J Vet Intern Med 1999;13:206–212

Peripheral Blood Lymphocyte Subpopulations and Immunoglobulin Concentrations in Healthy Foals and Foals with *Rhodococcus equi* Pneumonia

M. Julia B.F. Flaminio, Bonnie R. Rush, and Wilma Shuman

Infectious diseases are common in foals aged 1–5 months. The objectives of this investigation were to evaluate immunologic parameters in foals from birth to weaning to establish reference values for the proportion of circulating lymphocytes that were helper (CD4+) or cytotoxic (CD8+) T cells, or B cells; to measure serum immunoglobulin (IgM and IgG) concentrations; and to compare these immunologic parameters to values in foals with naturally occurring *Rhodococcus equi* pneumonia and in adult horses. Peripheral blood lymphocyte subpopulations were determined by flow cytometric analysis, and serum IgG and IgM concentrations were determined by radial immunodiffusion. Flow cytometric analysis of lymphocyte subpopulations suggested age-related changes in the cell-mediated immune system in horses. Absolute circulating CD4+ and CD8+ T lymphocytes and B cells increased linearly up to 3 months of age. Circulating B cell concentrations from birth to 6 months of age were greater than values in adult horses and the lymphocyte differences among the age groups are mainly due to variation in B lymphocytes. Both absolute and proportional B cell concentrations were greater in foals with *R equi* pneumonia than in healthy foals at the same age. The increase in absolute cell counts of each subpopulation was dependent on the increase of absolute peripheral blood lymphocyte count. Serum IgG concentration increased linearly from 1 to 3 months of age, and serum IgM concentrations increased from 1 to 6 months of age. These data suggest age-dependent cell-mediated and humoral development in young foals.

Key words: Equine neonate; Flow cytometer; Horses; Immune system; Lymphocytes in foals.

Susceptibility to infection is largely influenced by the immune status of the foal. *Rhodococcus equi* is a facultative intracellular organism that produces pneumonia in foals between 2 and 5 months of age, a time when maternally derived immunity is declining and foal immunity should be gaining competence and maturity.[1–4] *Rhodococcus equi* pneumonia is virtually nonexistent in horses over 6 months of age, suggesting age-dependent maturation of protective mechanisms.[5,6]

Monoclonal antibodies and flow cytometric analysis have been used routinely in the diagnosis of many primary and secondary immunodeficiency syndromes in human patients.[7–9] In human patients, peripheral blood total and percentage lymphocyte subpopulation values and the ratio between helper (CD4+) and cytotoxic (CD8+) T cells are used to indicate susceptibility to diseases such as *R equi* and *Pneumocystis carinii* pneumonia.[10–14] Absolute and percentage values for most lymphocyte markers differ substantially between children and adults, and among children from different age groups.[7,15,16] Although serum immunoglobulin concentrations have been determined in foals,[17,18] reference values for peripheral blood CD4+ and CD8+ T lymphocyte subpopulations have not been reported.

The objectives of this investigation were to evaluate immunologic parameters in foals from birth to weaning to establish reference values for the proportion of circulating lymphocyte subpopulations (CD4+, CD8+, and B cells), identify variability in serum immunoglobulin (IgM and IgG) concentrations, and compare these immunologic parameters to values in foals with naturally occurring *R equi* pneumonia and in healthy adult horses. Determination of reference values for these immunologic parameters may permit investigation of primary immunologic dysfunction in foals.

From the Department of Clinical Sciences (Flaminio, Rush), and the Department of Diagnostic Medicine and Pathobiology (Shuman), College of Veterinary Medicine, Kansas State University, Manhattan, KS.

Reprint requests: M. Julia B.F. Flaminio, DVM, MS, The James A. Baker Institute, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853; e-mail: mbf6@cornell.edu.

Received September 10, 1997; Accepted August 24, 1998.

0891-6640/99/1303-00010/$3.00/0

Materials and Methods

Healthy Foals

Fourteen clinically healthy Quarter Horse foals (4 males, 10 females) owned by the Kansas State University Horse Unit, Department of Animal Sciences, and two local horse producers were investigated from birth to weaning. The materials and methods of this investigation were approved by the Institutional Animal Care and Use Committee of Kansas State University. Vaccination and deworming programs followed a conventional schedule for pregnant mares and foals. Foals were introduced to a preventive medicine program at 3 months of age (n = 11) and at 5 months of age (n = 3). All the foals lived in the pasture with their respective dams up to weaning, which was by 5 months of age, and were supplemented with grass hay and 16% protein concentrate.

Foals with R equi *Pneumonia*

Fifteen blood samples from foals from 6 weeks to 2 months of age with *R equi* pneumonia were transported overnight on ice to the Kansas State University Veterinary Clinical Laboratory and to the Kansas State University Clinical Immunology and Flow Cytometer Laboratory. The diagnosis of *R equi* was made by bacterial culture of transtracheal wash or postmortem sample. The foals included 9 males and 7 females; affected breeds involved Quarter Horse (n = 3), Thoroughbred (n = 12), and Saddlebred (n = 1) foals; and the samples originated from Louisiana (n = 5), Kentucky (n = 9), Washington (n = 1), and Kansas (1). The severity of clinical signs, treatment, environment, and management were different among the individuals in this population.

