CIRCULATING IMMUNE COMPLEXES IN RABBITS SURVIVING RINDERPEST VIRUS INFECTION

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Summary. – Circulating immune complexes (CICs) precipitated from the sera of rabbits recovered from infection with rabbit virulent (lapinized) rinderpest virus (RPV) were found to contain RPV antigen and rabbit IgG. The CICs persisted for at least 77 days post infection. The CICs and the antigen isolated from them by chromatography on Sepharose-6B were found to be non-infectious. RPV-specific antibodies could be detected in the antisera raised in rabbits against the CICs and the isolated antigen. Furthermore, the antigenic content of CICs competed with the attenuated strain and the virulent isolate Hisar of RPV in competition ELISA. Identical CICs were not detected in the sera of calves vaccinated with RBOK strain.

Key words: rinderpest virus; circulating immune complexes; rabbits

Introduction

RPV of cattle, measles virus (MV) of humans and canine distemper virus (CDV) of dogs belong to morbillivirus genus of the family paramyxoviridae. Formation of CICs has been reported in MV infection (Ziola *et al.*, 1983; Salmi *et al.*, 1986) and platelet-associated CICs have been found in CDV infection (Krakowka *et al.*, 1987). In the case of RPV infection, formation of CICs has not been reported. The present study was undertaken to search for the formation of CICs in RPV infection.

Materials and Methods

Virus. Lapinized RPV (LRPV), Nakamura III strain, virulent for rabbits was used at its 998th rabbit passage in the form of spleenic tissue. Tissue culture rinderpest vaccine (TCRP), prepared with attenuated RBOK strain of RPV grown in primary calf kidney cell culture was used for vaccinating the calves.

Experimental animals. Adult male rabbits and cross-bred calves of either sex, aged 5 – 6 months were used.

Infection and vaccination. Eight rabbits were infected intravenously (iv) with 1 % suspension of LRPV in sterile PBS at a dose rate of 1 ml per rabbit. The rabbits which recovered from the infection were used in this study. Six uninfected rabbits which were kept separately constituted the control group. Six calves were vaccinated subcutaneously (sc) with 1 ml of TCRP.

Serum samples were collected at weekly intervals from the recovered and control rabbits from the 21st day post infection (p.i.) till the 77th day p.i. The vaccinated calves were bled 2 months post vaccination (p.v.); unvaccinated calves' serum constituted the control. Sera were either used fresh or kept at –20 °C before use. Sero-conversion was confirmed by neutralization test and ELISA.

Precipitation of CICs and separation of antigens and antibodies. CICs were precipitated from the sera with 3.5 % (w/v) PEG-6000 as described by Creighton *et al.* (1973). To separate the antigen and the antibody, the precipitates were disolved in 0.1 mol/l glycine-HCl pH 3.0 and fractionated on Sepharose-6B (Pharmacia), equilibrated with 0.05 mol/l PBS pH 7.4. PBS was used for elution, and the eluted fractions were monitored and collected by a fraction collector (Pharmacia). Fractions corresponding to A_{280} peak were pooled and concentrated by reverse dialysis against PEG-20 000. The peaks obtained were tested for their reactivity with antiserum species globulin in immunodiffusion. The method of Dische (1955) was used for detection of nucleic acids in the first peak. The protein content of PEG precipitated CICs (PPCICs) and Sepharose-6B peak I (SPI) was estimated by the standard method.

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Detection of RPV antigen in SPI. ELISA, as used by Lahiri and Thomas (1985) for detection of ribonucleoprotein complexes, was employed to detect the RPV antigen in SPI. Briefly, the wells of microtiter plate (Dynatech) were coated with 0.2 ml of SPI (from control or recovered rabbits), diluted to 5 µg/ml in 0.05 mol/l carbonate buffer pH 9.6. Gelatin (1 % in PBS) was used for blocking. Bovine anti-RPV hyperimmune serum (HIS) diluted 1:400 in PBS containing 0.05 % Tween-20 was incubated with coated antigen. Normal bovine serum (NBS) acted as control. Rabbit anti-bovine horseradish peroxidase (HRPO) conjugate (Dako) and O-phenylendiamine-H_2O_2 solution were used as conjugate and substrate, respectively. After stopping the reaction with 2N H_2SO_4, the absorbance at 492 nm (A_{492}) for each well was read in an ELISA reader (Dynatech). A two-fold or higher difference between A_{492} values corresponding to HIS and NBS was considered positive.

