Selected immunological factors in rabbits infected with four strains (Fr-1, Fr-2, SGM, Mał) of the RHD virus (rabbit haemorrhagic disease)

BEATA TOKARZ-DEPTUŁA[1], TOBIASZ DEPTUŁA[2]

[1]Chair of Microbiology and Immunology, Department of Natural Sciences, University of Szczecin, Poland; [2]Student of Medical Biotechnology, Faculty of Medicine II, Medical University in Poznan, Poland

Abstract

The aim of the experimental study was analyse the immunological response and clinical image, as well as anatomo-pathological lesions, and results of virusological studies in rabbits experimentally infected with selected strains of the RHD virus, originating from various biotopes in Europe.

Key words: *rabbit, the RHD virus, immunity.*

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Introduction

Since 1984, when information came from China about a disease whose etiological factor proved to be the RHD virus from the *Caliciviridae* family, the disease became a plague for wild and breed rabbits of meat and angora races, as well as mixed rabbits, and presently occurs almost in all countries worldwide [quote in 1-3, 17, 18]. The studies performed so far have allowed for defining the structure of the virus, the size of its capsid, physico-chemical properties, protein composition and the type of nucleic acid [quote in 1, 2, 4, 17, 18], as well as for learning about its replication method and the nucleotide sequence in the viral genome [quote in 2, 4]. Furthermore, Chinese scientists [quote in 1, 2, 4, 17, 18] as the only ones reported the possibility of replicating RHDV in cell cultures, which has not been so far repeated elsewhere. In turn, the studies in the area of immunity [1-18] report that immune reactions in rabbits in the first hours from infection with the RHD virus or after administration of specific vaccine, are principally related to non-specific immunity and refer to phagocyte activity of PMN cells, while immune responses in the hours preceding animal death are also related to non-specific humoral immunity and specific cellular immunity. Moreover, during the infection, changes to specific humoral mechanisms are recorded, measured with B lymphocytes activity, which is irregular during the infection with RHDV, manifested in earlier and final hours of the experiment [2, 3, 5, 14-16, 18].

The purpose of the study was to describe the changes to the selected non-specific cellular factors, as well as non-specific and specific humoral factors of immunity in rabbits experimentally infected with four strains (Fr-1,Fr-2,SGM and Mał) of the RHD virus. Also clinical symptoms were recorded, as well as anatomo-pathological (A-P) lesions and results of virusological studies.

Material and Methods

The study was performed on 45 rabbits of mixed breed and both sexes, clinically healthy, with body weight of 2.5-3.5 kg, belonging to conventional animals group CV III [19], in which no anti-RHD antibodies were found in the ELISA test [1]. The animals were infected with two French strains (Fr-1 and Fr-2), and two Polish strains (SGM and Mał), originating from naturally infected dead animals, prepared according to the aforementioned method [1], with haemagglutination titre from 5120 to 10240. The rabbits were divided into 5 groups. Four groups were administered the following: Group I – Fr-1 strain, Group II – Fr-2, Group III – Mał, and Group IV – SGM. Group V acted as control group for the four selected groups of infected animals. Animals were infected in the rear limb muscles with

Correspondence: Beata Tokarz-Deptuła, Department of Microbiology and Immunology, Faculty of Natural Sciences, University of Szczecin, Felczaka 3c, 71-412 Szczecin, Poland. Phone number: +48 91 444 16 05, Fax number: +48 91 444 16 06, Email: kuki001@interia.pl

lyophilisate of each strain of the RHD in the volume of 1ml of suspension (sterile distilled water). Rabbits in Group V were analogically infected with 1 ml of viral suspension. Blood for the tests was drawn from peripheral vein of rabbit ear at hour '0', namely just before administration of the antigen, and at 4, 8, 12, 24, 36, 48, 52, 56, 60 h of the experiment. In the case of animals where clinical symptoms of the disease were recorded, the experiment was finished earlier, as was in the case of their prior death.

In blood, the following were tested: adherence capacity (ZA) of PMN cells, absorption capacity (% of absorptive cells – %kp and absorption index – IP) of the model bacterial strain by such cells, as well as their cidal capacity in the NBT test (cytochemical method – spontaneous and stimulated test, and the spectrophotometric method) [1]. Furthermore, metabolic activity coefficient of granulocytes (WAMG) was calculated for the spontaneous test and stimulated test, as well as stimulation index [1]. Moreover, intracellular killing (TWZ) capacity by PMN cells was analysed in reference to the model bacterial strain, MPO activity in PMN cells, lysozyme (LZM) activity and concentration, as well as quantity of IgG and total Ig in blood serum [1]. Also, clinical changes were recorded, including mortality rate and A-P image of internal organs of dead animals, as well as results of virusological studies in the direct immunofluorescence test (IFb), with which the presence of the virus was detected in samples of internal organs of dead animals, as well as the presence of anti-RHD antibodies in serum was investigated using ELISA test [1].

