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MICROPHAGIC ACTIVITY IN HENS WITH MAREK'S DISEASE

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Abstract

Microphages represent the first line of defence against microbial aggression. The research aimed at evaluating phagocytosis during a Marek's disease episode in Rosso layers, showing ocular lesions, in spite of the vaccination at 1 day of age. Heparinized blood (50 IU/ml) was subjected to the in vitro carbon particle inclusion test on days 0 and 15 of the episode. Two microliters of an India ink were added to 0.5ml of blood; 0.15·ml of the mixture were transferred to 2·ml of saline and the rest was incubated for 30·min at 37°C and procedure repeated. Phagocytic activity index was calculated as the difference between the natural logarithms of the optical densities of the phagocytosis read spectrophotometrically (λ = 535·nm, d = 1·cm) after centrifugation (2500rpm for 10min), divided by time. The values indicated a significant (p = 0.0259) increase of the phagocytosis towards the end of the monitoring interval, thus indicating an evolving disease and underlining the probability of innate immunity being stimulated by the herpesvirus during the observation period. Specific cell-mediated immunity and functional levels of humoral immune effectors quantification could complete the pathogenic pattern in these birds.

Key words*: Marek's disease, hens, phagocytosis, spectrophotometry.*

INTRODUCTION

Polyneuritis described in 1907 by Marek has spread and continuously economically impacted on poultry breading ever since. IN spite of vaccination procedures implemented worldwide, the disease kept on emerging in various areas, leading to consideration of changes that might have occurred in the pathogenicity and pathogenesis of this herpes virus infection (Biggs, 2001). Recently, there have been several outbreaks of the disease in various regions, due to the evolving nature of MD virus (MDV), which necessitates the implementation of improved prophylactic approaches. It is therefore essential to better understand the interactions between chickens and the virus. The chicken immune system is directly involved in controlling the entry and the spread of the virus. It employs two distinct but interrelated mechanisms to tackle viral invasion. Innate defence mechanisms comprise secretion of soluble factors as well as cells such as macrophages and natural killer cells as the first line of defence. These innate responses provide the adaptive arm of the immune system including antibody- and cell-mediated immune responses to be tailored more specifically against MDV.

In addition to the immune system, genetic and epigenetic mechanisms contribute to the outcome of MDV infection in chickens.

The review discusses the current understanding of immune responses elicited against MDV and genetic factors that contribute to the nature of the response. Modern genetic analyses assisted the clarification to some extent of the susceptibility and also resistance mechanisms to the disease.

Nevertheless, neither Marek's disease pathogenesis nor the immune response of the host and the neoplastic changes are well understood.

MDV genome in various strains shares similarities and the gene content is similar but there is a quite high difference between the pathogenicity of these strains (McPhearson and Delaney, 2016).

Briefly, the pathogenesis of the MDV includes the inhalation of the contaminated dander, subsequent contact with the immune system in the lungs and within 24 hours from inhalation, with the immunocytes in the spleen, thymus, and bursa of the infected individual (Schat et al., 2008). The transfer of the viral particles to the lymphoid structures benefits of infected macrophage conveyance. Infected macrophages can further transfer the viral particle to B and T lymphocytes, but B cells could be also infected directly (Barrow et al., 2003).

The four cycles virulent MDV undertakes four stages, early cytolytic, latent, late cytolytic, and transformation (Osterrieder et al., 2006). Besides the involvement of the adaptive immune system's cells, the innate immune response of the host to challenge has been found to play a critical role in studies of inbred chicken lines with differing MD resistance (Kaiser, 2010). Chicken heterophils, acting as microphagic neutrophils in mammals, trap and destroy extracellular microbes, posing a physical barrier and helping avoid microbial spread (Chuammitri et al., 2009)

This study aimed to investigate the reciprocal relationship of white cell categories involved in the non-specific immune response, i.e., overall phagocytosis.

MATERIALS AND METHODS

Biological material. The investigations were carried out on a batch of Rosso layers (n = 65), aged 52 weeks, from a conventional farm, where the study was carried out and where they steyed till the end of the research period. Naturally occuring ocular signs ascribed to Marek's disease were observed in the experimental birds.

According to the technology, the chicken house was populated with replacement chickens aged 16 weeks, which were vaccinated at day one agains Marek's disease in the incubation station. The technology designed for layers was applied to the birds, involving teh start of the laying period at age of 18 weeks. Subsequently, the laying curve was calculated and it evolved according to the technological parameters planned.

During the technological cycle, the birds were kept on permenent litter, completed periodically with fresh wood shavings. They were fed and watered according to the needs of the category .

The hens were sampled twice at an 11 days' interval and the heparinized blood (50 IU/ml) was subjected to carbon particle inclusion and to a leukocyte blast transformation tests.

Carbon particle inclusion test (phagocytic activity). Phagocytic cells engulf inert particles such as carbon due to the defensive capacity of these cells. To obtain the carbon particle suspension, India ink was centrifuged at 6000 rpm for 60·min (Hettich Mikro 22R centrifuge, Germany). The collected supernatant was mixed in 2·µl aliquots with 0.50·ml of the blood (50 IU heparine/ml) sampled from each birds and 0.15·ml of the mixture were transferred immediately to 2·ml of saline. The remaining of the mixture for each blood sample was incubated 30·min at 37°C (Khokhlova et al., 2004). All tubes containing saline, blood and ink were centrifuged at 1500 rpm and the supernatants were read spectrophotometrically (l = 535·nm, d = 1·cm) (SUMAL PE2, Karl Zeiss, Jena, Germany). If the phagocytosis is in place, a decrease in absorbance with time should be observed as the carbon particles are phagocytized. Optical density values were converted in natural logarithms and the phagocytic activity index was then calculated as the difference between the ln at 0 and 30·min divided by time (30·min).

