

Immunosenescence in Horses

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The process of aging is associated with changes in both adaptive and innate immune responses in people, and similar changes appear to occur in aged horses. Excessive inflammation and impaired response to pathogen challenge may predispose the geriatric horse to many diseases of old age. Few age-specific recommendations are currently available for vaccination of the older horse. In addition, the effect of old age on risk of infectious disease is poorly documented. More work is needed to better understand the interactions of age, diet, vaccination, and disease risk. Author's address: Oklahoma State University, Department of Physiological Sciences, Center of Veterinary Health Sciences, Stillwater, OK 74078; e-mail: diannem@okstate.edu. © 2013 AAEP.

1. Introduction

Immunosenescence is the term that describes the changes that occur in the immune system with advanced age. Changes reported to occur in aged people include alterations in the composition of lymphocyte populations as well as blunted or dysregulated immune responses.¹ Immunosenescence is an important risk factor for morbidity and mortality of the aged and contributes to development of the infectious, neoplastic, and inflammatory diseases that afflict the elderly population. Current available evidence suggests that horses undergo similar age-related changes in immune function as do people.

2. Considerations of Aging Studies in Immunology

Scientific evaluation of age-induced changes in immune function is not straightforward. First, it is important to separate age-associated functional changes from disease-induced changes. In an aged population, this can be difficult because of the high prevalence of co-morbidities that contribute to chronic, low-grade inflammation. Ideally, a strict recruitment protocol should be used. In human im-

munological research, a screening procedure known as the SENIEUR protocol has been developed,² which combines a history, clinical examination, diagnostic blood work, and urine analyses to identify healthy aged individuals for study inclusion. No such standardized protocols have been adopted in veterinary gerontology. In studies of aged horses, it is important to consider hormonal status during the study period because several of the pituitary and adrenal hormones are strong modifiers of immune function. The profound effect of season on the output of pars intermedia hormones and the high prevalence of pituitary pars intermedia dysfunction (PPID) in the aged equine population^{3,4} can markedly confound a study investigating age-associated immune function in horses. Other important considerations when assessing immune function in aged horses include diet, endoparasite load, medications, nutritional supplements, exercise, season, transport, travel, housing, and environmental conditions of the study participants.

A second consideration when investigating age-related changes of immune function is how to define "aged." Age-induced changes may be missed if the

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study group is too young. Less intuitively, a study population that is too old may cause age-related immune defects to be missed because the selected population may have survived to extreme old age as the result of exceptional immune function.⁵ It is likely that biomarkers of aging differ with the progression of animals from old to very old age. Certainly, risk of specific diseases, frailty, or other impairments vary along the spectrum of aging. Finally, it is also important that the control population be a suitable age. For example, adult animals are needed in the control population if the goal of the study is to compare the immune function changes that develop in old age, whereas the control group would include younger animals if the goal was to assess the whole spectrum of age-associated immune changes.

3. Age-Associated Changes in Immune Function

Cell Populations

Age-associated alterations in lymphocyte subset populations have been documented in several species including people, dogs, and rodents.⁶⁻⁹ A robust decrease in naive T-cells, thought to be the result of thymic involution, is a consistent finding in aged people and rodents.¹⁰⁻¹³ In addition, it has been suggested the loss of naive T-cells may be the result of lifelong infection by viruses, especially herpes viruses such as cytomegalovirus or other chronic antigenic stimulants.^{14,15} Because of a lack of available antibodies that differentiate equine memory cells from naive cells, changes in naive T-cell populations have not been directly examined in aging in the horse.

Other findings in human leukocyte populations include alterations in total lymphocytes, CD4, CD8, and B-cells. The CD4:CD8 ratio, an indicator of whether lymphocyte populations favor an inflammatory or immunosuppressive bias, has been reported to be altered in human geriatric populations.¹⁶⁻¹⁸ Several studies have investigated lymphocyte populations in aged horses and ponies.¹⁹⁻²² Total lymphocyte populations decrease in aged horses.¹⁹⁻²¹ Other observations include a decrease in the total number of CD4, CD8, and B-cells²¹ and an increase in the percentage of CD4 lymphocytes in aged horses.²² In ponies, a decrease in total monocyte and eosinophil counts has been reported.²⁰ The CD4:CD8 ratio was found to be increased in aged horses,²¹ suggesting that a proinflammatory state occurs in old horses as in people.