The results of immunological studies were statistically analysed using *t*-Student test with $p < 0.05$, comparing the values obtained in infected groups with the ones obtained in the control group.

Results and Discussion

The results of immunological studies were described in Table 1, while clinical symptoms (A-P changes in internal organs), and results of virusological studies (IFb and ELISA tests) – in Table 2.

The analysis of the changes recorded (Table 1) evidences that the analysed strains of the RHD virus cause a different immunological response in rabbits, and their different mortality (Table 2). The assessment of the immunological image showed most changes, which maintained for longest, respectively, in animals infected with Fr-2, SGM, Mał and Fr-1 strain. They were more intense in the area of non-specific humoral immunity (MPO activity, LZM concentration and activity) (except for Fr-1 strain) than in the area of non-specific cellular immunity (ZA, IP, %kp, spectrophotometric NBT test, spontaneous and stimulated, stimulation index, spontaneous and stimulated WAMG, and TWZ). Changes to non-specific humoral immunity were manifested both with decrease and increase as regards concentration, and decrease in the area of LZM activity, while as regards MPO activity – principally increase was recorded. In the case of LZM activity, decrease in values was observed for all four analysed strains, whereas for Fr-2 strain the decrease was recorded from 4-56 h of the experiment, while in the case of SGM strain – only at 4 and 24 h, whereas at 36 and 56 h of the experiment increase in this parameter was observed. In turn, as regards LZM concentration, its increase was visible for Fr-2 strain (4-56 h) and Mał strain (8-56 h except for 24 h). In the case of SGM strain, in the observation period exclusively its decrease was recorded (8-60 h). No changes in this parameter were recorded for Fr-1 strain. As regards MPO activity, changes in the form of increase were visible exclusively in the case of Fr-2 strain (4-48 h), while decrease was recorded at 52 h for SGM strain, whereas for the remaining two strains – Fr-1 and Mał – no changes were recorded.

As regards non-specific cellular immunity, most changes occurred in spectrophotometric NBT test, and then in ZA, TWZ and stimulation index, whereas the changes were in the form of increase. The remaining tests (spontaneous and stimulated NBT test, spontaneous and stimulated WAMG) yielded less intense and short-lasting changes, characterised both with decrease and increase in values, except for stimulated WAMG test for Fr-2 strain, which recorded increase from 4 h to 52 h. In the case of spectrophotometric NBT test, all strains responded with increase falling between 4 h and 52-60 h of the experiment, except for Mał strain (increase just between 52 and 60 h). As regards ZA, changes were similar, yet lasted longer, as from 8, 24 and 36 h to 60 h of the experiment, and were the longest for Fr-2 and SGM strains, while the shortest for Mał strain. TWZ indicated increase in the case of the following strains: Fr-2 from 4 to 48 h, Mał from 8 to 36 h, and Fr-1 from 24 to 52 h, while SGM exclusively at 24 h, whereas from 48 to 60 h decrease in this parameter was recorded for this strain. Stimulation index was characterised with increase for Fr-2 strain between 4-48 h, for Mał strain at 36 and 60 h, for SGM strain at 48 h, while with decrease for SGM strain (from 52 h to 60 h), and Fr-1 strain at 8 h and 24 h of the experiment. IP indicated increase at 8 and 24 h for Fr-2 strain, and at 4 and 48 h for Mał strain. In turn, Fr-1 and SGM strains showed no changes to this parameter. %kp was most frequently recorded for SGM strain, and in the form of increase it occurred at 36 h, while in the form of decrease at 56 and 60 h. For Mał strain, the increase in this parameter occurred at 4 and 60 h, while for Fr-1 and Fr-2 strains no changes were recorded. Spontaneous NBT test indicated increase at single hours of the study, which referred to Fr-1 strain (12 and 24 h), SGM strain (8 and 24 h), and Mał strain (52 h). Stimulated NBT test revealed slightly more changes, although they only referred to increases and only for two strains: Fr-1 (12, 24 and 48 h), and Fr-2 (4, 8, 12 and 24 h). In the case of spontaneous WAMG, most changes referred to decrease (Fr-2 strain at 12, 48 and 56 h), while increase was observed for SGM (56 h and 60 h) and Mał strains (56 h). No changes in this parameter were recorded for Fr-1 strain. More intense changes were found in stimulated

