Quantitation of Immunoglobulin-Bearing Lymphocytes and Lymphocyte Response to Mitogens in Horses Persistently Infected by Equine Infectious Anemia Virus

KEITH L. BANKS AND J. B. HENSON

Department of Veterinary Pathology, Washington State University, Pullman, Washington 99163

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A defect in lymphocyte function could be responsible for persistent infection by the equine infectious anemia virus. The number of lymphocytes bearing surface immunoglobulin, as detected by immunofluorescence, and lymphocyte response to mitogens were the same in uninfected and equine infectious anemia-infected animals. A defect in T or B lymphocyte numbers or ability to respond to stimuli was not detected in this chronic virus disease.

Cellular immunity is an important host defense to some virus infections (4). Natural (4) and experimentally (M. S. Hirsch, Fed. Proc. 29:169–170, 1970) induced depression of cellular immune mechanisms often is followed by impaired resistance to virus infection. In vitro studies indicate that immune lymphocytes, independent of antibody and interferon, can destroy virus-infected cells (11). Other virus infections may result in a depression of cellular immune processes, including infection by mumps, (5), rubella (12), measles (15), infectious hepatitis (17), and lactic dehydrogenase virus (7).

Equine infectious anemia (EIA) is a lymphoproliferative disease, and the host is continuously viremic, although EIA virus antibody is present (9). One possible explanation of the persistence of the agent could be a virus-induced lymphocyte defect, subverting protective cellular immune processes.

Studies were conducted on peripheral blood leukocytes of uninfected and EIA-infected horses. Horses were experimentally infected by the EIA virus by inoculation of serum from other infected animals. Only the infected animals showed the clinical signs of EIA and had precipitating antibody against virus antigens (6, 9). Mononuclear leukocytes were isolated by the method of Boyum (3), washed three times, and resuspended at 10^7 to 5 × 10^8 cells/ml. The preparation contained 73 to 97% lymphocytes, with the remaining cells monocytes.

Cells were stained with fluorescent-labeled goat anti-equine gamma globulin to quantitate the number of immunoglobulin-bearing lymphocytes. Goat anti-equine immunoglobulin antiserum was prepared as previously described (2), and the gamma globulins were isolated by ammonium sulfate precipitation and adjusted with 0.5 M bicarbonate buffer (pH 9.5) to a concentration of 10 mg of protein/ml. Fluorescein isothiocyanate (200 μg per mg of protein) was added to the gamma globulin and mixed for 20 h, and the nonattached fluorescein was removed by dialysis. Immunoglobulin of preimmune goat serum was identically isolated and labeled to serve as one control. Both preparations had an F:PI ratio of approximately 14. Isolated cells (0.1 ml) and labeled antiserum (0.1 ml) were incubated together for 30 min at room temperature. Cells were then washed two times by layering over 30% newborn calf serum in Hanks balanced salt solution followed by centrifugation at 450 g for 5 min. The percentage of fluorescent cells was determined by using a Zeiss Ultraphot dark-field microscope with a 200-W HBO mercury lamp.

The number and characteristics of stained lymphocytes of eight uninfected and eight EIA-infected horses were very similar. At the time of examination, the length of infection of the horses with EIA was from 15 days to 4 years, with three of the horses in acute disease, while the other five were in quiescent periods of the disease. A mean of 27% of the uninfected horse
lymphocytes (range 23–35%) and 24% of the EIA-infected horse lymphocytes (range 18–29%) were stained (Table 1). The majority of the stained cells of both uninfected and EIA-infected horses had distinct aggregates of fluorescent material at one area of the surface membrane. The lymphocyte staining was shown to be specific for immunoglobulins by blocking the positive reaction with isolated horse immunoglobulins. Cells were not stained by fluorescein-labeled preimmune goat serum, and the labeled equine gamma globulins did not attach to horse polymorphonuclear cells or erythrocytes.

The lymphocyte response to phytohemmagglutinin (PHA) and pokeweed mitogen (PWM) was determined by cellular tritiated thymidine incorporation. The methods were exactly as previously described (1). Four concentrations of each mitogen were studied. The data are expressed as the stimulation index, which is determined by dividing the mean count per minute of tubes receiving the stimulant by the mean count per minute of tubes receiving no stimulants.

The response of lymphocytes from uninfected and EIA-infected horses to PHA and PWM is presented in Fig. 1 and 2, respectively. Lymphocytes averaged 54% of the cultured cells, and an average of 65% of both uninfected and EIA-infected cells was alive at the end of the 3-day period. At least nine EIA-infected horses and eight uninfected horses were examined at each mitogen concentration. Four EIA-infected horses were in acute disease when tested, and the length of infection varied from 12 days to 4.5 years. The response of lymphocytes of both groups was very similar at each mitogen concentration. No significant difference was observed in the range of responses and the mean stimulation index.

![Fig. 1. Proliferative response of lymphocytes from uninfected and EIA-infected horses to four concentrations of PHA. The stimulation index is determined by dividing the mean count per minute of cultures with PHA by the mean count per minute of cultures without PHA. The lines represent mean and standard errors for grouped data.](http://iai.asm.org/)
Two populations of horse lymphocytes were identified by immunofluorescence and found to be present in similar numbers in uninfected and EIA-infected horses. One group of cells had immunoglobulin attached, whereas the other group was consistently negative. In mice (14) and chickens (13), those cells bearing immunoglobulin are the thymus-independent lymphocyte or B cell, and the remaining cells are the thymus-dependent lymphocyte or T cell. The B cell is responsible for antibody production, sometimes in cooperation with the T cell (10), whereas the T cell is active in the cellular immune processes (16). The exact origin of the equine cells can only be implied from the studies in mice; however, the findings suggest that EIA infection does not result in a defect in the number of B or T lymphocytes. Lymphocyte transformation by plant mitogens can be used as an indication of the lymphocyte proliferative capability. Studies in mice, chickens, and man have shown that soluble PHA stimulates T cells, whereas PWM stimulates both T and B cells (8). The finding that EIA-infected horses respond normally to mitogens indicates that there is not a defect in B or T lymphocyte ability to respond to stimulation.

The mechanisms of viral persistence in EIA remain poorly understood, although one means of virus persistence could be an impairment of host defense. Antibody to EIA virus has been readily detected (6), and the total number of B and T lymphocytes and their ability to respond to stimuli are within normal ranges. This suggests that there is not a defect in lymphocyte function in EIA, although specific depression of lymphocytes sensitive to viral antigen could be occurring and not be detected by the experiments reported here.

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LITERATURE CITED

8. Janossy, G., and M. F. Greaves. 1971. Lymphocyte activation. I. Response of T and B lymphocytes to...