The immunity during immunization with the viral haemorrhagic disease (VHD) in rabbits

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Abstract

Results of investigations are presented obtained in rabbits experimentally infected with VHD (viral haemorrhagic disease) virus. In most cases the results involve multi-annual observations of the authors. The results point to participation and the role of immune phenomena in pathogenesis of the disease and document presence of immunotypes of the VHD virus.

Key words: rabbit, VHD virus, immunity

Introduction

In recent years, appearance of new severe infectious diseases of humans has been noted, the development of which is linked to germs of animal origin [1-5]. The diseases include effects of infections with coronaviruses, which not only have altered their tropism from alimentary to respiratory tract in animals [6], but, at present, have breached inter-specific barrier passing from animals to humans, e.g. Hendra, Nipah, SARS viruses (1-3). Such agents include also the virus of Borna disease, which originated from horses but has become assimilated in humans resulting in psychiatric syndromes [5]. This group of zoonoses is linked also to viruses of haemorrhagic fever of humans, including, i.a., Hantaan, Machupo or Ebola viruses [1, 4, 7, 8]. The latter infectious agents, resulting in lethal diseases of humans of an acute course provide no chances for studies on their pathogenesis. Therefore, monitoring of phenomena linked to interactions of the viruses with human macroorganism can only be surmised from studies which record alterations in experimental animals following their experimental infection with the agents and, in particular, the alterations linked to immune patterns [4, 7, 8]. Observations and studies of the type on human viruses remain, however, difficult to accept since the agents pose a significant hazard: they were classified by the Center for Disease Control and Prevention as agents of II and III category of biological factors, with the potential to be used by bioterrorists [1]. For this reason, studies are undertaken on experimental animals infected on purpose with typical for them microbes, yielding an analogous pattern of pathology to that seen in humans with haemorrhagic fevers [9, 10]. The infectious agents which yield almost identical changes to those noted in haemorrhagic fevers in humans include the viral haemorrhagic disease (VHD) virus, pathogenic only for rabbits [10, 11].

The Viral Haemorrhagic Disease (VHD), caused by a virus of Caliciviridae, was described for the first time in China in 1984. This virus, currently found on nearly all continents, induces a disease of a fulminant character, associated with a high mortality rate, e.g. 70-100% within 48-60 hours after exposure [10, 12, 13]. It is for this reason that numerous publications on the subject have appeared in the literature. However, only a few of them provide information on the pathogenesis of the disease. Therefore, an exploration of immune phenomena during the infection can significantly contribute to a better understanding of the pathogenesis of the VHD. Kęsy et al. [11] reported that VHD is a source of haemorrhagic fevers in humans, where the course of disease is characterized by acute or subacute presentation. The latter viruses can not be used in experiments due to their pathogenicity [4] and, hence, an exploration of immune phenomena in animal model, where the illness has a fulminant course, seems to be beneficial and necessary for practical and theoretical reasons [14-17].

