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Review

IMMUNE REACTIONS AGAINST THE RABBIT MYXOMA VIRUS

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ABSTRACT

The rabbit myxomatosis is caused by *a Leporipoxvirus* from the *Poxviridae* family. Extremely virulent strains kill the animals and no immunological reactions can be proved. Surviving individuals gain adaptive immunity that protects them from reinfection. In time virus is naturally attenuated and the resistance in the population is increased. There is not enough research about T- and B-lymphocyte immune reactions to the virus. This article deals with the scientific experience in the field of antibodies and their different protective effect. The authors have tried to analyze the data about the suppressive effects of the virus. Some of the virus proteins have also immunomodulatory activity. The molecular aspects of virulence and the oncolytic effects of myxoma virus are described. The future of myxoma virus research is directed towards its use as a part of anticancer therapy and the selection of rabbit breeds with higher antivirus resistance.

Key words: myxoma virus, resistance, immune reactions, oncolytic therapy

INTRODUCTION

Myxomatosis is among the most common and deadly diseases in rabbits. Infection is caused by a Myxoma virus (MYXV), family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Leporipoxvirus.* Domestic rabbit (Oryctolagus cuniculus) is the most sensitive species. Virus transmission occurs through blood-sucking insects or by direct contact. Outcome is often lethal. Two species of American wild rabbits are the natural hosts of the virus (1). The first documented description of infection was done after a spontaneous outbreak in laboratory rabbits in Uruguay in 1896 (2). During the 20th century the virus was purposely introduced in Australia for the reduction of rabbit overpopulation (3). Later it was also released in Europe. Nowadays disease is endemic for wild and domestic rabbits in different parts of the world, including Bulgaria (4).

Morbidity and mortality

Factors that influence morbidity and mortality include the strain virulence and the reactivity of the organism (5). In the beginning the lethal rate in highly virulent strains can reach 99%, but quickly goes down to 25% (6). This is due to the attenuation of the virus and the formation of innate resistance in the population. This phenomenon was observed in Australia in the 50s of the 20th century and was also demonstrated under laboratory conditions. In England Ross and Sanders (7) were able to induce the development of resistance in wild rabbits. Mortality was reduced from 56% in 1970, to 20% in 1974 and 17% in 1976. In the same time the isolated virus strains from these rabbits were pathogenic for 70-95 % of laboratory rabbits. There is an active process of coevolution between virus and host. It was also found that in wild rabbits the virus titer measured from the lymph node draining the place of inoculation was 10 to 100 times lower independent of virus strain (8). This lead to the conclusion that such a lymph node plays an important role in the control of virus replication and disease outcome. The basis of resistance is the better innate immunity mechanisms that grant a stronger cell-mediated immunity. The synthesis of Interferons and TNF-a induce strong antivirus immune answer. NK-cells also participate to reduce virus effects. However the virus causes gene mutations that suppress the innate immunity.

Role of antibodies in Myxomatosis

Mother antibodies protect newborn rabbits against the infection. Sobey and Conolly (9)

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found that 4-week old rabbits are protected while older animals get no immunity even if they have been born from mothers that have survived the disease. Young rabbits can also be protected for a period of 3 weeks by the intraperitoneal inoculation of homologous immune serum (10).

It was speculated that most rabbits in a wild population get infected in an early age when the titer of mother antibodies can still be measured but the clinical manifestations of myxomatosis are not profoundly exhibited. Rabbits post infection loose immunity and can be reinfected. Because reinfection occurs in different time intervals virus can circulate in the population all year round (11).

If IgM antibodies are found in the sera of 8-week old rabbits this means that they were soon infected but disease passed asymptomatically. This proves that the animals were partially protected from mother antibodies during infection. Having in mind the conclusions made by Fenner and Marshall (10) and Joubert et al. (12) that young rabbits are extremely sensitive to myxomatosis strains with different virulence, the observations published by Marchandeau et al. (13) confirmed that mother antibodies cannot totally protect from infection, but soften the manifestation of disease and do not interfere with immune answer. The same authors proved the circulation of IgG antibodies that are also of mother origin.

Immunopathologic effects of MYXV

Though there are many publications on Myxomatosis, little is known about the dynamics of pathogenesis and the influence of myxoma virus on the immune system. Important aspects are found in the research of Jeklova et al. (14). The CBC of experimentally infected rabbits with the highly virulent strains shows leucopenia between the 4^{th} and 6^{th} day post infection, lymphopenia between the 4^{th} and 11^{th} day, neutrophilia and monocytosis between the 9^{th} and 11^{th} day; however the overall leucocyte count is not increased. The lymph node draining the place of inoculation is enlarged; the count of T-lymphocyte subtypes (CD8+) is reduced and that of B- lymphocyte subtypes (CD4+) is increased. Similar results can be found in other lymphatic organs except mesentery lymph nodes. The contralateral lymph node and the spleen are enlarged on the 6^{th} day. Thymus atrophy is observed on the 9^{th} day, but virus infected cells can be found as early as the 4^{th} day in the medulla. The results of the lymphocyte proliferation assay (LPA) with phytohemagglutinin (PHA) show severe depression after 4-6 days of the lymphocytes in all of the investigated organs. Around the sixth day post infection IgM antibodies can be isolated and after the 11^{th} day – IgG. The conclusion from this in vivo experiment shows that the virus suppress the cell-mediated /CD8+/, as well as the humural immune answer /antibodies production/. Cell mediated immunity in MYXV as in all Poxviridae members is among the most important mechanisms for reduction of infection.