For detection of RPV antigen in SPI obtained after fractionation of 3.5 % PEG precipitates from vaccinated calves sera, rabbit anti-RPV HIS, normal rabbit serum (NRS) and swine anti-rabbit HRPO conjugate (Dako) were used.

Infectivity of PPCICs and SPI. The infectivity of rabbit PPCICs and SPI antigen was tested in rabbits. Three rabbits (group I) were inoculated iv with 1 ml of PPCICs diluted to 1 mg/ml in PBS, and three rabbits (group II) were inoculated similarly with SPI. Rabbits inoculated with LRPV (group III) served as control. Clinical symptoms and temperature responses were recorded and post mortem examinations conducted at death or on the 6th day p.i.

Detection of antibodies in antisera to PPCICs and SPI. The antisera to rabbit PPCICs and SPI were raised in rabbits. The reactivity of these antisera with the RBOK strain of RPV was tested in ELISA according to Sharma *et al.* (1983), except that the coating was done with a linear sucrose gradient purified virus diluted to 5 µg/ml in 0.05 % mol/l carbonate buffer pH 9.6.

Fractionation of SPI antigen. The SPI antigen was further fractionated on Ultrogel A-2 (LKB). The procedure used was identical to the fractionation on Sepharose-6B.

Competition ELISA. To find out the antigenic relatedness between the Ultrogel fractionated peaks and the RPV, competition ELISA was carried out according to Abu Elzein and Crowther (1982). The coating was done with the Ultrogel A-2 peak I (UPI) or II (UPII) and NRS was used for blocking. The UPI and UPII were competed with the attenuated RBOK strain and the virulent Hisar isolate for bovine anti-RPV antibody. Zero and 100 % competition controls were included in each test. The competition in % was plotted against the $\log_{10}$ of competitor concentration. Considering the competition line for homologous liquid phase virus as the standard, distance between this line and that for heterologous liquid phase virus at 50 % competition was measured. Antilog of these differences indicated the relative difference in the weight of the competitor to give equivalent competition to that of the homologous liquid phase virus.

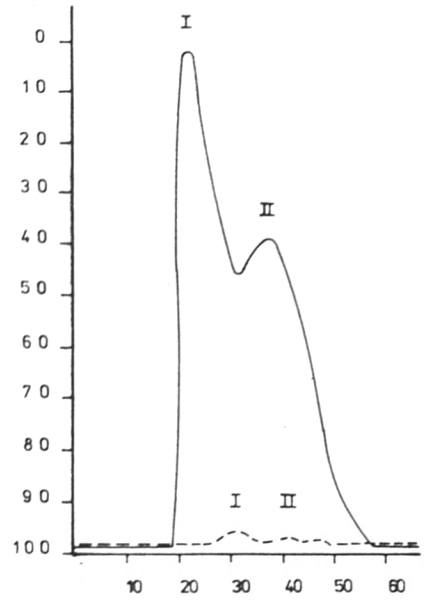

Fig. 1
Fractionation of PEG precipitates of rabbits' sera on Sepharose-6B
Recovered serum (continuous line), control serum (broken line). Abscissa: eluate including the void volume (ml); ordinate: transmittance (%).

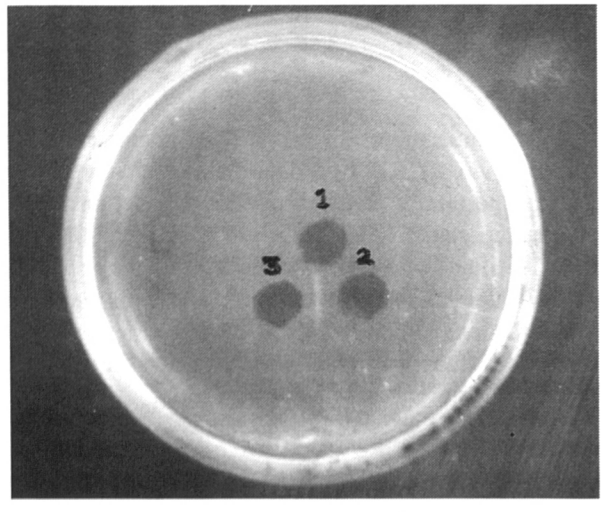

Fig. 2
Reactivity of Sepharose-6B fractionated peaks with anti-species globulin serum in immunodiffusion
Sepharose-6B peak I (1) and II (2), goat anti-rabbit IgG serum (3).