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Nonspecific immunity

Analysing the immune phenomena, we should note that they pertain only experimental infections and the evaluation of immunity following vaccination of rabbits against VHD virus infections. No such studies are available on naturally infected rabbits. Deng, JI, Li, Xu, and Du (18 and authors quoted in 19) demonstrated high concentrations of interferon in rabbits 12 h after administration of the inactivated vaccine and its low levels and augmented ingesting capacity of MN cells in rabbits experimentally infected with VHD virus just before death of the animals (42-48 h). On the other hand, the team of Deptuła [10, 20-26] experimentally infected rabbits with 100% lethal dose, using seven national strains (Kr-1, KGM, SGM, MAŁ, BLA) and two French strains (Fr-1, Fr-2) of VHD virus, evaluated immunity indices every 4 h and demonstrated that principal elements of immunity in the disease involved phagocytosis (capacity to adhere, to ingest and to intracellularly kill index bacteria) by PMN cells as well as activity of myeloperoxidase (MPO) in the cells as well as serum lysozyme (LZM) level. Moreover, as far as the range of deviations and their timing are concerned, the indices exhibited extensive variability, depending upon the type of employed strain. As a rule, the indices tended to increase at the beginning of the infection (4-8 h) and frequently persisted till 48-56 h while their fall was noted just before death of the animals (48-60 h). The studies proved also [20, 21, 23-25, 27] that Fr-2 strain was most immunogenic among the examined strains, followed by SGM, Fr-1, BLA, KGM, MAŁ, and Kr-1 strains. Most extensive differences between the viral strains were noted in the oxidising capacity of PMN cells, tested by the nitrotetrazolium blue (NBT) reduction , using spectrophotometric technique, which documented increase between 4-8 h and 52-56 h after infection as well as in concentration and activity of LZM. In the latter case, increase and decrease in the index developed, respectively, between hours 4 and 8 and hours 52-56 after infecting the rabbits. In the studies, the same authors [20, 21, 23, 24, 27-30] evaluated also immune indices in rabbits infected with four various doses (100%, 75%, 50%, or 25% lethal dose) of VHD virus, strain Fr-2 which, as mentioned earlier, proved most immunogenic among the 6 virus strains, and of Kr-1 strain, the weakest immunogen in the group. Analysis of the results showed that, independently of the virus dose, more extensive increase or decrease (more frequently) was noted in the case of Fr-2 strain. The most pronounced increases and decreases in studied indices were observed following 75% lethal dose of the virus (between 4 to 8 h and 120 h), 100% lethal dose (between 8 and 56 h), 50% lethal dose (between 4 and 36 h), and 25% lethal dose (between 4-8 h and 36 h). The alterations were most marked in cases of capacity to adhere, in NBT reducing capacity of PMN cells in the spontaneous, spectrophotometric and stimulated test (in a decreasing order). Slightly smaller alterations were observed in LZM activity, in the coefficient of granulocyte metabolic activity (WAMG) in the stimulated NBT test. In the case of Kr-1 strain, most pronounced increases and the most pronounced decreases were observed, in a decreasing order, in cases infected with 100% lethal dose (between 4-12 h and 48-56h), 75% and 25% lethal dose (between 4-8 and 36 h), and 50% lethal dose (4, 8, 12 and 36 h), and 25% lethal dose (between 4-8 and 36 h). The alterations were most marked in WAMG values in spontaneous and stimulated tests, in stimulation indices of NBT tests, in the level and activity of LZM and in the NBT test, spontaneous and stimulated one. The results demonstrated that the two compared VHD virus strains differed between each other, as shown already earlier, in the quality of evoked immune response, the quality of which does not always correspond to the mathematically calculated VHD virus dose.

The suppressive action of the two studied VHD virus strains on rabbit immune system, noted independently of the infecting dose and particularly evident just before death, proves that immune phenomena participate in pathogenesis of this suddenly developing viral infection, in agreement with the results of our earlier published studies. The results have been corroborated also by observations of pathologists [18, 31-40], who noted augmented activity of Kupfer cells and macrophage infiltrates in the spleen, lymph nodes, liver and lungs of rabbits experimentally infected with VHD. Participation of the factors in pathogenesis of the rabbit plague was confirmed also indirectly by results of Bulgarian authors [34], who showed that resistance could not be adoptively transferred with serum of VHD-immunised rabbits. According to the authors, this indicated that resistance in this type of infection is related to cell-mediated immunity.

Specific immunity

At present, the resistance is linked also to T and B lymphocytes and to their subpopulations as well as to serum IgG [10, 22, 26, 30, 41 and authors quoted in 10]. In rabbits experimentally infected with VHD virus as a rule increase was noted in levels of T (CD3, CD5) lymphocytes, Th (CD4) lymphocytes, Tc/Ts (CD8) lymphocytes, B (CD19) lymphocytes, activated T and B (CD25) lymphocytes and in serum IgG levels. The alterations in the indices, mainly in lymphocyte levels, developed most frequently 4 to 8 h after infection and persisted mostly to the 36th, 48th or 60th h. However, the changes varied depending upon the biotope from which VHD virus (strain type) originated and depending upon the infective dose of the virus.