Healthy Adult Horses

Ten clinically healthy adult Quarter Horses (n = 5) and Thoroughbreds (n = 5) from 5 to 15 years of age (6 geldings and 4 mares) owned by the Kansas State University, College of Veterinary Medicine, were used in this investigation. Vaccination and deworming pro-

grams followed a conventional schedule for adult horses. All the horses lived in dry lots, and were fed grass hay and 12% protein concentrate.

Blood Collection

Blood samples were obtained via jugular venipuncture using ethylenediaminetetraacetic acid (EDTA) collection tubes and tubes without anticoagulant. The samples from healthy foals were collected within 24 hours of birth and monthly up to 6 months of age. The samples from foals with *R equi* pneumonia were collected when clinical signs of respiratory disease were apparent and data were added to the study when *R equi* was confirmed as the etiologic agent. Samples from healthy adult horses were all collected on the same day. Blood samples were submitted to the Kansas State University Veterinary Clinical Laboratory for complete white blood cell count, via automated cell counter calibrated for equine blood, and differential cell count, via percent count on slide smear. Blood samples were also submitted to the Kansas State University Clinical Immunology and Flow Cytometer Laboratory for the determination of lymphocyte subpopulations (CD4+ and CD8+ T lymphocytes, and B cells), and serum IgG and IgM concentrations.

Phenotypic Analysis of Lymphocytes

Peripheral blood lymphocytes were separated from blood preserved in EDTA. Five milliliters of 0.01 M calcium- and magnesium-free phosphate-buffered saline solution (CMF-PBS) (Gibco, Grand Island, NY) were added to 5 ml of blood and mixed well by inversion. The 10-mL blood–CMF-PBS mixture was carefully layered onto 8 mL of Ficoll 400 (Histopaque-1077, Sigma, St. Louis, MO). The tube was centrifuged at 250 × *g* (Beckman, Palo Alto, CA) for 30 minutes at room temperature. The opaque interface was aspirated and washed 3 times with 10 mL of CMF-PBS.[19] The cells were diluted in 5 mL of CMF-PBS and counted using an automated cell counter (Elzone 180, Partide Data Inc, Elmhurst, IL). The proportion of subpopulations of blood lymphocytes was determined by flow cytometric analysis. Flow cytometer quality control was done by using CaliBrite™ Beads (Becton Dickinson, San Jose, CA) daily. Lymphocytes were labeled with primary-stage reagent, murine monoclonal antibodies to equine cluster of differentiation (CD) antigens CD4+ (clone HB61A), CD8+ (clone HT14A) T lymphocytes, and B cells (clone B29A) (VMRD, Pullman, WA). Autofluorescence, background fluorescence, and nonspecific binding were determined by using unstained cells, second-stage conjugate alone, and IgG1 and IgG2a isotype control antibodies, respectively. Second-stage conjugate was goat anti-mouse immunoglobulins conjugated to fluorescein isothiocyanate (Sigma Immunochemical, St. Louis, MO). The labeled leukocytes were analyzed with a FACscan flow cytometer (Becton Dickinson) with an argon ion laser tuned at 488 nm excitation wavelength. Fluorescence parameters from single cells were collected using a logarithmic amplifier after gating on the combination of forward light scatter and perpendicular light scatter to avoid cell aggregates and debris. Ten thousand events were collected and analysis was recorded by gating the lymphocyte population. Data were acquired in list mode and processed using Lysis II software (Beckman). The fluorescence distribution was displayed as a single histogram.

IgG and IgM Isotypes

Serum IgG and IgM concentrations were determined using commercial single radial immunodiffusion plates containing monospecific antisera in buffered agarose (VMRD). A standard curve was established by plotting the diffusion diameter (mm) of the reference standards versus their immunoglobulin concentrations (200, 400, 800, and 1,600 mg/dL for IgG, and 14, 28, 55, and 110 mg/dL for IgM).

Statistical Analysis

Comparisons of immunologic variables among the different ages, between healthy foals and foals with *R equi* pneumonia, and between foals and adult horses were made using one-way analysis of variance.[20] When significant ($P < .05$) differences were determined, post hoc comparisons were made with Student–Newman–Keuls multiple comparisons test of least squares means.

Results

All healthy foals in the study had serum IgG concentrations greater than 800 mg/dL within the first 24 hours of life. Total and differential blood cell counts were within the normal reference range in all adult horses and healthy foals throughout the investigation. All foals with *R equi* pneumonia had leukocytosis with neutrophilia, hyperfibrinogenemia, and normal lymphocyte values.

Total circulating lymphocyte counts increased linearly up to 5 months of age (Fig 1). Circulating lymphocyte counts at birth were lower ($P < .01$) than values from 1 to 6 months of age. Circulating lymphocyte counts at 1 month of age were lower ($P < .005$) than values from 3 to 6 months of age. Circulating lymphocyte counts at 2 months of age were lower ($P < .05$) than the values from 4 to 6 months of age. Circulating lymphocyte counts were greater at 2, 3, 4, 5, and 6 months of age ($P < .002$), and in foals with *R equi* pneumonia ($P < .01$) than values in adult horses.

