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Original article

Lymphocyte subpopulations and apoptosis of immune cells in rabbits experimentally infected with a strain of the RHD virus having a variable haemagglutination capacity

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Abstract

The paper describes the immunological response in the matter of percentage of T cells (receptor CD5+) and subpopulations (Th with receptor CD4+, Tc/Ts with receptor CD8+, T with receptor CD25+) and B cells with receptor CD19+, as well as the percentage of apoptotic granulocytes and lymphocytes, in rabbits experimentally infected with the Hagenow strain of the RHD virus. The material chosen for the experiment is special, as among all strains of RHD virus, there are only two strains which carry the variable haemagglutination capacity of red cells. The results of the study show that the Hagenow strain gives an untypical picture of T and B lymphocytes, whereas the results in inducing apoptosis seems to correspond with previous data, confirming the inclusion of apoptosis from 4 h p.i. and the intensity of the phenomenon being higher in granulocytes.

Key words: RHD, haemagglutination, lymphocytes subpopulation, granulocytes, apoptosis

Introduction

The RHD virus (rabbit haemorrhagic disease) in the applicable virological systematic is considered a homogenous virus (Annon 2011). In fact, its differentiation was evidenced in respect of such properties as haemagglutination capacity, properties resulting from differences in genetic code (mutations) (Niedźwiedzka 2008), as well as a capacity for creating antigenic variants (Niedźwiedzka 2008, Tokarz-

-Deptuła 2009). It is worth stressing that the haemagglutination capacity of red cells, related to the pathogenicity of strains of the RHD virus, is considered a property held by all strains of the virus. Nevertheless there are 5 strains of the RHDV (BLA, Rainham, Asturias, Frankfurt, whn-1) which have been described as non-haemagglutinating and moreover, there are 2 non-haemagglutinating strains that are antigenic variants (Pv97, 9905 RHDVa) and 2 strains of the RHD virus that are characterised with variable

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haemagglutination capacity, namely ZD and Hagenow strains (Niedźwiedzka 2008, Tokarz-Deptuła 2009).

Apart from a few Chinese studies carried out using the rosette method, with no strain characteristics (Fradelizi 1973, Chou 1977, Deng et al. 1989), there were studies determining 15 strains with haemagglutination capacity, including 12 haemagglutinating strains (Fr-1, Fr-2, SGM, MAŁ, KGM, PD, GSK, Kr-1, CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558) and 3 haemagglutinating antigenic variants of the RHDVa virus (Vt97, Triptis, Hartmannsdorf) and 4 non-haemagglutinating strains (BLA, Rainham, Frankfurt, Asturias) and 2 non-haemagglutinating RHDVa (Pv97, 9905 RHDVa), as well as 1 strain indicating variable haemagglutination capacity, ZD of the RHD virus (Tokarz-Deptuła and Deptuła 2003, Hukowska-Szematowicz et al. 2005, Hukowska-Szematowicz 2006, Hukowska-Szematowicz and Deptuła 2008ab, Niedźwiedzka 2008, Tokarz-Deptuła 2009, Niedźwiedzka-Rystwej and Deptuła 2010a). Results achieved so far as regards the number of T and B cells and their subpopulations indicate that the haemagglutination property in the typical strains of the RHD virus studied and antigenic variants of this virus (RHDVa) did not clearly affect the image caused in the area of such indices (Tokarz-Deptuła and Deptuła 2003, Hukowska-Szematowicz et al. 2005, Hukowska-Szematowicz 2006, Hukowska-Szematowicz and Deptuła 2008ab, Niedźwiedzka 2008, Tokarz-Deptuła 2009, Niedźwiedzka-Rystwej and Deptuła 2010a). The studied strain with variable haemagglutination capacity – ZD, in the area of T and B cells and their subpopulations (Th CD4+, Tc/Ts CD8+, T CD25+ and B CD19+), only indicated increases in the parameters studied, which were the most intensive in the area of Th lymphocytes with receptor CD4+ (Tokarz-Deptuła 2009).