The Fr-2 strain was shown to induce increase in B, T, Tc/Ts, Th, and in activated T and B lymphocyte levels. Employing 100% lethal dose of VHD virus, strain Fr-2, levels of B lymphocytes started to increase beginning at 4

h after infection, levels of T and Th lymphocytes – beginning at 12 h, and levels of activated T and B lymphocytes – starting at 24 h. The latter elevated levels persisted till 56th-60th h following the infection (levels of Tc/Ts lymphocytes were not examined). Infection with 75% lethal dose of the virus strain resulted in increased levels of T lymphocytes, activated T and B lymphocytes, Tc/Ts lymphocytes, B lymphocytes and Th lymphocytes starting at 4th-8th h and observed till 56th-60th h after the infection. Infection with 25% lethal dose of the virus strain was followed by a less intense increase, more frequently by a decrease, mainly in activated T and B lymphocyte level, An increase or a decrease in T, Tc/Ts, Th, B lymphocyte levels was basically observed in individual time points only and for any lymphocyte subpopulation did not persist longer than till 36 h after infection. The 50% lethal dose was followed by an even less intense increase than that observed after 25% lethal dose. The increase was noted just in the 24th h of the experiment and pertained only T, Tc/Ts and activated T and B lymphocytes.

Infection with 100% lethal dose of VHD virus, strain Kr-1 was followed by an increase in T, Tc/Ts, B, activated T and B and Th lymphocytes, persisting till 8th, 12th, 24th, or 36th h after infection. In cases of activated T and B lymphocytes, the increased levels persisted till 120th h of the experiment. Decreased levels were noted less frequently and were seen at 72 h for T lymphocytes, between hours 48 and 120 in the case of Tc/Ts lymphocytes and at 48 h in the case of B lymphocytes. Levels of activated T and B lymphocytes showed no decrease. Following infection with 25% lethal dose of the virus strain, increased levels of Tc/Ts lymphocytes were noted between hours 4 and 48, of B lymphocytes between hours 8 and 24, of Th lymphocytes between hours 36 and 48, of T lymphocytes only at 48 h after the infection. In the case of this strain, decreased levels were observed in activated T and B lymphocytes (hours 4, 8, and 36) and in Th lymphocytes (hours 8 and 12). However, the alterations were less pronounced and lasted shorter as compared to those registered following the 100% lethal dose. Nevertheless, the alterations were more pronounced as compared to those which followed infections with 75% lethal dose or 50% lethal dose of the strain, when increased or decreased levels of the cells were seen just in individual hours of the experiment. Following infection with 75% lethal dose, an increase (hours 12 and 24) or a decrease (hour 36) pertained mainly B lymphocytes, activated T and B lymphocytes (increased levels at 24 h and 36 h), Th lymphocytes (decrease at 12 h and increase at 36 h) and Tc/Ts lymphocytes (decrease at 36 h). Infection with 50% lethal dose of the strain was followed by alterations of shortest duration, as compared to effects of previously described doses, which involved increases in B lymphocytes at 4 h and 36 h, T and Th lymphocytes at 36 h, in activated T and B lymphocytes at 4 h and decreases in Th lymphocytes at 8 h and in T lymphocytes at 24 h. At this

dose of the virus strain, no significant alterations were noted in Tc/Ts lymphocytes.

Immunotypes of VHD virus

Results of our own studies on specific immunity in VHD-infected rabbits corroborated in part the data of Chinese authors [19] and those of Deng et al. (authors quoted in 10), who also noted elevated levels of T lymphocytes and of antibody-producing lymphocytes in VHD-immunised rabbits. However, it should be noticed that the authors have estimated levels of the cells using rosette tests and did so in a static model, failing to monitor dynamics of the alterations.

