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TRANSFORMING AND ONCOGENIC ACTIVITY OF SIMIAN SA 7 ADENOVIRUS DNA

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Transforming and oncogenic activity of whole Simian adenovirus SA 7 DNA (Ad SA 7) and of a mixture of its fragments produced by restriction endonucleases R. Bam HI and R. Sal I, was studied. Whole virus DNA was shown to transform rat kidney cells and rat embryonic fibroblasts and to induce tumor formation in newborn hamsters. Ad SA 7 DNA, hydrolyzed by R. Bam HI, possesses transforming activity; a mixture of DNA fragments produced by R. Sal I was oncogenic for hamsters.

KEY WORDS: DNA; transformation; oncogenic activity; restriction endonucleases.

Simian adenovirus SA 7 (Ad SA7) is known to possess high oncogenic and transforming activity [7, 8]. Physical mapping of the DNA of this virus by the use of several restriction endonucleases [3] has improved the chances of discovery of the region of the genome carrying the "oncogen."

The object of this investigation was to study the transforming and oncogenic activity of whole Ad SA 7 DNA and of DNA obtained by hydrolysis with restriction endonucleases R. Bam HI and R. Sal I.

EXPERIMENTAL METHOD

Ad SA 7 was grown on green guenon kidney cells [1]. The virus was purified by treatment with Freon-113 and centrifugation in a cesium chloride density gradient [6]. Ad SA 7 DNA was isolated by the detergent-phenol deproteinization method using pronase [4]. Isolation of the endonucleases and hydrolysis of the DNA by the enzymes were carried out as described previously [2].

A primary culture of kidney cells of 5-7-day WAG rats (KC) and a culture of WAG rat embryonic fibroblasts during the second half of pregnancy (EFC) were used for transformation. The EFC was used in the first subculture. The cells were cultured in 50-ml flasks with a seeding density of 180,000 cells/ml for KC and 150,000 cells/ml for EFC. The cells were grown in medium No. 199 with the addition of 10% calf serum. The cell culture was usually used on the 2nd day (70-80% monolayer). Transformation of the cells was carried out by Graham's method [5].

To study oncogenic activity 3-5 μg DNA in a volume of 0.03-0.05 ml was injected into day-old hamsters subcutaneously into the region of the dorsal surface of the neck.

EXPERIMENTAL RESULTS

On the addition of Ad SA 7 DNA to the flasks with the primary culture KC and EFC from WAG rats foci of transformation were formed. The foci appeared as white spots on the cell monolayer and became visible

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TABLE 1. Transforming Activity of Ad SA 7 DNA and a Mixture of Its Fragments after Hydrolysis by R. Bam HI

Material	No. of experiment	Mean no. of foci per flask in culture of	
		KC	EFC
Ad SA 7 DNA	1	2,3	3
	2	1,2	1
	3	1	Not determined
	4	3	» »
Ad SA 7 DNA hydrolyzed by R. Bam HI	1	1,2	Not determined

*In each experiment four flasks were used, and 1-2 μg Ad SA 7 DNA was added to each flask.

TABLE 2. Oncogenic Activity of Ad SA 7 DNA and a Mixture of Its Fragments Produced by Hydrolysis with R. Sal I

Material	No. of experiment	Frequency of appearance of tumors	Time of appearance of tumors
Ad SA 7 DNA	1	1/5	49
	2	1/5	53
	3	2/7	55
Ad SA 7 DNA hydrolyzed by R. Sal I	1	1/7	48
	2	2/5	73

*Numerator gives number of animals with an induced tumor, denominator total number of animals in experiments.

with the naked eye 3-5 weeks after transfection. Microscopic investigation showed that the foci of transformation consisted of groups of cells resembling epithelial cells with increased refractivity and with a tendency toward stratified growth. The number of foci in the positive experiments (Table 1) varied from one to three per flask after addition of 1-2 μg DNA. Transformation did not take place in control flasks treated with 10 μg DNA from chick erythrocytes. The cell cultures used (KC and EFC) did not differ significantly in their ability to undergo transformation. The efficiency of transformation of both cultures on average was one focus per 10^5 cells after addition of 1 μg DNA.