Results

All the 8 rabbits infected with LRPV developed clinical signs characteristic of RPV infectionn in rabbits, i.e. anorexia, pyrexia and diarrhoea. Five of these rabbits succumbed to disease; post mortem examinations confirmed death due to RPV. The CICs precipitated from the recovered rabbits' sera gave 2 peaks after fractionation on Sepharose-6B; similarly processed sera from the control rabbits gave

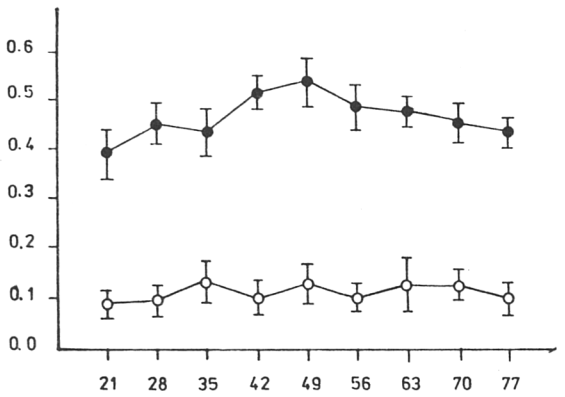

Fig. 3
Detection of RPV antigen in SPI from recovered rabbits' sera at different days p.i. by ELISA
Recovered serum (●), control serum (○). Abscissa: days p.i.; ordinate: A492 values corrected by subtracting those obtained with NBS.

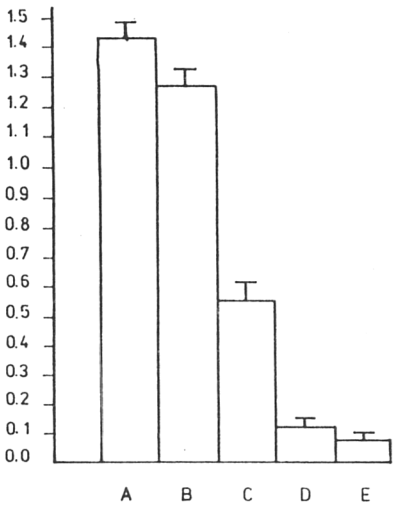

Fig. 4
ELISA reactivity of different rabbit sera with density gradient purified RPV strain RBOK
Sera diluted 1:400 in PBS-tween. Abscissa: anti-RPV HIS (A), anti-CIC serum (B), anti-Sepharose-6B peak I antigen(s) serum (C), healthy rabbit serum (D). No serum added conjugate control (E); ordinate: A_{492}.

very minor peaks (Fig. 1). In immunodiffusion, only the peak I from the infected rabbits was found to react with goat anti-rabbit IgG serum (Fig. 2). At this stage of our study the nature of the peak I was not known, so both its nucleic acid (DNA and RNA) and protein content was estimated. It was found to contain protein but no nucleic acids.

By ELISA, the RPV antigen could be detected in the SPI from the recovered rabbits' sera from the 21st till the 77th day p.i. (till the studies were made), whereas the corresponding minor peak (I) from the control rabbits' sera was negative for the RPV antigen (Fig. 3). The SPI from the sera of vaccinated calves was also found to be negative for the RPV antigen.

Table 1. Clinical observations and post-mortem findings in rabbits infected with rabbit PPCIC, SPI and LRPV

Group of rabbits	Rabbit No.	Infected iv with[a]	Clinical observations							
			Anorexia	Pyrexia	Diarrhoea on day					
					0	1	2	3	4	5
I	1408		only on 1st day	–	–	–	–	–	–	–
	1452	PPCIC (1mg)	only on 1st & 2nd day	–	–	–	–	–	–	–
	2752		–	–	–	–	–	–	–	–
II	1410		–	–	–	–	–	–	–	–
	2625	SPI	–	–	–	–	–	–	–	–
	2642	(1mg)	only on 1st day	–	–	–	–	–	–	–
III	0288	LRPV	3rd day onwards	+++ (day 2–5)	–	–	+	+++	+	+
	7444		2nd day onwards	+++ (day 2–5)	–	–	±	+	+	+
	7448		3rd day	+++	–	–	±	+	+	+++

