Acta virol. 37: 403–406, 1993

DETECTION OF ANTI-DNA ANTIBODY RESPONSES IN RABBITS INFECTED WITH RINDERPEST VIRUS BY ELISA

B. RATTAN*, B. SHARMA, R. P. BANSAL

Division of Virology, Indian Veterinary Research Institute, Mukteswar-Kumaon, Nainital, U. P., PIN-263 138, India

Received September 24, 1992; revised February 2, 1993

Summary. – ELISA was standardized to detect anti-DNA antibodies (ADAbs) in sera of rabbits infected with rinderpest virus (RPV). These antibodies were found in late (28th and 36th day post inoculation (dpi)) sera of rabbits infected with rabbit virulent lapinized RPV, but not in early (7th dpi) sera of the same rabbits. ADAbs were not found either in early or late sera of rabbits infected with attenuated RBOK strain of RPV. Rabbit anti-RPV hyperimmune serum was also found to be positive for ADAbs. Detection of these antibodies by ELISA proved DNA to be one of the target antigens for antinuclear antibodies induced in RPV infection.

Key words: rinderpest virus; anti-DNA antibodies; rabbits; ELISA

Induction of different types of auto-antibodies after virus infection and demonstration of virus-like structures in tissues of patients with autoimmune diseases led to the concept that viruses may act as trigger for autoimmunity. Among several candidate viruses which trigger autoimmunity, morbilliviruses had been of particular interest. In measles patients, lymphocytotoxic (Mottironi and Terasaki, 1970) and demyelinating (Yonezawa, 1971) antibodies have been demonstrated. Measles virus (MV)-antibodies (Filimonova *et al.*, 1982) and MV genomic RNA in lymphocytes (Andjaparidze *et al.*, 1989) have been reported in patients with autoimmune disorders. Another morbillivirus, RPV, has also been shown to induce the autoantibody production in the form of antinuclear antibodies (ANAs) (Fukuda and Yamanouchi, 1976, 1981; Imaoka *et al.*, 1988*a,b*). Based on immunofluorescence studies, Imaoka *et al.* (1988*b*) provided indirect evidence for DNA as one of the target antigens for ANAs induced by RPV. ELISA has been used to specifically identify ADAbs in human sera (Ali and Ali, 1983; Edberg and Taylor, 1986; Gripenberg and Kurki, 1986; Hoch and Schwaber, 1986). This is the first report on use of ELISA for detection of ADAbs in RPV infected rabbits' sera and on an unequivocal evidence that DNA is the target antigen for ANAs induced in this infection.

*Present address: National Research Center on Equines, Sirsa Road, Hisar-125 001 (Haryana), India

Healthy male rabbits (aged 3–4 months) were randomly divided into 3 groups. Group I (n = 3) rabbits were infected intravenously with 1 ml of $10^{4.76}$ $TCID_{50}$ of Vero adapted RBOK strain of RPV at 5th passage level. Group II (n = 3) rabbits were similarly inoculated with Vero adapted lapinized RPV (LRPV) at 8th passage level, and group III (n = 6) was uninfected control rabbits, kept separately. Serum samples were collected on 7th and 28th dpi. Group II rabbits were also bled on 36th dpi. Sero-conversion was confirmed by standard neutralization test and ELISA. Rabbit anti-RPV hyperimmune serum (RaRPHIS) was also included in this study.

Indirect ELISA was used to detect ADAbs in rabbits' sera. DNA used was purified from goat liver and assayed according to Sambrook *et al.* (1989). Only DNA preparations having absorbancy ratio (A 260/280 nm) of 2 or more were used. ELISA microtiter plates (Dynatech) were coated with 0.1 ml of 5 μg/ml of DNA diluted in 0.05 mol/l carbonate-bicarbonate buffer, pH 9.6 and incubated at 4 °C for 18 hrs. Before addition of each subsequent reagent, the wells were washed with Dulbecco's PBS containing 0.05 % Tween-20 (PBST). Blocking was effected with 0.1 ml of 2 % gelatin in PBST at 37 °C for 2 hrs. Then 0.075 ml of test sera diluted 1:200 in PBST was added in triplicate and the plates were incubated at 37 °C for 2 hrs. Swine anti-rabbit HRPO conjugate (Dakopatts, Netherlands) was used to probe the bound antibodies and to check for any reactivity of conjugate with coated DNA, without serum conjugate control was kept. O-phenylenediamine – H_2O_2 and 3 N H_2SO_4 were used as substrate and stop solutions, respectively. A_{492} was read in an ELISA reader and a 2-fold or higher difference between control and test sera was taken for positivity.