Absolute circulating CD4+ T lymphocyte concentrations increased linearly up to 3 months of age (Fig 2). Absolute circulating CD4+ T lymphocyte concentrations at birth were lower ($P < .05$) than values from 1 to 6 months of age. Absolute circulating CD4+ T lymphocyte concentrations at 1 month of age were lower ($P < .05$) than values at 3 and 6 months of age. Absolute circulating CD4+ T lymphocyte concentrations at 2, 3, 4, 5, and 6 months of age were greater ($P < .005$) than CD4+ values in adult horses. The percentage of circulating CD4+ T lymphocytes (Fig 3) slightly decreased by 4 months of age. The percentage of circulating CD4+ T lymphocytes at 1 month of age was greater ($P < .01$) than values from 4 to 6 months of age. The percentage of circulating CD4+ T lymphocytes was lower ($P < .001$) at 4, 5, and 6 months of age than the values in adult horses. Absolute and proportional circulating CD4+ T lymphocytes in foals within *R equi* pneumonia did not differ statistically from values in healthy foals at the same age and in adult horses.

Absolute circulating CD8+ T lymphocyte concentrations increased from birth to 3 months of age (Fig 2). Absolute circulating CD8+ T lymphocyte concentrations at birth were lower ($P < .005$) than values from 3 to 6 months of age. Absolute circulating CD8+ T lymphocyte concentrations were higher ($P < .01$) from 2 to 6 months of age than values in adult horses. Absolute circulating CD8+ T lymphocyte concentrations were greater ($P < .0005$) in foals with *R equi* pneumonia than CD8+ values in adult horses, but did not differ statistically from values in healthy foals at the same age. The percentage of circulating CD8+ T lymphocytes (Fig 3) was greater ($P < .05$) at 3, 4, and 6 months of age than CD8+ values in adult horses. The percentage of circulating CD8+ T lymphocytes was greater (*P*

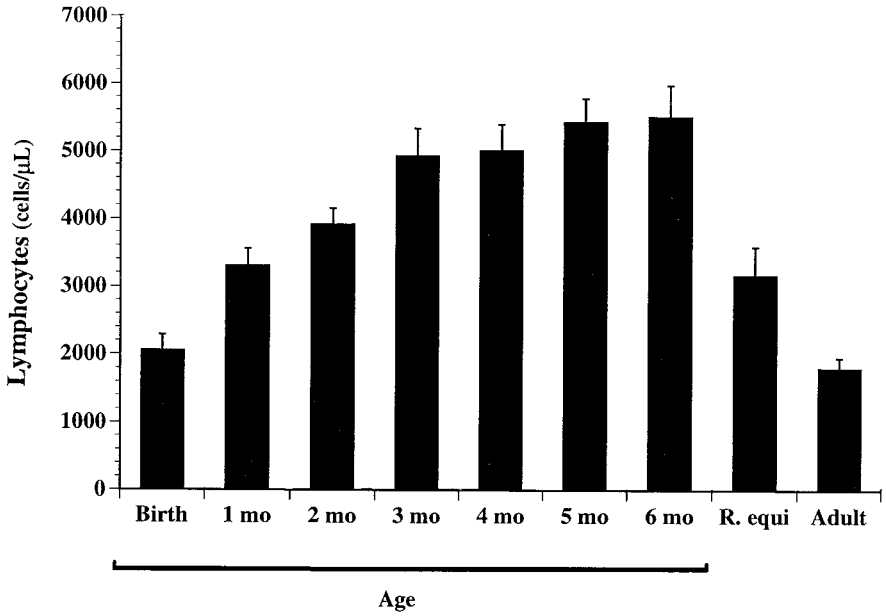

Fig 1. Peripheral blood total lymphocyte counts (cells/μL) in healthy foals from birth to 6 months of age, foals with *Rhodococcus equi* pneumonia, and healthy adult horses.

$< .005$) in foals with *R equi* pneumonia than the values in adult horses, but did not differ statistically from values in healthy foals at the same age.

The CD4+ : CD8+ ratio for foals from birth to weaning was 2.3 : 1, for foals with *R equi* pneumonia was 1.7 : 1, and for healthy adult horses was 3.6 : 1.

Absolute circulating B cell counts increased from birth to 6 months of age (Fig 2). Absolute circulating B cell concentrations at birth were lower ($P < .01$) than values from 2 to 6 months of age. Absolute circulating B cell concentrations at 1 month of age were lower ($P < .005$) than values from 3 to 6 months of age. Absolute circulating B cell concentrations in all foals from birth to weaning were greater ($P < .0001$) than values in adult horses. Absolute circulating B cell counts in foals with *R equi* pneumonia were greater than B cell counts in healthy foals of the same age ($P < .05$) and than B cell counts in adult horses ($P < .0001$). The percentage of B cells (Fig 3) was constant in foals from birth to weaning and was greater ($P < .0001$) than values in adult horses. The percentage of B cells in foals with *R equi* pneumonia was greater than the B cell values at the same age in healthy foals ($P < .0004$) and adult horses ($P < .0001$).