Statistical processing of the data. Averages of phagocytic indices for both samplings along with the standard deviations were calculated. Percentage increases over incubation time were estimated for each sampling and between samplings. The Excel program was used to calculate the significance of the differences between the two samplings by means of the t-Student test.

RESULTS AND DISCUSSIONS

In intensive farming technologies the occurrence of infectious disease impede on health and welfare but also on the productive performances of the birds. The severity of the disease outcome is further enhances under all aspects, health and economy of the business

when immune-suppressive viral infections are involved. Increased costs of prevention and control of such outbreaks add to the economic losses faced by the farmers.

Marek's disease herpesvirus in chickens is defined by lymphoma genesis in internal organs along with neurological damage has been associated to immune suppression (Calnek, 2001; Payne et al., 2004).

Studies were initiated to investigate if an increased level of genetic resistance could provide means of enhancing flock protection, during viral disease development, including Marek's disease. This research led to the development of genetic descriptors which could, in Marek's disease and not only, provide possibilities for the supplementary recognition of resistance genes to upkeep the advancement in obtaining disease-resistant lines of poultry. Some results indicated improved responses from various lines of chickens to live attenuated vaccines, being qualified as high or low responders and thus, initiating new possibilities to optimize the outcome of immune prevention by selection (Bumstead, 1998).

In prevention of Marek's disease it was essential to echelon the vaccines used inside the vaccination protocol based on B haplotype of the flock, since this regulates the immune response to Marek's disease (Pavizzi et al., 2004; Payne et al., 1976). The numerous other factors associated with raising technologies in poultry farming, which could exert an inhibiting influence by increase in corticosteroid levels due to stress, should alo be considered when evaluating the vaccination outcome (Baaten et al., 2004).

The significance of the post-vaccination antibody-mediated response in vaccine-induced protection is increased by the cell-mediated immunity (Sharma and Witter, 1975), considering the substantial contribution memory B cells have in a booster type response to the aggressor (Sharma et al., 1975). Previous research indicated the importance of the innate immune response, the intervention of the NK cell-mediated response being specifically stressed, following vaccination with HVT+ SB-1 (Heller and Schat, 1987). Vaccination against marek's disease can also induce cytotoxicity against viral antigen mediated by CD8+ T cells (Morimura et al., 1998). The antigen stimulation can change cytokine profiles subsequent to cellular changes that occur (OIE, 2010).

The present study aimed at monitoring the changes which occurred at global phagocytosis level in birds clinically diagnosed with Marek's disease using a test based on carbon particle inclusion by the circulating phagocytes.

As the individual values indicated, the phagocytosis increased over incubation time, from 0 to 30 minutes, as the inert particles were engulfed by the phagocytes. Nevertheless, the increased standard deviation for both samplings stood for a relative non-uniformity of the individual responses, while the values ranged during the first sampling from 0.043 to 0.098 (at 0 min) and from 0.02 to 0.073 (at 30 min) and during the second sampling from 0.088 to 0.456 (at 0 min) and from 0.019 to 0.11 (at 30 min).

Table 1 shows the comparison between the averages for the phagocytic indices along with the standard deviations. There was a 24.35% increase of phagocytosis during the first sampling incubation period, while the engulfment of carbon particles was more active during the second sampling, with a 27.20% increase from 0 to 30 min.

Table 1. Phagocytosis index in the experimental groups (X ± DS)

Specification	First sampling			Second sampling		
	ln0	ln30	ln0-30	ln0	ln30	ln0-30
Average	-2.71	-3.37	0.66	-1.55	-2.95	1.40
Stdev	0.28	0.33	0.36	0.59	0.46	0.46

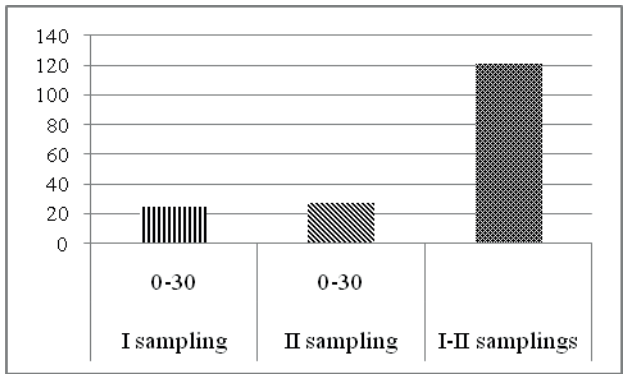

Figure 1. Percent of increase in phagocytic activity during the incubation period (0 to 30 min) within each sampling and the difference between the two samplings

The infection in Marek's disease takes place by respiratory route, subsequent to inhalation of contaminated dander/dust. The virus is engulfed by phagocytic cells (macrophages) at the respiratory system level. Due to the oaculiar anatomy of the avian respiratory components, including air sacs, the number of resident macrophages is smaller in this location. It was presumed that due to lesser numbers, the macrophage carriers of Marek's disease virus have to cross lung epithelial lining before being transported to lymphoid structures (Toth, 2000). No reference to the role of heterophiles as small phagocytes in transporting the virus within the blood stream is mentioned.

Non-the-less, there was a significant increase in overall phagocytosis from the first to the second sampling ($p = 0.02$) with the phagocytic activity increasing with 121.12% in 11 days (Figure 1), most probably due to the influence of the infection with the Marek's disease herpesvirus.

CONCLUSIONS

The statistically significant increase of the phagocytosis towards the end of the monitoring interval, recorded during this research indicated an evolving disease and underlined the probability of innate immunity being stimulated by the herpesvirus during the observation period. Quantifying functional levels of the adaptive cell-mediated immunity and humoral immune effectors could complete the immune-pathogenic pattern of MDV in these birds.

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