0.12	0.28*	0.18	0.25*	0.08	0.34*
		SD ±	0.09	0.06	0.04	0.07	0.02	0.06	0.10	0.08	0.02	0.66	0.01	0.05
	stimulated (l.b)	$\bar{x}$	0.33	0.63	0.25	0.52*	0.29	0.53*	0.39	0.53	0.20	0.50*	0.22	0.69*
		SD ±	0.15	0.06	0.06	0.04	0.04	0.05	0.20	0.05	0.06	0.06	0.03	0.05

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

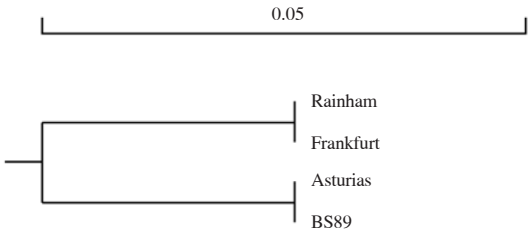

Fig. 1. Phylogenetic tree of 4 strains of RHD virus based on N-terminal fragment of gene coding VP60 structural protein

[6, 8, 12]. Analogical formation of strains into genogroups, conditioned by the time of strain identification, was obtained when comparing Czech [3] and Hungarian [10] strains. It is, however, hard to explain the present results leading to grouping of the Italian Bs89 strain from 1989 with Spanish Asturias from 2000, as this cannot be explained with either isolation time or origin, as previously recorded by Gall et al. [6] and Nowotny et al. [9] when analysing European strains. Perhaps this is linked to the fact that 'younger' strains, such as in this case Asturias strain from 2000, evolved from 'older' strains identified in the first years after the occurrence of the disease.

To conclude, it must be stated that the results of the study have proved that 4 analysed strains (BS89, Rainham, Asturias, Frankfurt) can be divided into two immunogroups: with high immunogenicity – non-haemagglutinogenic Frankfurt and Asturias strains, and with low immunogenicity – haemagglutinogenic BS89 strain and non-haemagglutinogenic Rainham strain. In turn, the genetic analysis of such strains also 'divided' them into two genetic group – one with non-haemagglutinogenic Asturias strain and haemagglutinogenic BS89 strain, and the other with non-haemagglutinogenic Frankfurt and Rainham strains. To conclude, it can be stated that 4 analysed strains of the RHD virus, despite creating 2 immunogroups and 2 genogroups, are of differing composition, which suggests that strain similarity in the genetic aspect does not go in line with their immunogenicity.

Acknowledgements

Paper financed from reseach grant of the Minister of Science and Upper Education no. N308 03832/3662.

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