In the case of apoptosis, studies in this area carried out by only five authors (Alonso et al. 1998, Jung et al. 2000, San-Miguel et al. 2006, Ni et al. 2009, Garcia-Lastra et al. 2010) referred exclusively to the assessment of apoptosis with histopathologic methods and the activity of caspase 3 in tissues (kidneys, lungs, liver, heart, spleen and lymph nodes) and cells (hepatocytes, eosinophils and macrophages) with undetermined strains of the RHD virus (Alonso et al. 1998, Jung et al. 2000, Ni et al. 2009, Garcia-Lastra et al. 2010) and with haemagglutinating strain AST/89 of the RHD virus (San-Miguel et al. 2006). Our own studies regarding the phenomenon of apoptosis of lymphocytes and granulocytes in peripheral blood of rabbits infected with the RHD virus, were carried out for 10 strains of the RHD virus, including 1 haema-

gglutinating strain, BS89, and 3 haemagglutinating variants, Vt97, Triptis and Hartmannsdorf, and for 3 non-haemagglutinating strains, Rainham, Frankfurt, Asturias, and 2 non-haemagglutinating variants, Pv97 and 9905 of RHDVa. The results of the studies revealed that the phenomenon occurred for all studied strains of the RHD virus between 4 and 36 h after rabbit infection, and was more intensive in granulocytes than lymphocytes (Niedźwiedzka 2008).

The purpose of the study was to analyse the volume of T cells (receptor CD5+) and subpopulations (Th with receptor CD4+, Tc/Ts with receptor CD8+, T with receptor CD25+) and B cells with receptor CD19+, as well as the percentage of apoptotic granulocytes and lymphocytes, in rabbits experimentally infected with the Hagenow strain of the RHD virus, which indicates the variable haemagglutination capacity of red cells, and which had not previously been studied in this respect.

Materials and Methods

The study was performed on 20 mixed-race rabbits of both sexes, weighting in the range of 3.20-4.20 kg, marked as conventional animals, coming from a licensed breeding farm (Annon 1987). During the experiment, the animals were kept at the vivarium, where zootechnical parameters were conformant to the standards recommended in Poland (Annon 2006). After transportation to the vivarium, the animals were subjected to a two-week adaptation period and were fed with full-portion rabbit feed at a quantity of 0.15-0.20 kg/day and had unlimited access to water. The rabbits used for the study were divided into a group of studied animals and a group of control animals (10 animals in each group). The animals in the infected groups were administered intramuscularly (muscles of the lower limb) the Hagenow strain of the RHD virus, which is a strain of variable haemagglutination capacity, suspended in 1 ml glycerol, while rabbits in control groups analogically received 1 ml glycerol. The Hagenow strain of the RHD virus obtained in Germany in 1990 reveals variable haemagglutination capacity, as the lack of it was observed in the case of this strain at a temperature of 4°C, whereas a positive result was obtained for this strain at room temperature (Schirrmeier et al. 1999). The strain of the RHD virus used for rabbit infection originated from naturally dead animals, and after being obtained from a naturally deceased rabbit and prepared in the form of liver homogenate, was used for experimental infection of rabbits from which, after death, the liver was sampled, which was then used for infection of the animals studied, by administering the liver tissue to

them in the form of 20% homogenate cleared by centrifugation at 3000 rpm, 10% chloroforming for 60 minutes and centrifugation again, and then suspension in glycerol at a 1:1 proportion (Niedźwiedzka-Rystwej and Deptuła 2010b). All the antigens of the RHD virus prepared had the same number of particles with a density of 1.31-1.34 g/dm³.

In the blood of rabbits, collected from the peripheral vein of the rabbit ear at 0, 4, 8, 12, 24 and 36 h from administration of the analysed strain, the percentage of T (CD5+), Th (CD4+), Tc/Ts (CD8+) lymphocytes, and lymphocytes with CD25+ receptor, as well as B (with CD19+ receptor) lymphocytes was determined, according to the method described by Deptuła et al. (Deptuła et al. 1998) using monoclonal antibodies (mouse anti-rabbit) (Serotec, USA). The samples tested were incubated for 45 minutes in ice, rinsed three times with Cell Wash (BD Biosciences, USA) by centrifugation 200 xg. 10 µl of rabbit antibodies were added to the cellular sediment, marked against mouse IgG with fluorescein isothiocyanate (FITC). After three rinses in Cell Wash, 2000 µl of lysing liquid removing erythrocytes (BD FACS Lysing Solution, BD Biosciences, USA) were added to the samples. After 10 minutes of incubation in the dark, at room temperature, measurements were made on a FACScan flow cytometer by Becton Dickinson (USA) using FACSDiva software.

