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BINDING OF SV40 TUMOUR ANTIGEN AND EPSTEIN-BARR-VIRUS NUCLEAR ANTIGEN TO ISOLATED ACID-FIXED NUCLEI

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Summary. — SV40 tumour antigen (TA) bound to methanol--acetic-acid-fixed interphase nuclei and metaphase chromosomes. This reaction was visualized by the anti-complement immunofluorescence test. The reaction was negative when the nuclei had been treated with deoxyribonuclease prior to the addition of antigen, or when TA-antibody negative serum had been used. In parallel tests TA and Epstein-Barr virus nuclear antigen were examined. Both the binding patterns and the optimum conditions for the reaction were similar.

Key words: *Polyomavirus*; *tumour antigen*; *cell nuclei*; *anti-complement immunofluorescence*

Introduction

The tumour or T antigen (TA) of SV40 (genus *Polyomavirus*) is a virus-coded, non-structural polypeptide present in the nuclei of cells transformed by (Black *et al.*, 1963) or infected with this agent (Rapp *et al.*, 1964). It can be detected by complement-fixation or indirect immunofluorescence tests by means of sera from animals either bearing tumours induced by SV40 (Black *et al.*, 1963; Rapp *et al.*, 1964) or, less regularly, infected with it (Vonka *et al.*, 1967; Tevethia, 1970). TA plays an essential role in both the productive infection and cell transformation. It has been recognized as an important control element in the synthesis and transcription of SV40 DNA during the productive infection (Tegtmeyer, 1972; Cowan *et al.*, 1973) and is also involved in both initiation and maintenance of the transformation (Kimura and Itagaki, 1975; Tegtmayer, 1975; Brugge and Butel, 1975). TA binds to double-stranded DNA (Carroll *et al.*, 1974). It has been shown that it binds preferentially at three sites of the SV40 chromosome, one of which appears to be identical with the origin of DNA replication (Jessel *et al.*, 1975; Reed *et al.*, 1975). This is consistent with its assumed regulatory function. Although several polypeptides have been recently recognized when analysing TA-anti-

body complexes by polyacrylamide gel electrophoresis (Prives *et al.*, 1977; Paucha *et al.*, 1978; Smith *et al.*, 1978), it is still usually referred in the singular. So it is in the precent report, because no attempts were made to separate the different TA components.

Epstein-Barr (EB) virus nuclear antigen (EBNA), apparently identical with the soluble (S) antigen detectable by complement-fixation, has also been recently characterized as a DNA-binding protein (Luka *et al.*, 1977). As an expression of this property, adding of the substance to EBNA-negative metaphase chromosomes results in a reconstitution of the EBNA reaction demonstrable by anti-complement immunofluorescence test (ACIF) (Ohno *et al.*, 1977). We utilized this observation for the development of an immunofluorescence microchromatography test for studying EBNA. Requiring minimal amounts of reagents, this test yielded the same results as chromatography on DNA cellulose columns (Hirsch *et al.*, 1978). More recently, utilizing the same technique, we have been able to demonstrate that EBNA binds more avidly to EBV DNA than to heterologous HSV DNA or various cell DNAs (Hirsch *et al.*, 1979). The aim of the present series of experiments was to find out whether the technique is also applicable to studying SV40 TA.

Materials and Methods

TA was extracted from SV40-transformed hamster TU-B cells following the procedure of Cikes *et al.* (1977) up to the centrifugation of crude extract at 100,000 × g. Pools of sera from hamsters bearing tumours induced by TU-B cells and possessing high levels of TA antibody demostrable in complement-fixation and in indirect immunofluorescence tests (Vonka *et al.*, 1967), and control negative sera from healthy animals were employed. EBNA was prepared from EBV genome-positive Raji cells as described (Hirsch *et al.*, 1978). EBNA-positive and EBNA-negative human sera were used for its monitoring. Nuclei from Ramos cells, an EBV genome-negative lymphoblastoid cell line, served as substrate. They were treated with methanol/acetic acid (3 : 1) and subsequently with 0.6 M NaCl (37 °C/60 min) to remove proteins bound to cell DNA. In addition, portions of the preparations were treated with deoxyribonuclease to destroy the target for DNA-binding reaction. ACIF was performed as described (Hirsch *et al.*, 1978), i.e. human complement and fluorescein isothiocyanate-labelled goat anti-human complement were used for the detection of both TA and EBNA.

