Characterization of Adenovirus Type 12 Tumour Antigen Produced in Chick Fibroblasts

(Accepted 16 June 1978)

SUMMARY

Tumour (T) antigen was characterized in non-permissive chick fibroblasts and permissive HEp-2 cells infected with Ad12 either in the presence or in the absence of cytosine arabinoside. Antiserum against T antigen specifically immunoprecipitated two polypeptides of apparent mol. wt. 50000 and 11000.

Type specific T antigen formation has been demonstrated in infected, interferon producing chick fibroblasts by immunofluorescence (Pusztai et al. 1977). The synthesis of adenovirus T antigen has been reported to be fairly insensitive to interferon action in human and monkey cells (Oxman et al. 1967). We have similarly found that the formation of Ad12 specific T antigen in chick cells was resistant to the effect of exogenous chick fibroblast interferon. However, interferon produced in chicken leukocytes inhibited the elaboration of T antigen, depending on the dose of interferon and the multiplicity of infection (Pusztai et al. 1977; Pusztai & Szabó, 1978). As one step towards our understanding of these observations, we have attempted to characterize the T antigen induced in chick cells infected with human adenovirus type 12. It has been claimed that the purified, native T antigen from Ad12 induced hamster tumour cells has a mol. wt. of 80000 (Raska et al. 1976). On the other hand sera from hamsters bearing Ad12 induced tumours were reported to immunoprecipitate several early polypeptides induced in Ad12 infected KB cells (Chinnadurai et al. 1977). In this communication we present further data on the properties of T antigen produced in Ad12-infected non-permissive chick fibroblasts and permissive HEp-2 cells.

Adenovirus type 12 (Huie strain, free of adeno-associated virus) was propagated, purified and titrated as described previously (Pusztai et al. 1977). Primary chick embryo fibroblasts and HEp-2 cells were cultured in Eagle's medium (MEM) containing 5% foetal calf serum and antibiotics. Monolayers of cells in 60 mm plastic Petri dishes were infected with 15 TCD50/cell of Ad12. Some monolayers of virus and mock-infected cells were incubated in the presence of 50 μg/ml of cytosine arabinoside (araC). Infected or mock-infected cells were labelled with 35S-methionine (40 μCi/ml) in methionine-free medium.

Cell extracts for immunoprecipitation were prepared by washing the cells twice with cold phosphate buffered saline and scraping the cells off in 1 ml of hypotonic buffer (20 mM-tris, pH 7-6; 10 mM-NaCl) and 2 mM-phenylmethylsulphonylfuoride (PMSF) – added as protease inhibitor. The samples were stored at -70 °C. Upon thawing, the samples were brought to 0-5% with NP40 and sonicated twice in an MSE PG100 type ultrasonic disintegrator at medium energy for 10 s. NaCl was added to a final concentration of 0-15 M and the samples centrifuged at 15000 g for 30 min at 4 °C.

Anti-tumour sera with T antigen specificity were obtained from hamsters bearing transplanted Ad12 induced tumours. The sera were screened by complement fixation test and those reacting only with T antigen were used for immunoprecipitation. To reduce non-specific immunoprecipitation the pooled sera were pre-incubated with normal cell extracts.

Immunoprecipitation tests were performed by incubating 900 μl of the cell extract with
Fig. 1. Autoradiograms after SDS PAGE analysis of extracts of adenovirus type 12 infected HEp-2 cells and chick fibroblasts and of immunoprecipitated polypeptides. HEp-2 cells and chick fibroblasts were infected at a multiplicity of 15 TCD50/cell and labelled with 35S-methionine 16 h and 24 h post infection, respectively. (a) Extracts of infected HEp-2 cells and (b) of infected HEp-2 cells in the presence of cytosine arabinoside; samples (a) and (b) were then treated with anti-T serum and marked as (c) and (d), respectively. (e) Extracts of mock or (f) virus infected chick fibroblasts treated with anti-T serum. (g) Extract of virus infected chick fibroblast treated with normal hamster serum. (h) Extracts of mock (h) and (i) virus infected chick fibroblasts. (j) Lysate of HEp-2 cells infected with Ad5 and labelled with 35S-methionine.