Table 1. Summary table of results of immunity studies in rabbits experimentally infected with four strains of the RHD virus

	Factors analysed	Analysed strains of the RHD virus			
		French (Fr-1)	**French (Fr-2)**	**Polish (SGM)**	**Polish (Mał)**
	adherence capacity (ZA)	↑ 36, 48, 52, 56	↑ 8, 24, 36, 48, 52, 56	↑ 24, 36, 52, 56, 60	↑ 36, 52
absorption capacity (zp)	absorption index (IP)	–	↑ 8, 24 ↓ 56	–	↑ 4, 48
	% absorptive cells (%kP)	–	–	↑ 36 ↓ 56, 60	↑ 4, 60
NBT test	spectrophotometric	↑ 4, 8, 12, 24, 48, 52	↑ 4, 8, 12, 24, 36, 48, 56	↑ 8, 12, 24, 36, 48, 52, 56, 60	↑ 52, 56, 60
	spontaneous	↑ 12, 24	–	↑ 8, 24	↑ 52
	stimulated	↑ 12, 24, 48	↑ 4, 8, 12, 24	–	–
	stimulation index	↓ 8, 24	↑ 4, 8, 12, 24, 36, 48	↑ 48 ↓ 52, 56, 69	↑ 36, 60
WAMG	spontaneous	–	↓ 12, 48, 56	↑ 56, 60	↑ 56
	stimulated	↑ 12, 48	↑ 4, 8, 12, 24, 36, 48, 52	↑ 52, 56	↑ 56
	intracellular killing test (TWZ)	↑ 24, 36, 48, 52	↑ 4, 12, 24, 36, 48	↑ 24 ↓ 48, 52, 56, 60	↑ 8, 12, 36
	MPO activity	–	↑ 4, 8, 24, 36, 48	↓ 52	–
lysozyme	concentration	–	↑ 4, 8, 12, 24, 36, 48, 52	↓ 8, 12, 24, 36, 48, 52, 56, 60	↑ 8, 24, 36, 48, 52, 56
	activity	↓ 12, 48, 52	↓ 4, 8, 12, 24, 36, 48, 52, 56	↓ 4, 24 ↑ 36, 56	↓ 8, 12, 48, 56
	IgG	↓ 48, 56	↑ 24, 36	↓ 48, 52	↑ 52
	Ig (ZST units)	–	–	↑ 36, 52	–

↓↑ *– statistically significant increase or decrease in the factor analysed as compared to the control group of rabbits; numbers indicate hours of the experiment; "– " means no change.*

WAMG, most of which were recorded – as previously mentioned – for Fr-2 strain (from 4 to 52 h). In the case of Fr-1, SGM and Mał strains, it only referred to single hours of the experiment.

What was interesting was the fact that in the case of all four strains of the RHDV (Fr-1, Fr-2, SGM and Mał), also rather significant changes to specific humoral immunity were recorded regarding the dynamics of serum immunoglobulins of G class, which fell between 24 and 56 h of the experiment. In the case of Fr-1 strain, decrease in IgG was observed at 48 and 56 h, for SGM – at 48 and 52 h, while increase was recorded for Fr-2 strain at 24 and 36 h, and for Mał strain at 52 h. In turn, changes recorded to total Ig quantity in ZST test in serum of rabbits proved insignificant, as manifested with increase at 36 and 52 h of the experiment exclusively for SGM strain.

The results of virusological studies performed using IFb test on samples of internal organs of all infected animals clearly point that the main organ homed by the RHD virus is the liver and the lungs, although also spleen. Low result for RHDV presence was obtained in the kidneys. Such distribution of the microbe testifies to its special predisposition for organs with rich blood supply, in which it causes irreversible thorough lesions. In turn, the analyses with ELISA test in experimental animals did not detect antibodies for the RHD virus.

Among four analysed strains of the RHDV (Fr-1, Fr-2, SGM and Mał), animal mortality (Table 2) was the highest, respectively, for Fr-2 strain (100%), SGM strain (95%), Fr-1 (90%) and Mał strain (80%). Small differences or a lack thereof was recorded for the four analysed strains as regards clinical symptoms and A-P lesions in internal organs.