The ELISA was found to be suitable for detection of ADAbs in RPV-infected rabbits' sera (Fig. 1). The highest content of ADAbs was found in 28th dpi sera of rabbits, infected with rabbit virulent LRPV and by 36th dpi these antibodies showed a slight decline. Early (7th dpi) sera from the same rabbits didn't have significant ADAbs. This is in agreement with the findings of immunofluorescence studies (Fukuda and Yamanouchi, 1981; Imaoka *et al.*, 1988*a*). ADAbs could not be detected in early or late sera of rabbits inoculated with the attenuated RBOK strain of RPV. Fukuda and Yamanouchi (1981) also could not detect ANAs in rabbits inoculated with another attenuated strain, the lapinized avianized (LA), of RPV. LRPV has been shown to cause marked destruction of T lymphocytes, leading to necrosis of the T-dependent area in the lymphoid

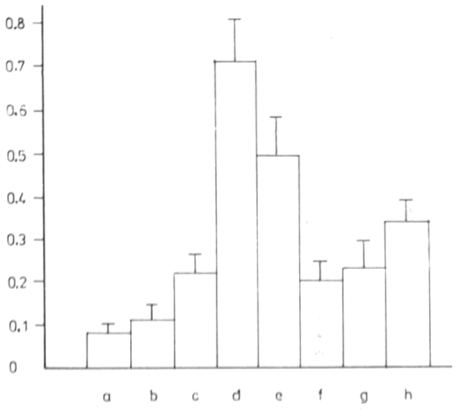

Fig. 1
Detection of anti-DNA antibodies in different rabbit sera by ELISA
Abscissa: a – without serum, conjugate control; b – healthy rabbit serum; c – early (7th dpi) serum from LRPV infected rabbits; d – late (28th dpi) serum from LRPV infected rabbits; e – late (36th dpi) serum from LRPV infected rabbits; f – early (7th dpi) serum from rabbits infected with RBOK strain of RPV; g – late (28th dpi) serum from rabbits infected with RBOK strain of RPV; h – RaRPHIS. Ordinate: A_{492}.

tissue (Yamanouchi *et al.*, 1974), whereas the attenuated LA strain has a much milder effect on T-cells (Fukuda and Yamanouchi, 1981). Evidence has been presented that a T-cell subpopulation is involved in control of proliferation of auto-reactive B-cells (Allison *et al.*, 1971; Carnaud *et al.*, 1977; Chused *et al.*, 1978). Thus, the necrosis of particular suppressor T-cells in the infection with the LRPV and not in the infection with the attenuated RBOK strain of RPV may be the reason for finding ADAbs only in rabbits infected with the LRPV. ADAbs detected in RaRPHIS may be produced, because this serum is also raised by repeated inoculation of rabbits with rabbit virulent LRPV. The agar gel precipitation test (AGPT) and counter immunoelectrophoresis (CIE) using RaRPHIS are commonly used for diagnosis of rinderpest (Scott, 1985). Thus, if the material sent for diagnosis of rinderpest is either improperly transported or is from a putrified carcase, there are chances of cell disruption and breakage of nuclei leading to release of cellular DNA, which may lead to false positive results in AGPT/CIE.

The use of ELISA for detection of ADAbs in sera of rabbits infected with RPV has not only obviated the need for cell substrates used in fluorescence studies, but it has also provided a direct evidence for DNA being one of the target antigens of ANAs induced in RPV infection of rabbits. Furthermore, ADAbs are diagnostic markers for systemic lupus erythematosus, an autoimmune disorder of humans, and are involved in its pathogenesis (Tan, 1988). The experimental induction of ADAbs in laboratory animals has proved difficult, but RPV infection of rabbits may provide a unique model for this phenomenon.