Serum IgG concentrations decreased from birth to 1 month of age, increased gradually from 2 to 3 months of age, and then slightly decreased at 4 and 5 months of age

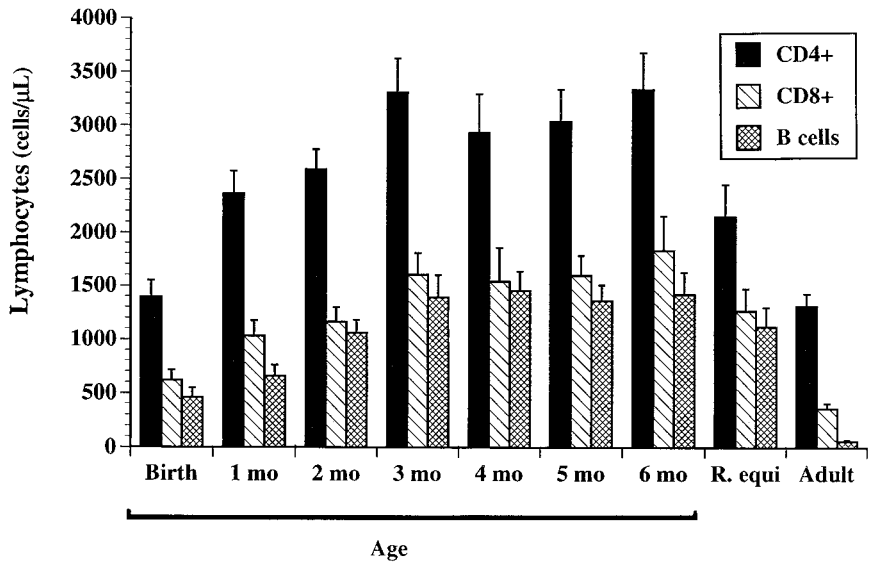

Fig 2. Peripheral blood lymphocyte subpopulation counts (cells/μL) in healthy foals from birth to 6 months of age, foals with *Rhodococcus equi* pneumonia, and healthy adult horses.

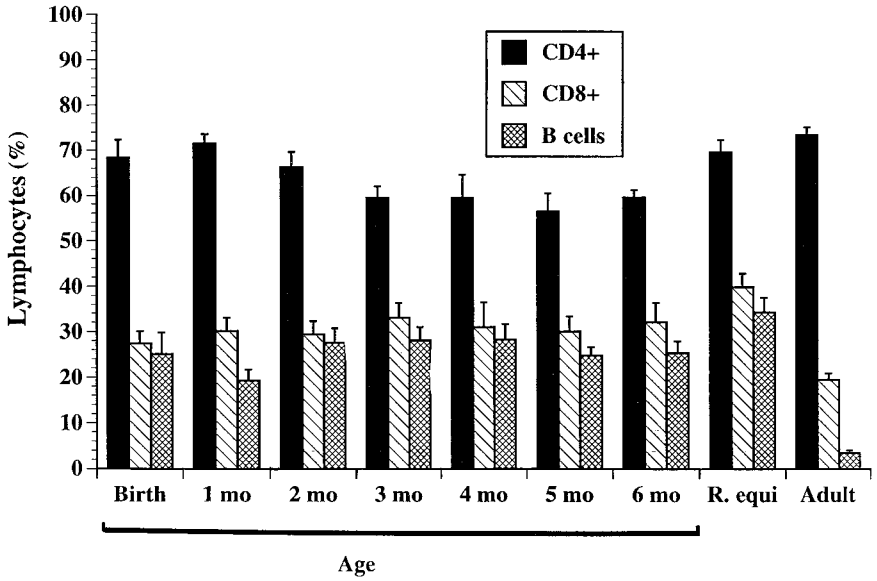

Fig 3. Peripheral blood lymphocyte subpopulations (%) in healthy foals from birth to 6 months of age, foals with *Rhodococcus equi* pneumonia, and healthy adult horses.

(Fig 4). Serum IgG concentrations at birth were greater ($P < .01$) than values at 1, 5, and 6 months of age. Serum IgG concentrations at 1 month of age were lower ($P < .05$) than values at 3 and 4 months of age. Serum IgG concentrations at 2 months of age were greater ($P < .005$) than values at 5 and 6 months of age. Serum IgG concentrations in foals with *R equi* pneumonia were greater ($P < .05$) than the values in adult horses, but did not differ statistically from values in healthy foals at the same age.

Serum IgM concentrations increased linearly from 2 to 6 months of age (Fig 5). Serum IgM concentrations at birth were lower ($P < .005$) than values at 5 and 6 months of age. Serum IgM concentrations at 1 month of age were lower ($P < .005$) than values at 4, 5, and 6 months of age. Serum IgM concentrations at 2 months of age were lower ($P < .01$) than values at 5 and 6 months of age. Serum IgM concentrations at 3 months of age were lower ($P < .01$) than values at 6 months of age. Serum IgM concentrations in foals with *R equi* pneumonia did not differ statistically from values in healthy foals at the same age and in adult horses.

