Sedimentation Behaviour of the Avian Tumor Virus Group Specific Antigen from the BAI Strain, a Virus Associated Myeloblast Cell

Hamster carrying tumors induced with Schmidt-Ruppin (S-R) strain of Rous sarcoma virus (RSV) develop an antibody which not only fixes complement with an antigen in the homologous tumor tissue but also reacts with an antigen of chicken cells injected with any one of the several avian sarcoma and leukosis viruses[1]. This group specific (GS) antigen is found in the soluble fraction of the cell[2]. However, BAUER et al.[3], by treating the purified BAI strain A (Myeloblastosis) virus with sodium dodecyl sulphate (SDS) and then reacting the separated components with hamster serum, have demonstrated the presence of this antigen in the virus. Thus, the so-called GS, soluble, cellular antigen is infact a viral structural protein, is evident from these studies. The present report describes the sedimentation behaviour of the soluble antigen of the BAI strain A virus associated myeloblast cell in comparison with an identical antigenic component of the virus. The results indicate an identical size of both GS viral and cell antigen.

Myeloblasts collected from leukemic chickens[4] and washed twice with medium 199 were stored at $-20\,^{\circ}C$ until needed. One ml of frozen and thawed myeloblasts diluted to 10 ml with $0.01\,M$ phosphate buffered saline (PBS), PH-7.4 were homogenized with a glass pestle. The homogenate was centrifuged to remove nuclear, mitochondrial and microsomal fractions[5]. The supernatant obtained after these centrifugations is the soluble fraction used in this study. Virus was purified by alternate low and high speed centrifugations[6] from chicken plasma containing 5×10^{11} virus particles/ml, as determined by ATPase assay[7]. Purified virus suspended in PBS was disrupted with SDS in 0.5% concentration. Excess SDS was removed by adding a drop of KCl and sedimenting the precipitate.

Microtechnique of SEVER[8] was used for complement fixation (CF) studies. Two-fold dilutions of antigen were incubated at $4\,^{\circ}C$ for 18 h, with 4 U of hamster serum and 2 U of complement. Sheep red blood cells sensitized with 2 U of hemolysin were added to the test mixtures and incubated at $37\,^{\circ}C$ for 30 min. The CF titre is expressed as the reciprocal of the highest antigen dilution giving 100% fixation. Serum pool obtained from hamsters bearing transplanted S-R tumors[9] used in this study had a CF titre of 64 or more and did not react with normal chick tissue. The serum was inactivated at $56\,^{\circ}C$ for 30 min.

Earlier studies have shown that one of the electrophoretic components of the virus split with SDS does fix complement with the hamster serum[3]. Sedimentation value of the complement fixing viral component was also determined. It was then of interest to see if the sedimentation behaviour of the CF antigen of the myeloblast cell is identical with the viral component. Both the myeloblast soluble fraction and SDS split virus products were run on gradient of 5 to 20% sucrose. SDS disrupted virus was diluted with PBS to contain a CF titer of 64 or more. 0.2 ml volume of each of these preparations was layered on sucrose gradients and spun for 24 h at 40,000 rpm in the spinco swinging bucket rotor SW 50. CF activity of both of these preparations peaked at the same distance from the meniscus (Figure). An approximate value[10] of 2.5 S was obtained for both components with human serum albumin as a reference. This value of the viral component is close to that obtained by analytical ultra centrifugation[3].

These studies indicate that the GS component found in the virus and the virus infected cells are not only identical in their complement fixing activity but also have identical sedimentation value. This additional physical similarity of the viral and cellular components further strengthens the view that the soluble cellular component is infact a viral structural constituent.

Zusammenfassung. BAI Virus Stamm A und mit diesem Virus infizierte Myeloblastzellen haben ein gemeinsames, gruppenspezifisches Antigen. Das aus dem Virus abgetrennte Antigen wurde mit dem aus den Zellen herausgelösten im Saccharose-Dichtegradienten verglichen. Sowohl für das Virus wie für das Zellantigen wurde der gleiche Sedimentationswert erhalten.

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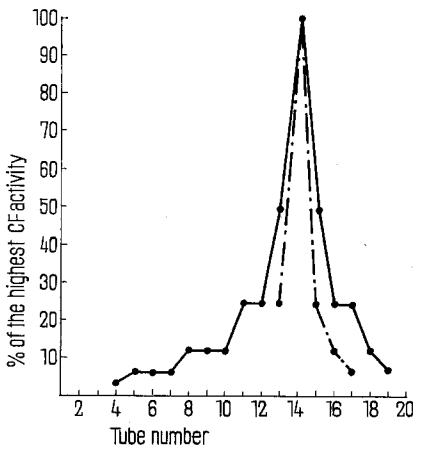

Sedimentation behaviour of the viral and cellular GS antigen. The solid line represents the broken virus and the dashed line myeloblast cell soluble fraction. Identical size of both the antigen preparations is indicated by their identical peak values of the CF activity.

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