Results

Already the first experiments indicated that the SV40 TA reacted with nuclei of Ramos cells; however, a simple indirect test was not sensitive enough for visualization of the reaction and ACIF had to be employed. Immunofluorescence of Ramos cell nuclei treated with either TA or with EBNA is shown in Fig. 1. Both substances gave a similar pattern of staining, including the staining of metaphase chromosomes. No fluorescence was seen either in preparations treated with control sera (T antibody-negative hamster and EBNA antibody-negative human serum, respectively) or in those which had been treated with deoxyribonuclease prior to the addition of TA or EBNA. These negative findings confirmed the specificity of the reaction and demonstrated that both TA and EBNA bound to DNA.

Table 1. Influence of ionic strength and pH on the reactivity of T antigen of SV40 (TA) and nuclear antigen of EBV (EBNA) with acid-fixed Ramos cell nuclei

Ionic strength	pH	Percentage of cells giving positive reaction after treatment with	
		TA	EBNA
0.05 M	5.5	0	0
	6.0	70–90	70–90
	6.5	>90	>90
	7.0	50	50
	7.5	±[1])	±
0.2 M	5.5	0	0
	6.0	30–50	30–50
	6.5	±	±
	7.0	0	0
	7.5	0	0

[1]) Weak fluorescence in some cells.

The results of some further experiments undertaken to specify the conditions of the reaction are summarized in Table 1. It can be seen that pH 6.0-6.5 was optimal for conversion of Ramos nuclei to a TA-positive state. No positive nuclei were observed at pH below 6.0 or above 7.5. Tests on the influence of different NaCl concentrations showed the strongest reactions in the range of 0.05 M to 0.1 M. At 0.2 M NaCl the reaction was weak and at a higher concentration no stained nuclei were observed. Parallel tests with EBNA demonstrated a close similarity in the behaviour of both substances.

Discussion

These data seem to indicate that the microchromatography immunofluorescence test originally introduced for EBNA is also applicable to SV40/ /DNA interaction studies; the findings are in agreement with data obtained by Carrol *et al.* (1974) on DNA-cellulose columns. The technique may be utilized for various purposes. It has been shown that the properties of SV40 TA formed by ts A mutants, including its affinity for DNA, are altered (Osborn and Weber, 1974; Kuchino and Yamaguchi, 1974; Tenen *et al.*, 1975, Alwine *et al.*, 1975); the present test can provide means for a rapid and simultaneous screening of many samples. Since it requires minimum amounts of reagents, it can also be utilized for studying the DNA binding properties of the various TA subcomponents. The ability of TA to bind to metaphase chromosomes has recently been demonstrated (D' Alisa and Gershey, 1978); immunofluorescence microchromatography may be useful in determining its binding pattern to chromosomes.

The nearly identical reactivity of EBNA and TA with DNA in the present system may be of interest. The significance of this observation is not understood at the moment.

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Explanations of Micrographs (Plate X):

Fig. 1. Immunofluorescence of SV40 TA and EBNA bound to acid-fixed nuclei of Ramos cells (×280).

A — TA antigen extracted from TU-B cells was diluted 1 : 3 in adsorption buffer consisting of 50 mM NaCl, 10 mM sodium phosphate, 1 mM β-mercaptoethanol and 5 % glycerol, pH 6.5. Methanol-acetic acid-fixed nuclei were treated with TA dilution (20 min/20 °C). The preparations were then treated with serum pool from hamsters bearing tumours induced by TU-B cells (1 hr/20 °C), with human complement (1 hr/20 °C) and with FITC-labelled goat anti-human-complement (β_{1C}/β_{1A} globulin) antibody (Hyland) (1 hr/20 °C). The inset shows metaphase chromosomes (×1040).

B — The same as *A* but normal hamster serum was used instead of TA-antibody positive serum.

C — The same as *A* but prior to TA addition, the acid-fixed nuclei had been treated with deoxyribonuclease (Sigma, DN-EP grade; 100 μg per ml of 0.15 M NaCl, 1 mM $MgCl_2$, 0.01 M Tris.HCl, pH 7.5, 30 min, 37 °C).

D — Methanol-acetic acid-fixed nuclei were treated (20 min/20 °C) with EBNA diluted 1 : 3 in the adsorption buffer. The preparations were then treated with EBNA antibody-positive human serum (1 hr/20 °C), with human complement (1 hr/20 °C) and with FITC-labelled goat anti-human complement antibody (1 hr/20 °C). The inset shows metaphase chromosomes (×1040).

E — The same as *D* but EBNA antibody-negative human serum was used instead of EBNA antibody-positive serum.

F — The same as *D*, but prior to the addition of EBNA the acid-fixed nuclei had been treated with deoxyribonuclease.