Assessment of apoptosis of lymphocytes and granulocytes was also made on the FACScan flow cytometer by Becton Dickinson with FACSDiva software (USA), using the set of reagents from a FAM Caspase Activity Kit (Imgenex Corp.) to detect general activity of the caspase pool: 1, 3, 4, 5, 6, 7, 8 and 9, where the study was performed according to the protocol provided by the manufacturer. Full blood was subjected to the action of BD Lysing Solution (Becton Dickinson, USA) and single rinsing with PBS to eliminate erythrocytes. Cells at a quantity of 300 µl, 10 µl of 30X FLICA reactant were added to the prepared suspension and incubated for 1 hour in darkness with air saturation with 5% CO₂ at 37°C, stirring every 20 minutes. After that time, 2 ml of 1X of washing buffer was added to the suspension. Samples were stirred, and then centrifuged at 400 x g for 5 minutes. The supernatant was removed, and the cells were suspended in 1 ml of 1x of the washing buffer, and the rinsing phase was then repeated. After the process of washing, 400 µl of 1X washing buffer was added to the cells, and incubated for 1 hour in darkness, in ice, and 2 µl PI (propidium iodide) were then added, after which the caspase activity of the coloured cells was measured using the flow cytometer.

Virusological studies, performed as control of animal condition, were carried out using the real-time

PCR method using a Light Cycler (Roche Diagnostics, Germany) on the liver cells of infected rabbits, after their death, and consisted of checking for the presence of the RHD virus in this organ, and thus evidence of animal infection. In phase one, rabbit liver, obtained *post-mortem*, was used for preparation of 20% homogenisate suspended in Trizol (Roche Diagnostics, Germany). RNA of the RHD virus was then isolated using commercial kits by A&A Biotechnology (Poland), according to the manufacturer's instructions. A complementary strand of nucleic acid (cDNA) was then obtained in reaction of reverse transcription on the matrix of viral RNA using 0.5 µl M-MLV Reverse Transcriptase enzyme (Invitrogen, USA). In this reaction, the following was used: 1 µl specific downstream starter with a concentration of 100 µM (TIB MOLBIOL, Germany), dNTPs (mix of dATP, DTP, DTP, dTTP) with a concentration of 25 mM each (Promega, USA), 0.5 µl inhibitor of RNA-se with a concentration of 40 u/µl (Promega, USA), 0.25 µl starter Oligo (dT)₁₈ with a concentration of 100 µM, 13.5 µl of water for molecular biology DEPC (Eppendorf, Germany), 2 µl 5x buffer for RT-PCR (Invitrogen, USA), 0.25 µl DTT with a concentration of 100 µM and 5 µl of viral RNA. cDNA obtained after reverse transcription was amplified using the real-time PCR technique with the use of intercalating fluorescent colourant SYBR[®]Green I using the LC FastStart DNA Master SYBR Green I reagents kit (Roche Diagnostics, Germany). The amplification reaction was carried out in a mix containing 2 µl of cDNA obtained after RT, added to 18 µl of reaction mix (2 µl FastStart DNA Master SYBR Green I, after 2 µl of relevant primers, 3.2 µl of MgCl₂ with a concentration of 5 mM and 8.8 µl water for molecular biology DEPC). Primers were designed in such a way so as to allow for identification of all ten studied strains of the RHD virus, and were designed on the basis of available information on ten analysed strains of the RHD virus sampled from the Gene Bank by TIB MOLBIOL, Germany – primer S with the sequence AAATAgTgggACTKCAACCAgTACCT with T_m=58.8°C and primer R with the sequence ggAgATRgggTTgTCRAYTgCAGAC with T_m=59.9°C. The reaction was carried out according to the following scheme:

I – pre-incubation phase 95°C for 10 minutes

II – amplification phase (45 cycles)

– denaturation 95°C for 10 seconds

– annealing 62°C for 10 seconds

– extension 72°C for 7 seconds

III – melting phase

– denaturation 95°C for 0 seconds

– annealing 65°C for 15 seconds

– melting 95°C for 0 seconds

IV – cooling phase 40°C for 30 seconds

Table 1. Lymphocytes T and B, their subpopulations and mortality of rabbits experimentally infected with the Hagenow strain of RHD virus.

