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Short communication

Lymphocytes T and B in rabbits infected with RHD virus

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Abstract

The aim of this study was to determine the differences in immunological response of animals infected with different antigenic variants of the virus - three haemagglutinating (Vt97, Triptis, Hartmannsdorf) and two non-haemagglutinating (Pv97, 9905 RHDVa). The specific immunological response was measured by the dynamics of changes in the amount of lymphocytes T (with CD5+, CD4+, CD8+, CD25+ receptor) and B (with CD19+ receptor).

The study showed differences in immunogenicity of the analysed RHDV antigenic variants, which allowed them to be divided into groups of: more immunogenic strains, including non-haemagglutinating 9905 RHDVa and haemagglutinating Vt97 and Triptis variants; and less immunogenic strains, including the haemagglutinating Hartmannsdorf variant and the non-haemagglutinating Pv97 variant. Such a result may indicate that the agglutination capacity of red blood cells might not be a factor impacting the number of T and B lymphocytes.

Key words: rabbit haemorrhagic disease, lymphocytes, antigenic variants

Introduction

Despite increasing knowledge concerning the variability of RHDV, a virus which is an etiological factor of rabbit plague, a dangerous viral disease of wild and breed rabbits worldwide, knowledge of the immunological response to antigenic variants of the RHD virus is still sparse. Therefore the aim of this study was to determine the differences in immunological response of animals infected with different antigenic variants of the virus. The specific immunological response was measured by the dynamics of changes in the amount of lymphocytes T (with CD5+, CD4+, CD8+, CD25+ receptor) and B (with CD19+ receptor), in rabbits experimentally infected with three haemagglutinating (Vt97, Triptis, Hartmannsdorf) and two non-haemagglutinating (Pv97, 9905 RHDVa) antigenic variants of the RHD virus. Additionally, data concerning the mortality rate of animals infected with the five studied strains were collected.

Materials and Methods

The study was performed on 100 mixed-breed rabbits of both sexes, typical for this kind of study as



previously described (Niedźwiedzka-Rystwej and Deptuła 2012). The blood of rabbits infected with different antigenic variants of RHDV was collected from the peripheral vein of the ear at 0,4,8,12,24,36 hours after administration of the analysed RHDVa strain, and the percentage of T (CD5+, CD4+, CD8+, CD25+) lymphocytes, and B lymphocytes (CD19+) was determined according to the procedure previously described (Niedźwiedzka-Rystwej and Deptuła 2012).

Results and Discussion

Analysis of the amount of lymphocytes T and B and their subpopulations in rabbits infected with different RHDV strains showed an increase in the percentage of analysed lymphocytes, especially in the time periods between: 4-8 h and 24-36 h after infection. The most changes were noted in the amount of lymphocytes with CD8+ receptor, and the least in the case of B lymphocytes with CD19+ receptor. The mortality of infected animals registered at the 36th hour of the experiment also varied between the experimental groups of animals. In groups of rabbits infected with haemagglutinating variants of RHDV the mortality rate was 100% in the case of infection with the Hartmannsdorf variant, 90% for the Triptis variant, and 30% for Vt97; the mortality of animals infected with the non-haemagglutinating variants Pv97 and 9905 RHDVa was 90%.

The results vary between analysed strains, yet due to the lack of immunological studies on RHDVa they can only be referred to the results obtained for strains of the RHD virus other than RHDVa. The available data describe 6 haemagglutinating strains, including 1 French Fr-2 strain (Tokarz-Deptuła 2009), 4 Czech strains: CAMP V-351, CAMP V-561, **CAMP** V-562, **CAMP** V-562 (Hukowska-Szematowicz and Deptuła 2008a,b), 1 Polish Kr-1 strain (Tokarz-Deptuła and Deptuła 2004), and 1 non-haemagglutinating Polish BLA strain of the virus (Hukowska-Szematowicz et al. 2005), which were assessed, as in this study, by the dynamic changes in the immunological response.

The results allow the investigated strains to be divided into two groups – high and low immunogenic. However, it should be stressed that the analysed viral variants are not distributed in these groups in relation to their haemagglutinogenicity (Tian et al. 2007). It is possible that the capacity of haemagglutination of the strains of the RHD virus is an effect of the structure of the VP60 protein. It should also be noted that the determination of the greatest number of changes in rabbits infected with the RHD virus concerned the subpopulation of lym-

phocytes with CD8+ receptor, which is consistent with the results obtained in studies on Ebola virus infections in humans (Warfield et al. 2005, Bradfute et al. 2008). Such observations were not confirmed in mice (Bradfute et al. 2007), where a decrease not only in the number of lymphocytes with CD8+ receptor was recorded, but also in lymphocytes with CD4+ receptor, B-cells and NK cells.

Additionally, our data on rabbit mortality caused by infections with the analysed RHDVa antigenic variants show that the haemagglutination capacity does not affect this parameter, as one of the haemagglutinating variants (Vt97) caused rather low mortality of 30%, while the remaining haemagglutinating (Triptis, Hartmannsdorf) and non-haemagglutinating (Pv97, 9905 RHDVa) variants caused a high mortality of 90-100%.

In conclusion, the results revealed that the three haemagglutinating (Vt97, Triptis, Hartmannsdorf) and two non-haemagglutinating (Pv97 and 9905 RHDVa) antigenic variants of the RHD virus differentially affect the immunological response measured by the dynamics of changes in the number of T and B lymphocytes and their subpopulations. The observed changes were mainly characterised by an increase in the investigated subpopulations of lymphocytes seen between the 4th-8th hour, and 24th-36th hour after infection. The most pronounced changes were noted in lymphocytes with CD8+ receptor. The differences in immunogenicity of the analysed RHDV antigenic variants allowed them to be divided into two groups of: 1) more immunogenic strains, including non-haemagglutinating 9905 RHDVa and haemagglutinating Vt97 and Triptis variants; and 2) less immunogenic strains, including the haemagglutinating Hartmannsdorf variant and the non-haemagglutinating Pv97 variant. It is worth noting that strains determined as antigenic variants (Capucci et al. 1998) have been determined only within their antigenicity on the basis of their reaction with antibodies, but their immunogenicity, that is the ability to impose an immunological response, has not been studied and therefore the term "antigenic variant" is not appropriate, since it exposes only antigenicity and not immunogenicity, and the latter seems to be a pivotal characteristic element of these strains. Furthermore, our observations regarding the pathogenicity of the five analysed strains, expressed as the mortality rate of infected animals, did not confirm the results of the authors (Capucci et al. 1998) who introduced the term "antigenic variants". They determined 90-100% mortality in rabbits infected with the RHDV strains described, while the present study showed that the mortality rate in rabbits infected with the Vt97 variant of the RHD virus was not more than 30%.





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