UDC 57: 61
CODEN: PDBI-A
YU ISSN 0031-5362
Conference paper

Lysis of autologous virus infected cells by direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity in lentiviral infection: equine infectious anemia

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Received September 26, 1989

The relapsing nature of lentiviral infections, particularly concerning restriction of viral gene expression, virus persistence and replication in the presence of host immune responses is most pronounced in equine infectious anemia (EIA) *(1)*. The immune response in EIA is complex, due to the fact that immunological functions are involved in both clinical disease and development of lesions and in most cases, eventual control of the disease process. Evidence suggests that the primary mechanism of control may be direct destruction of virus infected cells by cytotoxic T lymphocytes since antibody-dependent cell-mediated cytotoxicity (ADCC) is inhibited by a particular class of equine immunoglobulins, IgG (T), which are known not to interact with Fc-IgG receptor bearing cells following formation of antigen-antibody complexes *(3)*. However, the control of viremia by humoral and/or cell-mediated immune responses is not accompanied by elimination of latently infected cells, which persist indefinitely as a potential cause of recrudescence of acute disease. Thus, the objectives of our study were: (1) to establish the extent of the elimination of virus infected cells by direct cytotoxicity and ADCC during the acute and chronic stage of EIA; and (2) to determine the significance of the suppression of lymphocyte stimulation responses to mitogens and specific antigens by immune serum obtained from acutely and chronically infected horses.

TABLE 1

In vitro responses of peripheral blood lymphocytes (PBL) from noninfected horses and horses with acute or chronic form of equine infectious anemia (EIA) to mitogens and specific antigens in the presence of nonimmune horse serum (NS) and in the serum obtained in the acute (AS) or chronic (CS) form of the disease.

Source of PBL	Serum	Stimulator				
		None	ConA (1μg/well)	PWM (2μg/well)	Protein A (2μg/well)	Allogenic cells (10^5 cells/well)
		Mean CPM × 10^3 ± SD				
Non infected	NS	0.7±0.1	14.8±3.7	12.8±1.1	43.2±8.0	8.0±2.6
– horses	AS	0.6±0.1	15.3±2.0	12.5±1.3	25.0±9.2*	2.4±1.3*
N = 8	CS	0.3±0.1	9.5±1.8	12.0±1.1	11.4±3.8**	NT
EIA Infected horses	NS	0.3±0.1	5.1±2.1	2.1±0.9	8.8±3.2	5.2±1.3
– acute form	AS	0.7±0.1	2.7±0.8	1.7±0.7	2.9±1.7**	0.4±0.3**
of the disease	CS	0.6±0.3	3.3±0.1	1.9±0.6	2.0±0.9**	1.5±1.1**
N = 9						
– chronic form of	NS	1.1±0.2	8.2±1.5	5.5±1.1	32.9±7.5	10.2±2.3
the disease	AS	0.6±0.2	NT	NT	15.1±9.5*	2.0±0.8**
N = 12	CS	0.2±0.1	6.6±1.2	5.6±1.0	10.9±4.2**	NT

*$p < 0.05$ **$p < 0.01$ NT = Not tested

Presented at the 2nd Congress of Yugoslav Immunologists, Vrnjačka Banja, 29-05-6-06, 1989

TABLE 2

Cytotoxic activity of peripheral blood lymphocytes (PBL) from horses with acute or chronic form of equine infectious anemia (EIA) and noninfected animals against autologous and heterologous PHA lymphoblasts (LB) preincubated in nonimmune horse serum and in the serum obtained in the acute form of the disease

Source of effector PBL	Targets (% lysis)					
	Healthy control		Acute EIA		Chronic EIA	
	LB[a]	LB[b]	LB[a]	LB[b]	LB[a]	LB[b]
Noninfected horses	0/10[c]*	0/10*	0/10	0/10	NT	NT
EIA infected horses						
– acute form of the disease	0/11	0/11	2/11*	8/11*	NT	NT
– chronic form of the disease	0/8	0/8	0/8	0/8	0/19*	2/19*

[a]*Preincubated in nonimmune horse serum.*
[b]*Preincubated in the serum obtained in the acute form of EIA.*
[c]*No. of cytotoxic positive horses/No. of tested horses.*
**Autologous PHA lymphoblates.*
NT = Not tested

Ten horses were determined to be free of EIA virus by a slightly modified agar–gel immunodiffusion (AGID) test *(2)*. Nineteen of AGID test–positive horses, which had been naturally infected for 5 to 10 years, were classified as having the chronic form of the disease. Eleven horses were experimentally infected using Wyoming strain of EIA virus and after the first peak of pyrexia they were referred to as acutely diseased animals. PBL were isolated and the proliferation assays performed by the procedures described earlier *(5)*. Cell–mediated lympholysis (CML) and ADCC assays, based on the method previously described *(4)*, were employed. The presence of immune serum suppressed PBL responses to Protein A and allogeneic cells in horses with acute or chronic form of EIA, as well as in noninfected animals (Table 1). PBL from 8 of 11 acutely diseased horses and 2 of 19 chronically infected horses, demonstrated ADCC activity against autologous PHA lymphoblasts preincubated in the serum obtained during the acute phase of EIA (Table 2). CML was observed only in two horses with acute EIA, but the percent of lysis was rather low (6.5% and 8.4%, respectively). A decrease in the percent of lysis could be caused by the substitution of IgG with IgG (T) antibodies to EIA virus which bind to the T–cell receptors and block their interaction with class II MHC molecules on antigen presenting cells.

Acknowledgements.
We thank Prof. Dr. Berislav Jukić, Veterinariy Faculty, University of Zagreb for providing the AGID test results and Mrs. Ivanka Rumora for typing the manuscript.

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Presented at the 2nd Congress of Yugoslav Immunologists, Vrnjačka Banja, 29-05–6-06, 1989