Parameters	Values of parameters 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)	
Lymphocytes T (CD5+) (%)	\bar{x}	56.20	57.24	54.76	56.85	55.12	57.85	56.26	58.11	55.98	61.14*	55.07	57.82
	SD±	5.45	1.49	3.67	1.54	2.16	1.50	2.57	1.57	2.54	2.04	1.62	1.98
Lymphocytes Th (CD4+) (%)	\bar{x}	42.62	43.29	42.32	42.81	39.74	45.71*	38.88	43.56	38.26	47.19*	38.57	41.98
	SD±	2.41	1.54	3.21	1.32	3.00	1.97	3.59	2.06	3.45	2.59	3.50	1.16
Lymphocytes Tc/Ts (CD8+) (%)	\bar{x}	16.90	17.10	17.38	16.86	15.98	15.03	16.90	14.92	18.16	15.68	16.27	18.21
	SD±	1.02	1.05	0.73	0.97	0.57	0.72	0.96	1.03	1.12	0.95	1.02	0.33
Lymphocytes with CD25+ receptor (%)	\bar{x}	24.90	23.77	14.98	20.89*	16.54	23.94*	17.18	24.35*	19.30	24.64*	25.23	22.65
	SD±	1.13	1.21	1.13	1.09	1.28	2.05	1.89	1.94	1.50	1.22	2.93	1.43
Lymphocytes B (CD19+) (%)	\bar{x}	21.96	21.96	24.64	20.57	22.20	21.56	21.46	20.56	22.04	21.93	21.97	23.48
	SD±	2.80	1.03	2.95	1.71	2.76	1.38	1.75	1.56	2.08	1.32	3.00	1.84

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

Table 2. The apoptosis of granulocytes and lymphocytes in rabbits infected with the Hagenow strain of RHD virus.

Parameters	Values of parameters 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)	
The percentage of apoptotic granulocytes	\bar{x}	5.14	4.47	7.59*	4.15	7.11*	4.79	8.05*	4.99	10.95*	5.03	9.26*	4.27
	SD±	0.82	0.28	0.52	0.29	0.86	0.47	1.78	0.67	2.38	0.30	2.10	0.14
The percentage of apoptotic lymphocytes	\bar{x}	43.98	41.19	45.80*	38.57	46.22*	40.65	47.52	42.53	46.61	43.24	47.67	48.93
	SD±	2.90	2.30	4.11	2.69	2.86	2.25	2.07	1.91	2.86	2.63	4.07	2.41

Legend: \bar{x} – mean value; SD± – standard deviation; Z – infected animals, K – control animals; () – number of animals, * – statistically significant change

Virusological studies using the serological method were carried out in all animals in the infected and control groups before commencement of the experiment in order to detect the presence of antibodies to the RHD virus using ELISA test commercial kits manufactured by Instituto Zooprofilattico Sperimentale Brescia, Italy.

The results of the immunological studies were subjected to statistical analyses using the t-Student test with $p=0.05$, and presented as statistically significant values in Tables 1-2.

Results

Analysis of the results in the area of the percentage of T cells with receptor CD5+ and their subpopulations (Th CD4+, Tc/Ts CD8+, T CD25+)

and B cells with receptor CD19+ (Table 1) indicates that the results regarding this strain of the RHD virus are different from the strains of the RHD virus previously studied, including the ZD strain with variable haemagglutination capacity. As regards the percentage of T cells with CD5+ receptor, a decrease in this ratio was observed for the Hagenow strain of the RHD virus at 24 h from infection. In the case of Th lymphocytes with CD4+ receptor, the decrease was recorded at 8 and 24 h from virus administration, whereas in the case of the percentage of T cells with CD25+ receptor, decreases were visible at 4, 8, 12 and 24 h from infection. For the percentage of Tc/Ts lymphocytes with CD8+ receptor and B cells with CD19+ receptor, no changes were recorded.

In the case of the percentage of apoptotic granulocytes and lymphocytes (Table 2) in rabbits infected with the Hagenow strain of the RHD virus, it

must be concluded that the strain caused apoptosis from 4 h from animal infection, and the process lasted until their death at 36 h, and was more intensive in reference to granulocytes.

Virusological studies carried out in blood serum before the commencement of the experiment did not reveal the presence of anti-RHDV antibodies, whereas virusological studies using the real-time PCR method performed on liver tissue sampled *post mortem* from animals infected during the study confirmed the presence of a copy of the RHD virus in this tissue.