Discussion

Absolute circulating CD4+ and CD8+ T lymphocytes and B cells increased linearly up to 3 months of age. At

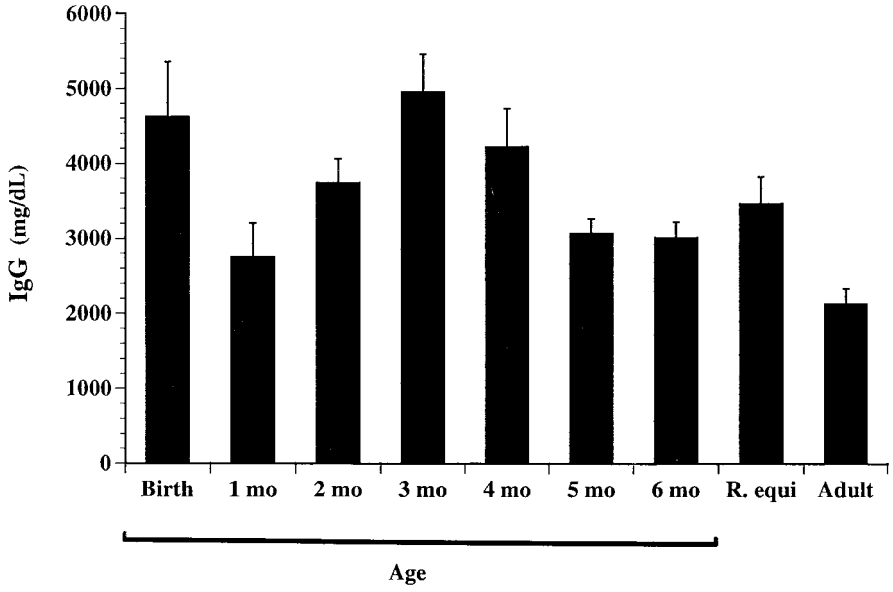

Fig 4. Serum immunoglobulin G (IgG) concentrations (mg/dL) in healthy foals from birth to 6 months of age, foals with *Rhodococcus equi* pneumonia, and healthy adult horses.

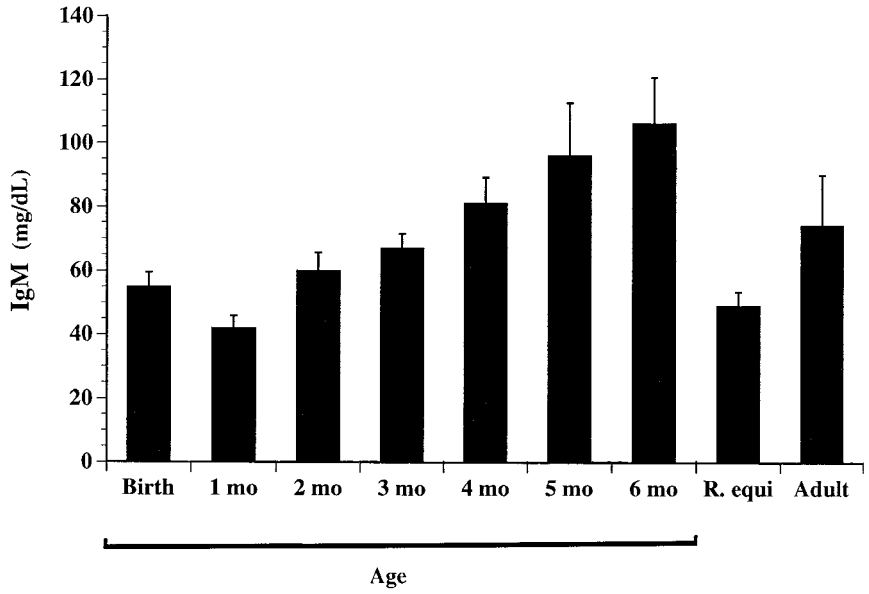

Fig 5. Serum immunoglobulin M (IgM) concentrations (mg/dL) in healthy foals from birth to 6 months of age, foals with *Rhodococcus equi* pneumonia, and healthy adult horses.

birth, absolute CD4+ and CD8+ T lymphocyte values were similar to values in adult horses, and absolute values in foals from 1 to 6 months of age were greater than values in adult horses. Circulating B cell concentrations from birth to 6 months of age were greater than values in adult horses. Therefore, an age-related increase occurs in the absolute T lymphocyte subpopulations that is dependent on the change of absolute circulating lymphocytes and not exclusively dependent on the percentage of each lymphocyte subpopulation. The decrease in B lymphocyte concentration seems to be dependent on the decrease of the percentage of this cell type. The significant increase in total lymphocyte count in foals in the first months of life may also suggest active immunity against first exposure to different microorganisms in the environment and development of immunologic memory. The results of this investigation are similar to the findings in humans, wherein there is a progressive increase and then decline occurs in absolute numbers of total lymphocyte, CD4+ and CD8+ T cell, and B cell concentrations with increasing age.[7,15,16] The results of this investigation differ from the values obtained in a previous study of neonatal foals, wherein approximately 5% of circulating mononuclear cells were B lymphocytes in the first days of life. The mean percentage of B lymphocytes increased from 20 to 40 days of age, when the values stabilized (12–15%), and the average values of circulating T lymphocytes ranged from 5.2 to 16.1%, which seemed independent of the age of the foals.[21] It is possible that the variance in the results is related to the different methods used in the studies.

