Periodate‐lysine‐paraformaldehydeで固定した鶏白血病ウイルスの型特異抗原の安定性

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Brief Note

Stability of Avian Leukosis Virus Type-specific Antigen in the Cell after Fixation in Periodate-lysine-paraformaldehyde

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Stability of avian leukosis virus type-specific antigen in chicken embryo fibroblasts after fixation in periodate-lysine-paraformaldehyde was tested by the indirect immunoperoxidase method. Antigen that had been steeped in 0.015M Tris-HCl saline buffer solution containing 20% bovine serum and 30% glycerin was preserved at −20°C, 4°C and room temperature without loss of its antigenicity as long as 16 weeks. It was also stable against heat treatment at 56°C, acid treatment and ethyl ether treatment.

The indirect immunoperoxidase (IIP) method is a very useful tool for detection of antigens of antibodies against avian leukosis viruses (ALVs).[2] The type-specific antigen of ALV was readily detected in ALV-infected chicken embryo fibroblasts (CEF) shortly after fixation in periodate-lysine-paraformaldehyde (PLP) by the IIP method. This paper deals with stability of the type specific antigen of ALV in infected CEF after fixation in PLP.

The ALV used was RAV-1 (subgroup A). It had been isolated from BH-RSV-1 by the end-point dilution method.[5] Stock virus was prepared in CEF cultures with C/O and C/E phenotype and stored at −80°C. These cultures were prepared from embryos obtained from chickens of lines 15I and BK which had been kept under ALV-free conditions[5] in such manner as described previously.[4] Primary CEF cultures were inoculated each with 10^7 resistance-inducing units (RIU) of RAV-1 and incubated in an incubator at 38°C under 5% CO_2. After 3 to 5 days, they were transplanted into 60-mm plastic petri dishes (Falcon #3002) containing coverslips. Three or 4 days after transplantation the coverslips with infected cells were fixed in PLP, which consisted of 0.01M sodium m-periodate, 0.075M lysine, and 2% paraformaldehyde in 0.0375M sodium phosphate buffer solution at pH 7.4.[1] They were used for the tests.

Anti-RAV-1 chicken serum was prepared in chickens of BK line by 3 repeated intravenous injections with 10^7 RIU of RAV-1 each. The antibody titer of the serum was 1,000 to homologous virus by the neutralization test. The serum used for the IIP test was a 1: 250 dilution in phosphate buffer saline solution (PBS).

Horseradish peroxidase (HRPO)-conjugated rabbit anti-chicken IgG was prepared by the method described previously.[3]

The staining procedure of fixed CEF on the coverslip has also been described.[2] Briefly, after washing the coverslip with cold PBS, chicken serum was added to CEF. Then, CEF were kept in an incubator at 38°C for 60 minutes and subjected to two 5-minute cycles of serial washing with PBS, borate buffer saline solution and PBS containing Tween 20 (Sigma) (washing procedure). After that, HRPO-conjugated

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rabbit anti-chicken IgG was applied to CEF, which were kept again in the incubator at 38°C for 60 minutes. After the second incubation, CEF on the coverslip were washed by the washing procedure and placed in a diaminobenzidine mixture, which consisted of 0.5 mg/ml diaminobenzidine tetrahydrochloride and 0.01% peroxide in 0.05M Tris-HCl buffer solution (pH 7.6). They were kept at room temperature for 20 minutes, washed once with cold PBS and three times with distilled water, and observed the results of staining by the microscope.

The buffer solution used for the preservation test (preservation buffer) was 0.015 M Tris-HCl saline buffer solution (pH 7.4) containing 20% bovine serum and 30% glycerin.

The preservation test was performed in the following manner. CEF fixed in PLP on the coverslip were steeped into the preservation buffer and stored at room temperature, 4°C, −20°C, or −80°C. At 1, 2, 4, 8, 12, and 16 weeks of storage, they were stained by the IIP method.

As a result, the antigenicity was preserved without any loss during the test period at every temperature, except −80°C at which the preservation buffer froze and the cell sheet peeled off from the coverslip.

The stability of the antigen against some physicochemical treatment is shown in Table 1. The antigenicity was not changed at all by acid treatment at pH 3.0 or 2.5 at room temperature for 30 minutes. It was decreased a little by heat treatment at 56°C for 30 minutes, but increased a little by ethyl ether treatment at 0°C for 30 minutes. The reason for the augmentation of antigenicity by ethyl ether treatment remained to be elucidated.

Thus, type-specific antigen of ALV, which could be used for the IIP method to detect antibody against ALV,[2] could be stabilized by fixation in PLP and preserved as long as 16 weeks under moderate conditions. This result indicates that the IIP method with PLP-fixed ALV-infected CEF is a very useful tool for detection of type-specific antibody against ALV in laboratory studies and in field surveys. (August 10, 1981)

Table 1. *Stability of avian leukosis virus type specific antigen in the cell after fixation with periodate-lysine-paraformaldehyde*

Treatment	RAV-1*1	Normal*1
pH 3.0,*2 room temp., 30 min.	++*5	−
pH 2.5,*2 room temp., 30 min.	++	−
56°C, 30 min.*3	+	−
Ethyl ether,*4 0°C, 30 min.	+++	±
Non-treatment, room temp., 30 min.	++	−

*1 RAV-1: RAV-1 infected chicken embryo fibroblast, Normal: Non-infected chicken embryo fibroblast.
*2 Steeped in 0.17M glycine-HCl buffer solution.
*3 Steeped in 0.015M Tris-HCl saline buffer solution (pH 7.4).
*4 100% ethyl ether
*5 +~+++: Degrees of positive reaction, ±: Suspicious reaction, −: No reaction.

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