Discussion

The results of the study as regards the percentage of T cells with CD5+ receptor and their subpopulations, as well as B cells with receptor CD19+, can be compared with the studies on 15 strains with haemagglutination capacity, including 12 haemagglutinating strains Fr-1, Fr-2, SGM, MAŁ, KGM, PD, GSK, Kr-1, CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558, and 3 haemagglutinating antigenic variants Vt97, Triptis, Hartmannsdorf, 4 non-haemagglutinating strains (BLA, Rainham, Frankfurt, Asturias), and 2 non-haemagglutinating RHDVa (Pv97, 9905 RHDVa), and to 1 strain indicating variable haemagglutination capacity ZD of the RHD virus (Tokarz-Deptuła and Deptuła 2003, Hukowska-Szematowicz et al. 2005, Hukowska-Szematowicz and Deptuła 2008ab, Niedźwiedzka 2008, Tokarz-Deptuła 2009, Niedźwiedzka-Rystwej and Deptuła 2010a).

Analysis of the results for the percentage of T cells with receptor CD5+ on the Hagenow strain of the RHD virus with a decrease at 24 h post infection, indicates that the ZD strain did not show a decrease in this respect. Moreover, an identical result to the present studies was obtained for the haemagglutinating KGM strain of the RHD virus (Tokarz-Deptuła 2009), and non-haemagglutinating Asturias strain of the RHD virus (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2010a). A similar change falling at 12 h p.i. was recorded for the non-haemagglutinating variant 9905 RHDVa (Niedźwiedzka-Rystwej and Deptuła 2010a). Analysis of the percentage of Th lymphocytes with receptor CD4+, where the Hagenow strain of the RHD virus indicated a decrease at 8, 24 h after virus administration, indicates that the ZD strain of the RHD virus with variable haemagglutination capacity did not confirm the image obtained for the Hagenow strain of the RHD virus (Tokarz-Deptuła 2009). Furthermore, in the area of haemagglutinating strains, similar results were obtained for the KGM strain (decrease at 24 h

(Tokarz-Deptuła 2009) and for haemagglutinating antigenic variant Vt97 (decrease at 24, 36 h) (Niedźwiedzka 2008), while for non-haemagglutinating strains, similar values were recorded for the Rainham strain (decrease at 12,24,36 h) (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2010a) and Asturias strain (decrease at 12, 24 h) of the RHD virus (Niedźwiedzka 2008), as well as for non-haemagglutinating variant 9905 RHDVa of the RHD virus (Niedźwiedzka 2008).

The lack of changes recorded in the case of the percentage of Tc/Ts lymphocytes with receptor CD8+ is not confirmed by the results obtained for the ZD strain of the RHD virus, which indicated changes in this respect in the form of an increase at 48 and 52 h after virus administration (Tokarz-Deptuła 2009). The result obtained in the present study confirms the data obtained for haemagglutinating strain CAMP V-351 (Hukowska-Szematowicz et al. 2005, Hukowska-Szematowicz 2006, Hukowska-Szematowicz and Deptuła 2008ab), CAMP V-562 (6,7), CAMP V-558 (Hukowska-Szematowicz 2006, Hukowska-Szematowicz and Deptuła 2008a), BS89 (Niedźwiedzka 2008) and haemagglutinating variant Hartmannsdorf of the RHD virus (Niedźwiedzka 2008) and for the non-haemagglutinating Frankfurt strain of the RHD virus (Niedźwiedzka 2008). In the case of the percentage of T cells with receptor CD25+, the decrease recorded in the present study at 4, 8, 12, 14 h for the Hagenow strain of the RHD virus is not identical with results obtained for the ZD strain of the RHD virus, and is only slightly similar to haemagglutinating strain PD of the RHD virus, where changes were noted at 4, 8 and 12 h from virus administration (Tokarz-Deptuła 2009). However, the results obtained are not confirmed for non-haemagglutinating strains. In the case of lack of changes to the percentage of B cells with receptor CD19+ for Hagenow strain of the RHD virus, there is a similar lack of changes in this respect indicated by the strain with variable haemagglutination capacity, ZD of the RHD virus (Tokarz-Deptuła 2009), and identical results were obtained for haemagglutinating strain CAMP V-561 (Hukowska-Szematowicz 2006, Hukowska-Szematowicz and Deptuła 2008ab), CAMP V-558 (Hukowska-Szematowicz 2006, Hukowska-Szematowicz and Deptuła 2008a) and haemagglutinating variants Vt97 and Hartmannsdorf of the RHD virus (Niedźwiedzka 2008), as well as for the non-haemagglutinating Asturias strain (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2010a) and non-haemagglutinating variant 9905 RHDVa (Niedźwiedzka 2008).