Table 2. Clinical symptoms, anatomo-pathological changes (A-P) and results of virusological studies in direct immunofluerescence test (IFb) in rabbits infected with four strains of the RHD virus

RHD virus strain	Number of dead animals at particular hours of the experiment							% of deaths at 60 h of the experiment	Clinical symptoms	A-P changes	IFb results		ELISATest
	12	24	36	48	52	56	60						
Fr-1	–	–	–	–	–	–	18	90	Dejection, lack of appetite, sneezing, bloody rhinorrhoea, discharge of foam from mouth, purulent conjunctivitis	Typical of the RHD virus, recorded principally in the liver and spleen, in the lungs and partially in the kidneys	++++ ++ ++ +	liver lungs spleen kidneys	No anti- RHDV antibodies detected
Fr-2	2	–	–	16	–	–	2	100	Rhinitis and sneezing, squealing 5-6 hours before death		++++ ++++ +++ +	liver lungs spleen kidneys	
SGM	1	–	–	–	–	–	18	95	Sneezing, dejection, dyspnoea, conjunctivitis, convulsions 5-6 hours before death		++++ ++++ +++ +	liver lungs spleen kidneys	
MAŁ	–	–	–	–	–	–	16	80	Rhinitis and sneezing, excitation, bloody rhinorrhoea, wheezing 5-6 hours before death		+++ +++ ++ +	liver lungs spleen kidneys	

++++ – very intensive fluorescence in the IFb test; +++ – intensive fluorescence in the IFb test; ++ – weak fluorescence in the IFb test; + – very weak fluorescence in the IFb test.

The comparison of the presently obtained results with observations by other authors is rather difficult, as there are none in this area – apart from the studies by Polish authors, including my own [1-18]. Although some works by Chinese authors [20-25 and quote in 1, 2, 17, 18] regarding the infection and vaccination of rabbits with the RHD virus, present absorption capacity of MN cells coming from peritoneal cavity, the quantity of PMN cells and IFN concentration in peripheral blood of rabbits, and the number of T and B lymphocytes, and in some of such papers the authors have evidenced that the number of PMN cells recorded in 24-hour intervals (from 24 h to 120 h) and the quantity of IFN tested every 6 hours (from 6 h to 48 h), they do not show the differentiation between particular strains of the RHD virus. In turn, when comparing the presently obtained results to the studies by Polish authors [1-18 and quote in 1, 2, 17, 18], it can be concluded that in the case of rabbit infection with the RHD virus, the changes are similar and principally observed in non-specific cellular immunity factors (increase in values at early hours of the experiment) and non-specific humoral immunity (decrease at final hours – just before the death of animals). It must also be added that the currently recorded image of changes for four strains of the RHDV is also conformant to the hypothesis [1-18] stating that, at present, the principal role in the control of infection with this virus is also played by mechanisms of non-specific both cellular and humoral immunity, and not just the specific cellular immunity factors, as it had been assumed so far. It must be concluded that the present results of immunological studies, pointing to significant differences in the increase and decrease in values of the immunological factors analysed, must be linked to different dynamics of immunological response and different stimulation of the immune system by the analysed strains of the RHDV, which would testify to their varied immunogenicity. This is conformant, as mentioned before, with the studies by Polish authors [1-18 and quote in 1, 2, 17, 18], which also indicate that strains from different biotopes are characterised with different response of the immune system of rabbits.

The time and number of deaths in rabbits infected with four strains of the RHDV (Fr-1, Fr-2, SGM and Mał), pointing to their different virulence, confirm earlier studies [1, 2, 3, 17, 18], which evidenced that various strains cause different mortality, ranging from 60 to 100%, and falling at 36 to 48-60 h from infection. It must also be added that the recorded time of death and mortality rate among rabbits infected with four analysed strains of the RHDV coincides with the changes in the immune system, which was also evidenced in earlier observations [1, 2, 3, 17, 18]. Furthermore, the recorded clinical symptoms, in the aspect of the time of occurrence, and A-P lesions, confirm earlier studies [1, 2, 17, 18] and data provided by other authors [quote in 1, 2, 17, 18], indicating that no significant differences were detected in this aspect for strains of the RHD virus originating from different biotopes.

Conclusions

1. The four analysed strains of the RHD virus (Fr-1, Fr-2, SGM and Mał) only differ with immunogenicity and partly with virulence.
2. Non-specific cellular and humoral immunity constitute an important defence mechanism in rabbits infected with the RHD virus, and it seems that the decrease in the quantity and activity of the factors analysed is the cause of death in such animals.

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