The proportion of T lymphocyte subpopulations was constant among age groups throughout this study, although the proportion of CD4+ T lymphocytes from 4 to 6 months of age was lower than the values in adult horses. The proportion of B cells was significantly higher in foals than values in adult horses. These findings agree with those in the human literature, wherein percentages of B cells are higher in newborn infants than in adult humans,[16] and the percentages of CD4+ and CD8+ T lymphocytes and B cells are relatively constant in all age groups of infants. The lymphocyte differences among the age groups are mainly due to variation in B lymphocytes. Using the percentage values for lymphocyte subpopulations in children may be misleading because children have significantly higher total lymphocyte values than adults.[7] The use of absolute values of lymphocyte subpopulations in foals is reinforced by this investigation.

The sum of the percentages of lymphocyte subpopulations in healthy foals and foals with *R equi* pneumonia was greater than 100%. This was not observed in adult horses. The presence of CD4+/CD8+ double-positive T lymphocytes has not been confirmed in horses; however, the double expression of T cells has been well described in pigs, humans, and cattle.[22–24] Double-positive T lymphocytes are nonactive immature cells originating from the thymic cortex, which may reach the blood stream in young animals.[25] This study could not confirm the existence of double-positive T lymphocytes in young foals. Because monocytes and macrophages may express CD4 and CD8 binding,[26,27] we may speculate that these cells contribute to high counts of the sum of lymphocyte subpopulations above 100%. The expression of a pan-granulocyte/monocyte surface molecule was not measured in this investigation to observe the percent of monocytes present in the analyzed gated area, although this possibility should be considered when evaluating lymphocyte subpopulation counts in young foals. Further studies are necessary to indicate the differences between adult horses' and foals' cellular expression and to develop a better understanding of the lymphocyte phenotyping subpopulation counts in the young foals.

Foals with *R equi* pneumonia had similar or higher total and percentage CD4+ and CD8+ T lymphocyte and B cell concentrations in peripheral blood than adult horse values. Evaluation of CD4+ and CD8+ lymphocytes in foals with clinical *R equi* pneumonia (from 6 weeks to 2 months of

age) did not identify differences in the total and percent T lymphocyte subpopulation values compared to healthy foals of same age. Both absolute and proportional B cell concentrations were greater in foals with *R equi* pneumonia than the values in healthy foals at the same age. We suspect that the period of susceptibility to infection likely occurs within the first weeks of life, which may be a consequence of inadequate passive transfer or maturation of T cell activity. When clinical signs are detected at 6 weeks to 2 months of age, T lymphocyte subpopulation values and ratios seem to be within normal limits.

Total and percent CD5+, CD4+, and CD8+ lymphocyte values are lower in bronchoalveolar lavage from normal young foals than in adult horses, and lymphocyte subpopulations increase to adult values in the first 3–10 weeks of age.[28] The increase in lymphocyte subpopulations reported in the lungs of young normal foals follows the increase in peripheral blood in a similar pattern to this study. It is possible that introduction of lymphocytes in peripheral blood and in tissues may occur concomitantly in young foals. Little information is available to explain the regulation of migration, distribution, and production of lymphocyte subpopulations in the different tissues.[29] The susceptibility of the foal lung to *R equi* may be due to a relative state of immunodeficiency to intracellular pathogens, peripherally in the blood or locally in the lung.[4] In rats and humans, an age-dependent variation exists in the composition and development of immunocompetent cells in specific organs, such as the respiratory and intestinal mucosa.[30,31] On the other hand, foals are able to combat equine herpesvirus-1 and recover from disease before mechanisms of immunity, such as circulating antibody, are detectable.[32–34] This may occur due to functional cell-mediated immune mechanisms within the respiratory system at this age. The pathogenicity of the organism should be considered when studying the development of the immune system in young foals.

Antibody and both CD4+ and CD8+ T lymphocytes likely play a role in protection against *R equi* infection. In one experimental model, selective depletion of CD8+ lymphocytes in individuals with *R equi* infection resulted in progression of clinical disease.[5,6,35–37] In a different experimental model, mice lacking CD8+ T lymphocytes were able to clear *R equi*, whereas those lacking CD4+ T lymphocytes developed persistent infection.[38,39] Immune clearance of *R equi* may require the Th1 lymphocyte response. Interferon gamma and interleukin-2, products of Th1 response, activate macrophages for their killing activity and stimulate phagolysosomal fusion. The CD8+ T lymphocytes may play a supportive role in the clearance of intracellular bacteria, with direct cytotoxicity of infected cells and release of cytokines.[35,40]

Maternal immunoglobulin-derived IgG and IgM were expected to decrease in the first month of life because of consumption during protection against environmental pathogens and catabolization. Immunoglobulin G peaked by 3 months of age, and values decreased gradually to adult values by 5 months of age. Immunoglobulin M increased linearly during the investigation and reached concentrations similar to those in adult horses by 6 months of age. The linear increase in serum IgM concentration may be associated with primary and secondary exposure to environmental antigens of foals in their first 6 months of life. Serum IgG concentrations were less pronounced after 4 months of age, which may indicate secondary exposure to antigens.