Changes regarding apoptosis in granulocytes and lymphocytes in peripheral blood of rabbits infected

with the Hagenow strain of the RHD virus are similar to the results obtained for 1 haemagglutinating BS89 strain and 3 haemagglutinating variants Vt97, Triptis and Hartmannsdorf, and 3 non-haemagglutinating strains, Rainham, Frankfurt and Asturias, and 2 non-haemagglutinating variants, Pv97 and 9905 of RHDVa (Niedźwiedzka 2008). Since, in the studies on apoptosis, an increase in the percentage of apoptotic granulocytes and lymphocytes was recorded, it can be concluded that this confirms the stimulation of apoptosis phenomenon in immune cells in peripheral blood, which conforms to previous results in this area (Niedźwiedzka 2008).

The absence of anti-RHDV antibodies in rabbits before the commencement of the experiment and the presence of a copy of the RHD virus in liver tissue in rabbits after infection, indicates that the RHD virus is the only cause of animal death.

In conclusion, the Hagenow strain of the RHD virus, as one of two strains with variable capacity for red blood cell haemagglutination, represents an untypical image in the percentage of T and B cells and their subpopulations (T, Th, Tc/Ts, T with receptor CD25+). This dissimilarity is expressed only by decreases in the parameters analysed, which is not recorded for other observed strains of the RHD virus, including those with variable haemagglutination capacity. Moreover, the ZD strain with analogical haemagglutination capacity, causes only increases in the parameters analysed. The image of changes obtained for T cells with receptor CD5+ and their subpopulations (Th with receptor CD4+, Tc/Ts with receptor CD8+, T with receptor CD25+), for the Hagenow strain of the RHD virus is the most similar to the image obtained for the non-haemagglutinating Asturias strain of the RHD virus. The image of changes as regards the percentage of B cells with receptor CD19+, caused by the Hagenow strain, which did not indicate any changes, is similar to the ZD strain – the second among strains with variable haemagglutination capacity. It can therefore be concluded that the picture obtained in the area of the percentage of T cells and their subpopulations (Th, Tc/Ts, T with receptor CD25+) and B cells can suggest suppressive action of the Hagenow strain of the RHD virus. Taking into account the different results obtained for the ZD strain of the RHD virus, it cannot be clearly concluded whether this is caused by a biological property of variable haemagglutination capacity. In the case of apoptosis, the results obtained for the Hagenow strain of the RHD virus are similar to those obtained for all previously studied strains, namely BS89, Vt97, Triptis, Hartmannsdorf, Rainham, Frankfurt, Asturias, Pv97 and 9905, since all of these, as with strain analysed in this study, stimulate the process of apoptosis of

granulocytes and lymphocytes in the peripheral blood of rabbits infected with these strains starting from 4 h to 24-36 h from virus administration, namely until death, which confirms the important role of apoptosis in the course of infection with this virus.