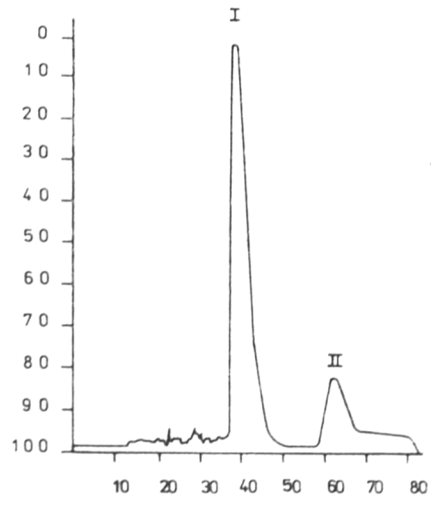

Fig. 5
Fractionation of the Sepharose-6B peak I antigen from recovered rabbit serum by molecular exclusion chromatography on Ultrogel A-2
Abscissa: eluate including the void volume (ml); ordinate: transmittance (%).

fectious. The reactivity of the antisera to PPCICs and SPI antigen with the RBOK strain of RPV in ELISA is shown in Fig. 4. Both the antisera were found to have anti-RPV antibodies; antiserum to the PPCICs gave a strong reaction which was comparable to that of the anti RPV-HIS, which indicated the viral (RPV) specificity of the CICs.

The SPI antigen could be resolved into 2 peaks by fractionation on Ultrogel A-2 (Fig. 5). The results of the competition of Ultrogel A-2 peaks with an attenuated strain and a virulent isolate of the RPV (Table 2) indicated antigenic similarity between the Ultrogel peaks and the RPV, as competitors showing antilog difference of <100 are considered to be strongly related (Abu Elzein and Crowther, 1982). This further proved that CICs contain RPV antigen(s).

Discussion

The present study indicated that persistent CICs are formed in rabbits recovered from infection with the LRPV.

Table 1. Clinical observations and post-mortem findings in rabbits infected with PPCIC, SPI and LRPV (continued)

Group of rabbits	Ra bbit No.	Infected iv with[a]	Mortality	Post-mortem lesions in			
				Spleen	Caecum	Peyer's patches	Sacculus rotundus
I	1408		survived	–	–	–	–
	1452	PPCIC (1mg)	survived	–	–	–	–
	2752		survived	–	–	–	–
II	1410		survived	–	–	–	–
	2625	SPI	survived	–	–	–	–
	2642	(1mg)	survived	–	–	–	–
III	0288		died on 5th day	+++	++	+	++
	7444	LRPV	survived	+++	+	+	+
	7448		died on 5th day	++	++	++	+++

[a]All materials used for infection dissolved in PBS. One mg corresponds to protein content. PPCIC=PEG-precipitated CIC; SPI = Sepharose-6B peak I antigen; LRPV = 1% spleenic tissue from a rabbit infected with LRPV at 998th passage; (+++) = severe; (++) = moderate; (+) = mild; (±) = semi-solid faeces; (–) = not found.

Table 2. Antigenic relationship between Ultrogel A-2 fractionated peaks and RBOK strain and Hisar isolate of RPV in competition ELISA

Solid phase coated antigen	Differences in competitor weights[a] with	
	RBOK strain	Hisar isolate
UP I	83.18	15.14
UP II	18.20	91.20

UP I = Ultrogel A-2 peak I; UP II = Ultrogel A-2 peak II. [a]Antilog of $\log_{10}$ difference in competition lines (at 50% competition) between homologous antigen and heterologous liquid phase virus.

The clinical observations and post mortem findings of rabbits infected with the rabbit PPCICs and SPI (Table 1) indicated that the PPCICs and the SPI antigen are non-in-

The PEG precipitates from the recovered rabbits' sera could be fractionated into two peaks, one of which (peak II) contained the rabbit IgG, indicating that what we got in the

precipitates was something bound to the rabbit IgG (an immune complex), and that the antigenic content of these immune complexes (ICs) lays in the peak I. As no nucleic acid could be detected in the peak I, the possibility of DNA-anti-DNA antibody or RNA-anti-RNA antibody ICs was ruled out. The proteinaceous nature of this peak was confirmed and the RPV antigen could be detected in it in sera samples from the 21st to the 77th day p.i., suggesting the persistence of CICs for at least 77 days p.i. In MV infection, the persistence of CICs up to 3 months has been reported (Ziola *et al.*, 1983). Several investigators (Cordier *et al.*, 1977; Moretta *et al.*, 1978; Mingari *et al.*, 1978) have shown that following the binding of the ICs to the IgG Fc-receptor on mononuclear cells, a process of dissociation proceeds and the cells from which the ICs are dissociated, become incapable of binding the IgG type ICs again or acting as effectors, suggesting that the IgG Fc-receptor is rapidly shed. If an analogous phenomenon operates in rabbits, the CICs may not find enough IgG Fc-receptor bearing cells (which can phagocytize these) to bind and lead to the persistence of IgG type CICs in the circulation.