Foals that do not receive an adequate amount of maternally transferred antibodies may be more susceptible to *R equi* infection during their first exposure. If the number of bacteria overwhelms their phagocytic and killing capacity, the disease may develop. Specific antibodies against *R equi* may block the initial stages of cellular infection, alter the route by which bacteria enter the macrophage, and decrease inhibition of phagolysosomal fusion.[41] The presence of antibodies via maternal passive transfer or hyperimmune plasma transfusion must occur before the bacterial exposure to result in protection against *R equi* organisms.[42] Immunologic activation is required for the effective killing of *R equi* by alveolar macrophages. Nonexposed foals are able to phagocytize the nonopsonized organism but unable to kill it, whereas *R equi*-exposed foals acquire this capacity.[43]

The humoral immune system seems to be well established by 3 months of age, wherein serum IgG concentrations are greater than concentrations in adult horses. It has been suggested that immunizing blood mares to provide passive protection to neonates can bypass the problems of immunologic immaturity in the neonate. However, this practice may delay antibody production in the first year of life.[44] Higher serum IgG concentrations are found in colostrum-deprived foals than in naturally nursed foals between 3 and 5 months of age.[45] Humoral response to vaccination and natural challenge likely is competent at birth; however, very high maternally derived immunoglobulin concentrations in early life possibly may delay the foal's response to natural challenge and vaccination. This study did not investigate specific immune response to vaccination or environmental challenge in healthy foals or foals that developed *R equi* pneumonia. Further investigations are necessary to determine the most appropriate vaccination program for a given organism in mares and foals.

Acknowledgments

We thank Mrs Elizabeth M. Szczurek-Raub and Dr Matthew J. Sylte, DVM, MS, for their technical support. This study was supported by a grant from the American Quarter Horse Association.

References

1. Perryman JE. Immunological management of young foals. Compend Cont Educ Pract Vet 1981;3:S223–S228.

2. Buergelt CD, Hines SA, Cantor G, et al. A retrospective study of proliferative interstitial lung disease of horses in Florida. Vet Pathol 1986;23:750–756.

3. Prescott JF, Ogilvie TH, Markham RJI. Lymphocyte immunostimulation in the diagnosis of *Corynebacterium equi* pneumonia in foals. Am J Vet Res 1980;41:2073–2075.

4. Prescott JF. Immunodeficiency and serious pneumonia in foals: The plot thickens. Equine Vet J 1993;25:88–89.

5. Ellenberger MA, Kaeberle ML, Roth JA. Equine cell-mediated immune response to *R equi.* Am J Vet Res 1984;45:2424–2427.

6. Kaufmann SHE. CD8+ T-lymphocytes in intracellular microbial infections. Immunol Today 1988;9:168–174.

7. Heldrup J, Kalm O, Prellner K. Blood T and B lymphocyte sub-

populations in healthy infants and children. Acta Paediatr 1992;81: 125–132.

8. Series IM, Pichette J, Carrier C, et al. Quantitative analysis of T and B cell subsets in healthy and sick premature infants. Early Hum Dev 1991;26:143–154.

9. Rosen FS, Cooper M, Wedgwood RJP. The primary immunodeficiencies. Review article. Med Prog 1995;333:431–440.

10. Emmons W, Reichwein B, Winslow DL. *R equi* infection in the patient with AIDS: Literature review and report of an unusual case. Rev Infect Dis 1991;13:91–96.

11. Harvey RL, Sunstrom JC. *R equi* infection in patients with and without human immunodeficiency virus infection. Rev Infect Dis 1991;13:139–145.

12. Duncan RA, von Reyn F, Alliegro GM, et al. Idiopathic CD4+ T-lymphocytopenia—Four patients with opportunistic infection and no evidence of HIV infection. N Engl J Med 1993;328:393–398.

13. Masur H, Oguibene FP, Yarchoan R, et al. CD4+ counts as predictors of opportunistic pneumonia in human immunodeficiency virus (HIV) infection. Ann Intern Med 1989;111:223–231.

14. Phair J, Munoz A, Detels R, et al. The risk of *Pneumocystis carinii* pneumonia among men infected with human imunodeficiency virus type 1. N Engl J Med 1990;322:161–165.

15. Stern DA, Hicks MJ, Martinez FD. Lymphocyte subpopulation number and function in infancy. Dev Immunol 1992;2:175–179.

16. Erkeller-Yuksel FM, Deneys V, Yuksel B, et al. Age related changes in human blood lymphocyte subpopulations. J Pediatr 1992;120:216–222.

17. McGuire TC, Crawford TB. Passive immunity in the foal: Measurement of immunoglobulin classes and specific antibody. Am J Vet Res 1973;34:1299–1303.