25 μl of the appropriate hamster antiserum. After 150 min at 25 °C, 0.8 ml of the IgG fraction of rabbit anti-hamster IgG (Miles Laboratories) was added and incubated overnight at 4 °C. The samples were then centrifuged and the pellet was washed twice with buffer (20 mM-Tris, pH 7.6; 150 mM-NaCl; 0.5 % NP40). The final pellet was solubilized in sample buffer (Russell & Blair, 1977). The labelled polypeptides from cell lysates and immunoprecipitates were analysed by SDS-polyacrylamide gel electrophoresis (Russell & Blair, 1977). The apparent molecular weights were determined with the previously calibrated Ad5 polypeptides as references on the gel.

Monolayer cultures of either HEp-2 or primary chick fibroblast cells were infected with type 12 adenovirus, and labelled with 35S-methionine for 180 min at 16 h and 24 h after infection. Mock-infected cells were labelled in a similar fashion. Extracts of infected or mock-infected cells were prepared and immunoprecipitated with either normal hamster serum or anti-T serum. The precipitates were analysed by SDS-polyacrylamide gel electrophoresis. The anti-T serum precipitated a polypeptide with an apparent mol. wt. of 50000 and a smaller polypeptide of mol. wt. approx. 11000 from the infected HEp-2 cell extract (Fig. 1c). The same result was obtained in the presence of 50 μg/ml araC (Fig. 1d). In the infected chick fibroblast extract the anti-T serum precipitated the same polypeptide of mol. wt. 50000 (Fig. 1f). However, there appeared to be considerably less of this protein in chick than in HEp-2 cells. The smaller polypeptide was also detected. The faint band cannot be seen in the photograph. These polypeptides were not observed in the immunoprecipitate when
normal hamster serum was employed (Fig. 1 g). Similarly, none of the T antigen polypeptides could be detected when mock-infected chick fibroblast extract was precipitated by anti-T serum (Fig. 1 e).

Thus the infection of the permissive HEp-2 cells or the non-permissive chick fibroblasts with the highly oncogenic type 12 adenovirus resulted in the production of the same proteins with apparent mol. wt. of 50,000 and 11,000 which could be selectively precipitated by our antiserum against T antigen.

Ambiguous data have been published concerning the properties of Ad12-specific T antigen. Chinnadurai and others (1977) immunoprecipitated four polypeptides from extracts of Ad12 infected KB cells with sera from hamsters bearing Ad12 induced tumours. None of these polypeptides was similar in size to that of T antigen produced in HEp-2 or chick cells and noted here although they observed two faint bands of mol. wt. 45 to 47,000 and 35,000.

Raska et al. (1976) have isolated and purified Ad12-specific T antigen from tumour and transformed cells of hamster origin. This native T antigen had a mol. wt. of 80,000, determined on Sephadex G-100. The 1000-fold purified T antigen preparations still revealed four to seven polypeptide species under the denaturing conditions of SDS-polyacrylamide gel electrophoresis, depending on the purification procedures applied (Biron & Raska, 1977). Interestingly enough, each of these purified T antigen preparations contained the 50,000 mol. wt. polypeptide. We assume that the size of the major component of Ad12 specific T antigen is 50,000 and that our observation results from highly specific tumour sera which we have used. We have also observed a small polypeptide of mol. wt. 11,000 associated with Ad12 T antigen. As the Ad2 specific T antigen also contains a polypeptide of smaller size (Gilead et al. 1976), the possibility exists that native T antigen represents a complex of different polypeptides. However, it cannot be ruled out that the smaller peptides represent fragments of the major species.

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Note added in proof. While this article was in preparation, a paper was published (Biron, K. K., Morrongiello, M. P., Rašková, J. and Ráska, K. Jun., 1978. Virology 85, 464–474) which reported results similar to ours concerning the mol. wt. of Ad12 specific tumour antigen.

REFERENCES


Short communications


(Received 28 April 1978)