It should be added that another aspect of our own data on non-specific immunity [17, 20, 21, 26, 27] and on specific immunity [22, 25, 26, 28, 30, 41 and authors quoted in 10] in rabbits experimentally infected with seven VHD virus strains, originating from various biotopes in Poland (BLA, KGM, SGM, MAŁ, Kr-1) or in France (Fr-1, Fr-2) is their distinct immune reactivity, which points to their distinct antigenicity and indicates existence of immunotypes of VHD virus. The hypothesis has in part been confirmed by the data of other authors [32], who demonstrated differences in haemaglutinating properties among Korean and Mexican strains of the virus. Investigators from France [42] described eight distinct genetic varieties of the VHD virus, related to various sites of origin. Polish investigators [43], on the other hand, examined genotypic variability of 8 strains (isolates) of VHD originating from Poland and France and demonstrated extensive genetic stability of the virus. Within the region of non-structural proteins, the variability ranged between 1.1% and 2.8% in the four Polish strains (SGM, KBM, PD, LUB) and between 5.8% and 7.5% in the two French strains (PLF – Fr-2, Fr-1) and three Polish strains (GSK, ŻD, BLA). In most of the studied isolates (strains) nucleotide variability in the region of VP60 capsid protein varied between 0.2% and 1.1%.

Presenting immune phenomena in VHD virus-infected rabbits in the context of pathogenesis of rabbit plague, studies of an Italian investigator (authors quoted in 44) should be mentioned. The author suggested that immunity in VHD-infected rabbits may include factors such as colony stimulating factor (CSF) for the cells of immune system, tumour necrosis factor-beta (TNF-β) and closer undefined lymphokines. Also the Chinese investigators [authors quoted in 43] pointed to a significant role of the transfer factor, isolated from spleens of VHD-infected rabbits. Our team [24, 25] examining a large group of rabbits, experimentally infected with VHD virus, demonstrated activating and stimulating effects of TNF-α, which practically manifested by prolongation of the animals life by 8 to 12 h.

Interpretation of serologic results

It should also be mentioned that detection of antibodies in laboratory and free-living rabbits presents interpretation difficulties. The antibodies result most probably [19, and authors quoted in 12] from stimulation of the rabbits with the non-pathogenic RCV virus (rabbit calicivirus), related to VHD virus. The data, provided by Bulgarian authors [34] point to other, closer undefined immune phenomena in rabbits naturally infected with VHD virus, which probably develop due to interactions between the inactivated (contained in the vaccine) and the viable VHD virus.

Conclusion

Summing up the results on immune phenomena in rabbits experimentally infected with VHD virus it should be noted that alterations in immunity represent one of principal causes of the dynamic and rapid development of the process. The alterations shape course of the disease, create pathogenic mechanisms and are mainly linked to changes in phagocytosis executed by PMN and, in part, by MN cells, to changes in concentration and activity of LZM (increase or decrease takes place, depending on strain, in the first hours of infection or, more frequently, a decrease takes place just before death of the rabbits), to altered numbers of T, B lymphocytes and in their subpopulations (Th, Tc/Ts, T and B lymphocytes)(increase in the course of almost entire infection, depending upon the strain and dose of the virus, and a short lasting decrease at the terminal stage of the infection). It should also be added that the recorded, beginning at already 36 h, increase in serum IgG in rabbits experimentally infected with various doses of VHD virus [10, 20, 21, 26] indicates not only the role of antibodies but suggests also a new, more rapid pathway of their synthesis (e.g., by participation of HSP proteins in the pathway) in the acutely developing viral infection.

The presented immune pattern in rabbits experimentally infected with VHD virus seems to prove that altered immunity in the animals is of fundamental importance for health and survival of the animals and determines pathogenesis of the disease.

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