The PPCICs and the SPI antigen isolated from them were found to be non-infectious. Infectious ICs have been reported in different infections, e.g. lactic dehydrogenase virus infection in mice (Notkins *et al.*, 1966), lymphocytic choriomeningitis in mice (Oldstone and Dixon, 1967), aleutian disease of mink (Porter *et al.*, 1980), but in all of them non-neutralizing antibodies participate in the ICs. One of the reasons for the non-infectious nature of the ICs in the RPV infection may be that the ICs are formed with neutralizing antibodies, as non-neutralizing antibodies have not been detected in RPV infection. The absence of RNA in the SPI pointed out that the ICs formed in the case of RPV infection may not contain the complete infectious virions; in the case of MV only the haemagglutinin protein (Perrin and Oldstone, 1977) and in human immunodeficiency virus only the p24 protein (Schupbach *et al.*, 1984) have been shown to form ICs.

Antisera to the PPCICs and the SPI antigen were raised in the species from which they were isolated (rabbits). Both these sera were found to contain anti-RPV antibodies, which proved the RPV antigen(s) to be the antigenic content of the CICs. Bartfeld *et al.* (1989) used similar approach to prove the antigenic content of the CICs observed in amyotrophic lateral sclerosis to be the enteroviral antigen.

In our study the SPI was resolved into two peaks by chromatography on Ultrogel A-2. In competition ELISA, both these peaks competed with the virulent Hisar isolate and the attenuated RBOK strain of RPV, which not only confirmed the antigenic content of the ICs to be the RPV antigen(s), but it also indicated that the SPI antigen consisted of two fractions.

The virus-specific CICs could not be detected in the vaccinated calves. The CIC formation may be associated with the disease caused by a virulent virus, just as anti-nuclear antibodies (ANAs) are produced in rabbits in infection with LRPV but not with the attenuated lapinized avianized (Fukuda and Yamanouchi, 1981) and RBOK (Rattan *et al.*, 1993) strains of RPV. Furthermore, Darsley *et al.* (1988) found that the LRPV infection is immunosuppressive; whereas the attenuated RBOK strain is not immunosuppressive, it rather slightly enhances both the humoral and cell-mediated immune responses (CMIR). For the persistence of the CICs, the immunosuppression may become operative in the case of LRPV–infected rabbits, and on the other hand normal or slightly boosted humoral and CMIR may get rid of the CICs when formed in case of the calves vaccinated with the RBOK strain. Similar studies on the cattle recovered from rinderpest would clarify this point.

The formation of the CICs in the infection with MV is well documented and the present study indicates occurrence of them in rabbits infected with RPV. MV antibodies (Shirodaria *et al.*, 1979; Filimonova *et al.*, 1982) and MV genomic RNA in lymphocytes (Andjaparidze *et al.*, 1989) have been reported in patients with autoimmune disorders. The autoimmune disorders like systemic lupus erythematosus, chronic glomerulonephritis and rheumatoid arthritis are accompanied by the CIC formation. The ANAs have been established as the diagnostic markers of the autoimmune disorders (Tan, 1988). The LRPV has been shown to induce the ANAs (Fukuda and Yamanouchi, 1981; Imaoka *et al.*, 1988; Rattan *et al.*, 1993), but the involvement of RPV in autoimmune disorders has not been reported. So far there have been no reports regarding the induction of the ANAs in MV infection. A positive correlation of these factors suggests a possible inter-relationship between morbilliviruses, CICs, ANAs and autoimmune disorders.

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References

Abu Elzein, E.M.E., and Crowther, J.R. (1982): Differentiation of foot-and-mouth disease virus strains using a competition enzyme linked immunosorbent assay. *J. virol. Meth.* **3**, 355–365.

Andjaparidze, O.G., Chaplygina, N.M., Bogomolova, N.N., Koptyaeva, I.B., Nevryaeva, 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.