Acknowledgements. We thank the Director of this Institute for providing the necessary facilities.

References

Ali, A., and Ali, R. (1983): Enzyme linked immunosorbent assay for anti-DNA antibodies using fluorogenic and colorigenic substrates. *J. immunol. Meth.* **56**, 341–346.

Allison, A. C., Denman, A. M., and Barnes, R. D. (1971): Cooperating and controlling functions of thymus derived lymphocytes in relation to autoimmunity. *Lancet* **2**, 135–140.

Andjaparidze, O. G., Chaplygina, N. M., Bogomolova, N. N., Koptyaeva, I. B., Nerryaeva, E. G., Filimonova, R. G., and Tareeva, I. E. (1989): Detection of measles virus genome in blood leucocytes of patients with certain autoimmune diseases. *Arch. Virol.* **105**, 287–291.

Carnaud, C., Charreier, J., and Bach, J. F. (1977): Adult thymectomy promotes the manifestation of autoreactive lymphocytes. *Cell. Immunol.* **28**, 274–283.

Chused, T. M., Moutsopoules, H. M., Sharrow, S. O., Hansen, C. T., and Morse, H. C., III (1978): Mechanism of autoimmune disease in New Zealand black mice, pp. 177–191. In N. R. Rose, R. E. Bigazzi and N. L. Warner (Eds): *Genetic Controls of Autoimmune Disease,* Elsevier, North-Holland and New York.

Edberg, J. C., and Taylor, R. P. (1986): Quantitative aspects of lupus anti-DNA autoantibody specificity. *J. Immunol.* **136**, 4581–4587.

Filimonova, R. G., Tareeva, J. E., Khaletskaya, E. V., Fadeeva, L. L., and Nevryaeva, E. G. (1982): Antibodies to virus antigens in patients with glomerulonephritis and other renal diseases. *Ter. Arkh.* **7**, 32–35 (in Russian).

Fukuda, A., and Yamanouchi, K. (1976): Autoimmunity induced in rabbits by rinderpest virus. *Infect. Immun.* **13**, 1449–1453.

Fukuda, A., and Yamanouchi, K. (1981): Comparison of autoimmunity jinduction with virulent and attenuated rinderpest virus in rabbits. *Jap. J. med. Sci. Biol.* **34**, 149–159.

Gripenberg, M., and Kurki, P. (1986): Demonstration of human autoantibodies by quantitative enzyme immunoassay. *J. immunol. Meth.* **92**, 145–159.

Hoch, S., and Schwaber, J. (1986): Specificity analysis of human anti-DNA antibodies. *J. Immunol.* **136**, 892–897.

Imaoka, K., Kanai, Y., Nishikawa, Y., Yoshikawa, Y., and Yamanouchi, K. (1988*a*): Induction of antinuclear antibodies in rabbits by infection with rinderpest viruses with different degree of virulence. *Jap. J. vet. Sci.* **50**, 115–118.

Imaoka, K., Yoshikawa, Y., Kanai, Y., and Yamanouchi, K. (1988*b*): Characterization of antinuclear antibodies induced in rabbits by rinderpest virus infection. *Arch. Virol.* **102**, 111–118.

Mottironi, V. D., and Terasaki, P. I. (1970): Lymphocytotoxins in disease. I. Infectious mononucleosis, rubella and measles, pp. 301–308. In P. I. Terasaki (Ed.): *Histocompatibility Testing,* Munksgaard Copenhagen.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989): *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor.

Scott, G. R. (1985): Rinderpest in the 1980s, pp. 145–174. In R. Pandey (Ed.): *Progress in Veterinary Microbiology and Immunology,* I. S. Karger, Basel.

Tan, E. M. (1988): Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44**, 93–151.

Yamanouchi, K., Chino, F., Kobune, F., Fukuda, A., and Yoshikawa, Y. (1974): Pathogenesis of rinderpest virus infection in rabbits. I. Clinical signs, immune response, histological changes and virus growth patterns. *Infect. Immun.* **9**, 199–205.

Yonezawa, T. (1971): Demyelinating antibodies in measles. *Progr. neurol. Res.* **15**, 460–464.