18. MacDougall DF. Immunoglobulin metabolism in the neonatal foal. J Reprod Fertil 1975;23:739–742.

19. Akens MK, Holznagel E, Franchini M, et al. Comparative analysis of equine lymphocyte subsets in whole blood and gradient-purified samples. Vet Imunol Immunopathol 1997;58:231–237.

20. Ott RL. An Introduction to Statistical Methods and Data Analysis, 4th ed. Belmont, CA: Wadsworth Publishing Co; 1993:769–998.

21. Frymus T, Schollenberger A. Circulating B and T lymphocytes in foals during first five months of life. Zentralbl Veterinarmed 1979;26:722–728.

22. Pescovitz MC, Sakopoulos AG, Gaddy JA, et al. Porcine peripheral blood CD4+/CD8+ dual expressing T-cells. Vet Immunol Immunopathol 1994;43:53–62.

23. Blue ML, Daley JF, Levine H, et al. Coexpression of T4 and T8 on peripheral blood T cells demonstrated by two color fluorescence flow cytometry. J Immunol 1985;134:2281–2289.

24. Mathy NL, Walker J, Lee RP. Characterization of cytokine profiles and double-positive lymphocyte subpopulations in normal bovine lungs. Am J Vet Res 1997;58:969–975.

25. Fridkis-Hareli M, Mehr R, Abel L, Globerson A. Developmental interactions of CD4 T cells and thymocytes: Age-related differential effects. Mech Ageing Dev 1994;73:169–178.

26. Denny TN, Stein D, Mui T, et al. Quantitative determination on surface antibody binding capacities of immune subsets present in peripheral blood of healthy adult donors. Cytometry 1996;26:265–274.

27. Grunig G, Triplett L, Canady LK, et al. The maternal leucocyte response to the endometrial cups in horses is correlated with the developmental stages of the invasive trophoblast cells. Placenta 1995;16: 539–559.

28. Balson GA, Smith GD, Yager JA. Immunophenotypic analysis of foal bronchoalveolar lavage lymphocytes. Vet Microbiol 1997;56: 237–246.

29. Westermann J, Pabst R. Lymphocyte subsets in the blood: A diagnostic window on the lymphoid system? Immunol Today 1990;11: 406–409.

30. Jecker P, Ptok M, Pabst R, et al. Age dependency of the composition of the immunocompetent cells and the expression of adhesion molecules in rat laryngeal mucosa. Laryngoscope 1996;106:733–738.

31. Insoft RM, Sanderson IR, Walker WA. Development of immune function in the intestine and its role in neonatal diseases. Pediatr Clin North Am 1996;43:551–571.

32. Chong YC, Duffus WPH. Immune responses of specific pathogen free foals to EHV-1 infection. Vet Microbiol 1992;32:215–228.

33. Lunn DP, Holmes MA, Gibson J, et al. Hematological changes and equine lymphocyte subpopulation kinetics during primary infection and attempted re-infection of specific pathogen free foals with EHV-1. Equine Vet J 1991;12:35–40.

34. Gibson JS, O'Neill TO, Thackray A, et al. Serological responses of specific pathogen-free foals to equine herpesvirus-1: Primary and secondary infection, and reactivation. Vet Microbiol 1992;32:199–214.

35. Ellenberger MA, Kaeberle ML, Roth JA. Equine humoral immune response to *R equi.* Am J Vet Res 1984;45:2428–2430.

36. Nordmann P, Ronco E, Nauciel C. Role of T-lymphocyte subsets in *R equi* infection. Infect Immun 1992;60:2748–2752.

37. Takai S, Kitajima H, Tamaa Y, et al. Humoral antibody response to the antigens of virulent *R equi* in foals. J Equine Sci 1994;5:121–126.

38. Ross TL, Balson GA, Miners JS, et al. Role of CD4+, CD8+ and double negative T-cells in the protection of SCID/beige mice against respiratory challenge with *R equi.* Can J Vet Res 1996;60:186–192.

39. Kanaly ST, Hines SA, Palmer GH. Failure of pulmonary clearance of *R equi* in CD4+ T lymphocyte deficient transgenic mice. Infect Immun 1993;61:4929–4932.

40. Hines SA, Kanaly ST, Byrne BA, Palmer GH. Immunity to *R equi.* Vet Microbiol 1997;56:177–185.

41. Speert DP. Macrophages in bacterial infection. In: Lewis CE, McGee JO, eds. The Macrophage. Oxford, UK: Oxford University Press; 1992:215–263.

42. Chaffin MK, Martens RJ, Martens JG, et al. Therapeutic effects of immune plasma in foals with *Rhodococcus equi* pneumonia. Equine Vet J 1991;21(Suppl):23–29.

43. Hietala SK, Ardans AA. Interaction of *Rhodococcus equi* with phagocytic cells from *R equi*-exposed and non-exposed foals. Vet Microbiol 1987;14:307–320.

44. Insel RA, Amstey M, Woodin K, et al. Maternal immunization to prevent infectious diseases in the neonate or infant. Int J Technol Assess Health Care 1994;10:143–153.

45. Bernoco MM, Liu IKM, Willits NH. Hemolytic complement activity and concentrations of its third component during maturation of immune response in colostrum-deprived foals. Am J Vet Res 1994;55:928–933.