Cytokine and Acute-Phase Protein Profiles in the Aged Horse

Serum cytokine profiles in the aged human typically are those of a pro-inflammatory phenotype.^{23,24} Aged horses show similar cytokine profiles with increased gene expression of tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-1 β , IL-8, interferon (IFN)- γ , IL-15, and IL-18^{22,25} and increased proin-

flammatory: anti-inflammatory cytokine ratios including IL6:IL10 and TNF- α :IL10 ratios.²⁵ Serum cytokine concentration of TNF- α was increased in aged horses in one study²² but not another.²⁵ Several confounding factors might affect serum TNF- α concentration including concurrent illness, inflammation, or season of sample collection. The role of other serum cytokines in aging of horses has not been extensively examined because of the limited availability of valid assays. The effect of age on serum acute phase protein concentrations is not yet known, although assays validated for use in horse are now available for the measurement of serum amyloid A and C-reactive protein.

PBMC Cytokine Response to Stimulation

Several studies have evaluated the impact of aging on the responsiveness of equine peripheral blood mononuclear cells (PBMC) after immune stimulation. Inflammatory response after stimulation of whole blood or PBMC from healthy aged people results in a greater release of proinflammatory cytokines than that observed in adults.^{26,27} Similar studies have revealed an increase in TNF- α and IFN- γ production from PBMC after stimulation in aged horses.^{22,25}

Lymphocyte Function

One of the most consistent findings in immunosenescence is a decrease in the ability of lymphocytes to proliferate. Studies in rodents and people have shown impaired lymphocyte proliferation may involve a decrease in serum IL-2 concentration or IL-2 receptor expression.^{11,28,29} However, a decrease in lymphocyte proliferation has also been reported independent of these two factors, suggesting defects in intracellular signaling may also occur with aging.³⁰ Horses also have an age-associated decrease in lymphocyte proliferation^{20,22,31} that is not responsive to IL-2 supplementation and not associated with a change in lymphocyte IL-2 receptor expression.²⁰ This suggests that, similar to what has been reported in people, the age-associated defect in lymphocyte division in horses may be caused by alterations in intracellular signaling.

Neutrophil Function

Although immunosenescence affects primarily adaptive immunity, changes in innate immunity occur as well. Innate immunity includes the response of neutrophils and macrophages as a first-line defense against pathogens. Age-associated changes in neutrophil function that have been reported in people and rodents include decreases in phagocytosis, oxidative burst, chemokinesis, and chemotaxis. Neutrophil numbers appear to be maintained.³²⁻³⁵ In healthy aged horses, neutrophil adhesion, oxidative burst, and phagocytosis were all found to be unchanged, whereas chemotaxis was increased, compared with that observed in healthy adult horses.³⁶ In contrast, preliminary data suggest that horses

with PPID have impaired neutrophil function, perhaps contributing to the increased frequency of bacterial diseases such as abscesses and sinusitis. Because of the difficulty making an early diagnosis of PPID, it may be necessary to consider all aged horses at higher risk for neutrophil impairment unless proven otherwise.

Vaccine Responsiveness

One of the major health concerns regarding immunosenescence in people is a failure of the geriatric population to develop adequate titers after vaccination, particularly after influenza virus immunization.³⁷ As a result, morbidity and mortality caused by influenza infection is greatest in the very old. The response of aged horses to influenza vaccines has also been reported to be less robust than that of young or adult horses in several studies.^{19,37} Muirhead et al³⁸ reported a decrease in immunoglobulin subtypes immunoglobulin (Ig)G α and IgG β in aged horses, although single radial hemolysis titers suggested both adults and aged horses had an adequate response to be protective. With the use of a different vaccine, Horohov et al²⁰ reported a 10-fold decrease in resultant titers in aged ponies, compared with adults. Response to naive antigenic challenge was also examined in horses >20 years of age. Contrary to what has been reported in primates,^{39–41} the magnitude of a primary antibody response did not decline with age.³⁸ When a rabies vaccine was administered to rabies vaccine-naïve aged horses, the antibody titer after both the initial and second immunization did not differ from that of adult horses. However, in this study, 80% of both the control and aged population had low serum selenium concentrations, which could result in a suboptimal vaccine response in both groups. Further studies are needed to clarify the ability of the aged horse to respond to naive and amnestic vaccine challenge and to assist in the development of ideal vaccination protocol for geriatric horses.

Although the scientific literature is sparse, aged horses appear to be at greater risk of development of neurologic disease after West Nile infection compared with human adults.⁴² Although not necessarily the result of immune impairment, in an experimental model of neuropathogenic equine herpes virus (EHV-1) infection aged horses appear more susceptible to develop neurologic signs.⁴³ Enhanced risk of infectious disease may be minimized by a complete and appropriately administered vaccination schedule.

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