References

- Alonso C, Oviedo JM, Martin-Alonso JM, Diaz E, Boga JA, Parra F (1998) Programmed cell death in the pathogenesis of rabbit hemorrhagic disease. *Arch Virol* 143: 321-332.
- Annon (1987) Information and training materials of the Laboratory Animals Section, General Assembly of the Association of Agriculture Engineers and Technicians, In: *Materiały informacyjno-szkoleniowe Sekcji ds. Zwierząt Laboratoryjnych ZG Stowarzyszenia Inżynierów i Techników Rolnictwa, Warsaw*, pp 26-77 (in Polish).
- Annon (2006) Regulation of the Minister of Agriculture and Rural Development of 10 March 2006 on detailed conditions for maintenance of laboratory animals in experimental units, breeding units and suppliers (*Polish Journal of Laws of 2006, No. 50, item 368*), (in Polish).
- Annon (2011) International Committee on Taxonomy of viruses www.icvtonline.org.com
- Chou CT (1977) Specific surface marker of rabbit B lymphocyte – rabbit bursal equivalent lymphocyte antigen. *Cell Immunol* 28: 334-336.
- Deng RT Xu YF, Yu NX (1989) Dynamic observation of RTLA and RABELA cells in the initial stage post immunisation with RHD vaccine (in Chinese). *Shanghai J Anim Sci Vet Med* 5: 26-28.
- Deptuła W, Kostrzewa A, Stosik M, Tokarz-Deptuła B, Wiktorowicz K (1998) Subpopulations of peripheral blood lymphocytes in rabbits. *Nowiny Lekarskie* 67: 377-382.
- Fradelizi DP (1973) Rabbit T-lymphocyte specific surface marker – rabbit thymus specific antigen (RTLA). *Cell Immunol* 24: 484-486.
- Garcia-Lastra R, San-Miguel B, Crespo I, Jorquera F, Alvarez M, Gonzalez-Gallego J, Tunon M (2010) Signalling pathways involved in liver injury and regeneration in rabbit hemorrhagic disease, an animal model of virally-induced fulminant hepatic failure. *Vet Res* 41: 2.
- Hukowska-Szematowicz B, Tokarz-Deptuła B, Deptuła W (2005) Lymphocytes T and their subpopulations in peripheral blood in rabbits experimentally infected with two strains of VHD virus (viral haemorrhagic disease). *Pol J Environ Stud* 14: 550-555.
- Hukowska-Szematowicz B (2006) Immunological-genetic characteristics of chosen strains of RHD (rabbit haemorrhagic disease) virus. Doctoral thesis, Szczecin, Poland (in Polish).
- Hukowska-Szematowicz B, Deptuła W (2008a) Peripheral blood lymphocytes in rabbits infected with Czech strains, CAMPV-562 and CAMPV-558 of RHD virus. *Centr Eur J Immunol* 33: 8-13.
- Hukowska-Szematowicz B, Deptuła W (2008b) Dynamics of peripheral blood lymphocytes in rabbits experimentally infected with two Czech strains of RHD virus. *Bull Vet Inst Pulawy* 52: 23-29.

- Jung JY, Lee BJ, Tai JH, Park JH, Lee YS (2000) Apoptosis in rabbit haemorrhagic disease. *J Comp Pathol* 123: 135-140.
- Ni Z, Wei W, Liu GQ, Chen L, Yu B, Yun T, Hua JG, Li SM (2009) Studies on the apoptosis of RK13 cells induced by rabbit hemorrhagic disease virus. *Bing Du Xue Bao* 25: 316-317.
- Niedźwiedzka P (2008) Immunological profile and phenomenon of apoptosis in rabbits experimentally infected with strains of RHD virus (rabbit haemorrhagic disease) with different biological features. Doctoral thesis, Szczecin, Poland (in Polish).
- Niedźwiedzka-Rystwej P, Deptuła W (2010a) Cytometric analysis of lymphocytes T and B in rabbits infected with non-haemagglutinogenic strains of RHD virus (rabbit haemorrhagic disease) – Rainham, Frankfurt and Asturias. *Pol J Vet Sci* 13: 157-162.
- Niedźwiedzka-Rystwej P, Deptuła W (2010b) Non-specific immunity in rabbits infected with 10 strains of the rabbit haemorrhagic disease virus with different biological properties. *Centr Europ J Biol* 5: 613-632.
- San-Miguel B, Alvarez M, Culebras JM, Gonzalez-Gallego J, Tunon MJ (2006) N-acetyl-cysteine protects liver from apoptotic death in an animal model of fulminant hepatic failure. *Apoptosis* 11: 1945-1957.
- Schirmeier H, Reimann I, Köllner B, Granzow H (1999) Pathogenic, antigenic and molecular properties of rabbit haemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterization of antigenic variants. *Arch Virol* 144: 419-435.
- Tokarz-Deptuła B, Deptuła W (2003) Dynamic alterations in peripheral blood lymphocytes in rabbits experimentally infected with VHD (viral haemorrhagic disease) virus – Polish strain Kr-1. *Pol J Vet Sci* 6: 67-69.
- Tokarz-Deptuła B (2009) Immunity phenomena in rabbits infected with the RHD (rabbit haemorrhagic disease) virus. *Pol J Env Stud* 7: 1-81.