The study of the oncogenic activity of Ad SA 7 DNA showed that it was high (Table 2). Tumors appeared 49-55 days after subcutaneous injection of 3-5 μg DNA into newborn hamsters (Table 2). Morphologically, they were undifferentiated sarcomas.

Hydrolysis of Ad SA 7 DNA by restriction endonucleases R. Bam HI and R. Sal I did not affect its transforming or oncogenic activity. Enzyme R. Bam HI is known to hydrolyze Ad SA 7 DNA with the formation of seven fragments [3]. The resulting mixture of fragments was shown to induce transformation of the primary KC culture (Table 1). Under the influence of restriction endonuclease R. Sal I, Ad SA 7 DNA is split into six fragments [3]. Injection of a mixture of these fragments into newborn hamsters induced the formation of tumors, which appeared on average with the same frequency and at the same times as after injection of whole DNA (Table 2). Fragmentation of DNA was carried out under conditions of exhaustive hydrolysis; the completeness of hydrolysis of DNA was verified by electrophoresis. The minimal quantity of DNA detectable by this method was 0.01 μg. Since 1 μg was used in the transformation experiment, possible contamination with unfragmented DNA could not exceed 1%.

Hence, both whole Ad SA 7 DNA and a mixture of its fragments produced by specific restriction endonucleases R. Bam HI and R. Sal I, possessed transforming and oncogenic activity in these experiments.

It is intended next to study the transforming and oncogenic activity of individual fragments of Ad SA 7 DNA obtained by the action of R. Bam HI and R. Sal I. In that way the position of the minimal region responsible for these processes in the genome can be determined. We have now obtained cell lines transformed by Ad SA 7 and virus DNA. Determination of homology between the DNA of the transformed cells and virus DNA by the hybridization method will also help to solve this problem.

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ROLE OF T- AND B-LYMPHOCYTES IN HETEROGENEITY OF CELL-MEDIATED REACTIONS TO BACTERIAL ANTIGENS IN MAN

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Blood leukocytes from 30 patients with allergy to tuberculin and bacterial antigens were treated with antithymus (ATS) or anti-immunoglobulin serum (AIGS), after which the leukocyte migration inhibition (LMI) test was carried out with these antigens. ATS abolished LMI by tuberculin and sometimes by bacterial antigens (staphylococcal, streptococcal, etc.). AIGS frequently abolished LMI caused by bacterial antigens but not by tuberculin. In other cases treatment with any serum abolished LMI by antigens or, conversely, it was abolished only by treatment with both antisera in turn. The type of lymphocytes (T or B) determining the reaction to the same antigen in the secondary immune response differed in different patients and also differed in the same patient for different antigens. Five types of interaction between lymphocytes and antigen in the LMI test were distinguished.

KEY WORDS: T- and B-lymphocytes; cell-mediated reaction; antithymus and anti-immunoglobulin serum; immune response to bacterial antigens; hypersensitivity of delayed type (HDT).

To induce an immune response to thymus-dependent antigen, interaction between two types of lymphocytes and macrophages is necessary [7]. The role of these cells in hypersensitivity of delayed type (HDT) reactions, developing in patients, is less clear. Stimulation of human and animal lymphocytes by an antigen to which they exhibit HDT causes the formation of biologically active substances (mediators) in vitro, especially a factor inhibiting migration (MIF) of macrophages and polymorphs [1, 2, 9]. By abolishing the function of T- or B-lymphocytes by treatment with specific antisera and by analyzing MIF formation attempts were made to discover which lymphocytes react, and by means of which receptors, with bacterial antigens and tuberculin during the secondary immune response in vitro.

EXPERIMENTAL MATERIAL AND METHOD

To block receptors of T-lymphocytes or to eliminate these cells from the suspension of leukocytes it was treated with monospecific rabbit antiserum against human thymus (ATS). The antiserum was obtained by the method in [4] after immunization of rabbits (two or three cycles) with thymus cells from healthy fetuses, which had died as a result of complications during labor. The rabbits were immunized with thymocytes (50-100 million per injection, 50-80% of the cells were viable), treated with rabbit antiserum against human serum proteins, and 1-2 days later they received an intravenous injection of 1-2 ml of the same antiserum in order to suppress synthesis of antispecific antibodies and so to increase the specificity of the ATS. The antisera

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