Bartfeld, H., Dham, C., Donnefeld, H., Ollar, R.A., deMasi, M.T., and Kascsak, R. (1989): Enteroviral related antigen in circulating immune complexes of amyotrophic lateral sclerosis patients. *Intervirol.* **30**, 202–212.

Cordier, G., Samarut, C., and Revillard, J.P. (1977): Change of Fc-receptor related properties induced by interaction of human lymphocytes with insoluble immune complexes. *J. Immunol.* **119**, 1943–1948.

Creighton, W.D., Lambert, P.H., and Miescher, P.A. (1973): Detection of antibodies and soluble antigen-antibody complexes by precipitation with polyethylene glycol. *J. Immunol.* **111**, 1219–1227.

Darsley, M., Kinuthia, S.W., Jackson, F., and Murray, P.K. (1988): Rinderpest: Immunology. Annual report of the Institute for Animal Health, Berkshire, England, p. 19.

Dische, Z. (1955): Colour reactions of nucleic acid components. In E. Chargaff and J.N. Davidson (Ed.): *The Nucleic Acids. Chemistry and Biology.* Vol. I., Academic Press, New York, pp. 285–288.

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

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

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

Krakowka, S., Cork, L.C., Winkelstein, J.A., and Axthelm, M.K. (1987): Establishment of central nervous system infection by Canine distemper virus: breach of the blood brain barrier and facilitation by antiviral antibody. *Vet. Immunol. Immunopathol.* **17**, 471–482.

Lahiri, D.K., and Thomas, J.O. (1985): The fate of heterogenous nuclear ribonucleoprotein complexes during mitosis. *J. Biol. Chem.* **260**, 598–603.

Mingari, M.C., Moretta, L., Moretta, A., Ferrarini, M., and Preud' homme, J.L. (1978): Receptors for IgG and IgM immunoglobulins on human T lymphocytes: Mode of re-expression after proteolysis or interaction with immune complexes. *J. Immunol.* **121**, 767–770.

Moretta, L., Mingari, M.C., and Romanzi, C.A. (1978): Loss of F_c receptors for IgG from human T lymphocytes exposed to IgG immune complexes. *Nature* **272**, 618–620.

Notkins, A.L., Mahar, S., Schelle, C., and Goffman, J. (1966): Infectious virus-antibody complex in the blood of chronically infected mice. *J. exp. Med.* **24**, 81–89.

Oldstone, M.B.A., and Dixon, F.J. (1967): Lymphocytic choriomeningitis: production of antibody by 'tolerant' infected mice. *Science* **158**, 1193–1195.

Perrin, L.H., and Oldstone, M.B.A. (1977): The formation and fate of virus antigen-antibody complexes. *J. Immunol.* **118**, 316–322.

Porter, D.D., Larsen, A.E., and Porter, H.G. (1980): Aleutian disease of mink. *Adv. Immunol.* **29**, 261–286.

Rattan, B., Sharma, B., and Bansal, R.P. (1993): Detection of anti-DNA antibody responses in rabbits infected with rinderpest virus by ELISA. *Acta virol.* **37**, 403–406.

Salmi, A., Lund, G., Yiola, B., and Reunanen, M. (1986): Circulating immune complexes in patients with subacute sclerosing panencephalitis. *Clin. Immunol.* **41**, 16–25.

Schupbach, J., Sarngadharan, M.G., Blayney, D.W., Kalyanaraman, V.A., Bunn, P.A., and Gallo, R.C. (1984): Demonstration of viral antigen p24 in circulating immune complexes of two patients with human T cell leukaemia/lymphoma virus (HTLV) positive lymphoma. *Lancet* **1**, 302–305.

Sharma, B., Joshi, R.C., Lahiri, D.K., and Bansal, R.P. (1983): Evaluation of enzyme linked immunosorbent assay for rinderpest. *Indian J. anim. Sci.* **53**, 1293–1295.

Shrirodaria, H., Norrby, E., McCullough, K.C., Carpentar, W.C., and Orvell, C. (1979): Measles virus specific antibodies from multiple sclerosis and rheumatoid arthritis patients. *Infect. Immun.* **25**, 408–416.

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

Ziola, B., Lund, G., Meurman, O., and Salmi, A. (1983): Circulating immune complexes in patients with acute measles and rubella virus infections. *Infect. Immun.* **41**, 578–583.