Immunosuppressive and immunomodulatory activity of MYXV

In the last few years scientific interest to the virus is increased not only due to its significance as an infectious disease, but also due to the observation that some of the virus proteins show immunosuppressive and immunomodulatory effects.

The full DNA sequence of the myxoma virus is already known (15). Some of the genes code the synthesis of proteins with immunosuppressive and immunomodu-latory function (16, 17). Knockout of these genes can lead to virus attenuation. More than twenty of these proteins are already classified, but research in the field of their role in pathogenesis continues. Immunomodulatory proteins lead to the inhibition of the host immune defence like apoptosis of diseased cells, activation of cytotoxic T-lymphocytes and NK-cells, disturbed synthesis of proinflammatory cytokines, etc. Different pox viruses show different immunomodulatory proteins because the ways for cell infection are not the same (18).

MYXV prompt the synthesis of proteins that link TNF and chemokines, inhibit proinflammatory cascades and stimulate the cell proliferation throughout epidermal growth factors (19). The virus proteins that are expressed on the cellular membrane suppress the activation of macrophages and T-lymphocytes (20, 21). Some of the myxoma virus proteins suppress the synthesis of interferons which play a crucial part in the antivirus protection (22). One of this (M007) that is a product of infected cells is an IFN-γ receptor homolog. Another protein (M013) prevents the production of proinflammatory cytokines. Deletion of M007 and M013 genes leads to virus attenuation. Another mechanism that influences immune defence is inhibition of apoptosis of infected cells. Such an effect was proofed for five of the MYXV proteins - M002, M004, M005, M011, M013 (19).

Cell-mediated immune answer is characterized by recognition of MHC- I class from the CD8+ lymphocytes and destruction of the infected cells. M153 protein inhibits the expression of MHC- I class molecules (23, 24). The same protein suppress also ALCAM 1 (Activated Leukocyte Cell Adhesion Molecule; CD166), which disrupt the recognition of the infected cells from T-cells (25). Other important immunosuppressive protein is M141. It causes suppression of the activation of macrophages and T-lymphocytes (20), while M001 inhibit the influx of monocytes in the locus of penetration of the virus. This resume includes only a part of the information that is nowadays available for known myxomavirus proteins.

In recent years the importance of glycans in regulation of immune responses are studied. MYXV is one of the rare viruses that encodes an α2,3-sialyltransferase through its M138L gene and that enzyme is proved to be one of the virulence factors that contributes to immunosuppression (26)

Application of MYXV – vaccine vector and oncotherapy

There is no treatment against myxomatosis so hygiene and immunoprophylaxis remain the most important measures for disease prevention. Experiments with alive Shope fibroma virus vaccines give unsatisfactory result, so scientific attention is concentrated at attenuated myxoma virus strain (27) and bivalent vaccines against myxomatosis and haemorrhagic disease (28).

The Poxviridae family belongs to the DNA viruses that are able to cause strong immune reaction, integrate foreign DNA and remain stable, so they can be used as vector vaccines against a broad spectrum of pathogens. One important characteristic of myxomavirus is the fact that it is pathogenic to leporids only which makes it suitable as a vaccine vector in other species. A vaccine against feline calici virus infection was successfully tested. It contained an apathogenic laboratory attenuated myxomavirus that was able to express a calicivirus capside protein (29). Myxoma virus can be also used as vaccine vector for new successful vaccination strategy against bluetongue (30).

A modern perspective in the field of oncotherapy is the use of oncolythic viruses that can destroy cancer cells sparing the normal cells (31). Among poxviruses the myxomavirus demonstrates the highest oncolythic potential. Good oncolythic results were observed in human glioma treatment (32), in vivo pancreatic adenocarcinoma cells lysis (33), and lysis of cancer cells in bone marrow autograft in acute myeloid leukemia (34).

Myxomavirus was found capable to cause cell death to feline cancer cell cultures (35). The susceptibility of different feline neoplastic cells is being studied.

Another application of myxomavirus is the use of some factors like serin-protease inhibitor (Serp-1) that has antiinflamatory effects. It is secreted by virus infected cells and can be tried as a therapy for human arthritis (36).

CONCLUSION

The research of myxomavirus influence to immune reactivity and its immunomodulatory effects will help scientists to understand the molecular mechanisms of pathogenesis and the way resistance is created in rabbit populations. The answer to these questions will give also new possibilities to myxomavirus medical application.

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