

# Influence of Vaccination with A/PR 8/34 (HO N1) Influenza Virus on the Oncogenic Activity of Polyoma Virus in Newborn Wistar Rats

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## SUMMARY

Vaccination with the A/PR 8/34 (HO N1) strain of influenza virus can decrease significantly the oncogenic activity of polyomavirus in newborn Wistar rats. This effect was regularly observed when animals were vaccinated within 24 hr after birth, whereas vaccinations performed on Days 3 and 10 failed to influence the oncogenic potential of polyoma virus.

In 3 series of experiments, influenza virus vaccination resulted in significantly decreased tumor rates, and, in a 4th series, in reduced tumor growth only.

Heat inactivation of influenza virus failed to abolish its influence on the oncogenicity of polyoma virus.

In most of the experiments, the antibody response to influenza virus was enhanced by inoculation of animals with polyoma virus. In contrast, polyoma virus antibody titers were, in some experiments, decreased, and, in others, not influenced by vaccination with influenza virus.

## INTRODUCTION

The oncogenic activity of polyoma virus in animals can be influenced by inoculation with nononcogenic microorganisms (1, 8, 10, 11). Vaccination with BCG<sup>3</sup> has been reported (1, 10, 11) to reduce or enhance the growth of polyoma virus induced tumors in hamsters, mice and rats. Inhibition or enhancement of tumor growth by BCG vaccination has been found to depend on the dosage and route of vaccination with BCG and the tumor size at the time of vaccination (1, 10, 11). Furthermore, nononcogenic adenoviruses types 1 and 5 and a low oncogenic variant of polyoma virus C·H<sup>0</sup> did greatly enhance the oncogenic potential of polyoma virus in newborn mice (8).

The experiments described in this paper were designed to determine whether vaccination with A/PR 8/34 (HO N1) influenza virus has an influence on the oncogenic activity of polyoma virus in newborn Wistar rats (2-4). For this

purpose, newborn rats were inoculated with graded doses of polyoma virus and vaccinated with influenza virus, and the tumor rates (kidney sarcomas) and kidney weights were determined. Furthermore, the antibody response to influenza virus and polyoma virus was recorded.

## MATERIALS AND METHODS

**Polyoma Virus.** The Stuart Eddy strain of polyoma virus was used. Virus was produced in secondary mice embryo cultures and treated with receptor-destroying enzyme purchased from Philips-Duphar B.V., Petten, The Netherlands, as described previously (3, 4).

The infectivity titers were determined on Micro Test II No. 3040 tissue culture plates, purchased from Falcon Plastics, Oxnard, Calif.; secondary mice embryo cells were used (3, 4). Infectivity titers were expressed in terms of TCID<sub>50</sub>/0.2 ml. Hemagglutinin titers were determined as previously described (3, 4). Titers were expressed in terms of hemagglutinin units/0.025 ml. The values of log TCID<sub>50</sub>/hemagglutinin unit of the virus suspensions used for inoculation of animals ranged from 4.00 to 4.32.

**Influenza Virus.** The egg-adapted influenza virus strain A/PR 8/34 (HO N1) was used. Virus was purified by adsorption onto and elution from BaSO<sub>4</sub> purchased from E. Merck, Darmstadt, Germany (6), and was suspended in 0.15 M NaCl solution, buffered at pH 7.0 with 0.01 M phosphate.

The hemagglutinating activity was measured by means of the photometric HCU method (6). Titers were expressed in terms of HCU's (6).

The egg infectivity titers were determined on 11- to 12-day-old eggs, as described by Hirst (9), and were expressed in terms of EID<sub>50</sub>. The value of log EID<sub>50</sub>/HCU was 4.44.

In some experiments, virus was inactivated by heating at 37° for 2 hr.

**Animal Experiments.** Newborn Wistar rats purchased from Zentralinstitut für Versuchstierzucht, Hannover, Germany, were used. Animals were inoculated s.c. with 0.3 ml of polyoma virus within 24 hr after birth.

The dose of polyoma virus given per animal was expressed in terms of hemagglutinin units/0.025 ml × 0.3 ml. As an example, if an animal received 0.3 ml of polyoma virus suspension containing 48 hemagglutinin units/0.025 ml, the virus dose was scored as 576 hemagglutinin units.

Animals were sacrificed 40 days after inoculation. The

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<sup>3</sup> The abbreviations used are: BCG, *Bacillus Calmette-Guérin*; TCID<sub>50</sub>, tissue culture-infective dose (virus dose just sufficient to infect 50% of cultures); HCU, hemagglutinin concentration unit; EID<sub>50</sub>, egg-infective dose (virus dose just sufficient to infect 50% of eggs); ACU, antibody concentration unit.

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kidneys were tested histologically for tumors as described previously (3, 4). In addition, the weight of the kidneys with and without tumors was determined. Kidneys were fixed prior to weighing by means of 1.6%, w/v, formaldehyde dissolved in buffered saline (0.15 M NaCl solution buffered at pH 7.4 with 0.067 M phosphate).

Vaccinations with influenza virus were carried out i.p. (0.1 ml). Unless otherwise stated, vaccinations with both viruses were done simultaneously. The influenza virus doses were expressed in terms of HCU × ml given per animal.

**Antibody Titrations.** Sera were drawn 40 days after infection with polyoma virus and were treated with M/90 KIO<sub>4</sub> purchased from E. Merck. Antibody titers against A/PR 8/34 influenza virus were determined by use of the photometric ACU method (5) and were expressed in terms of ACU units (5).

Polyoma virus antibodies were titered by means of hemagglutination inhibition test as described previously (3, 4). Titers were expressed in terms of the reciprocal highest serum dilutions yielding complete inhibition of 4 hemagglutinating doses of polyoma virus.

All groups to be compared were tested for antibody in the same test.

**Statistical Analysis.** Differences between the means of the logarithms of antibody titers and the means of kidney weights were tested for significance by use of a *t* test ( $p < 0.05$ , unless otherwise stated).  $\chi^2$  analysis was used for testing tumor rates for significant differences ( $p < 0.05$ ).

## RESULTS

The influence of vaccination with influenza virus on the oncogenic activity of polyoma virus was examined in 4

series of experiments (Series A to D). The results obtained in Series A, B, and C are given in Table 1. The doses of polyoma virus (hemagglutinin units) given per animal are listed in Column 2, and the corresponding doses of influenza virus (HCU × ml) are listed in Column 3. Column 4 shows whether active or heat-inactivated influenza virus was used, and Column 5 states the time at which vaccinations were performed.

The ratios of the number of animals with tumors to the number of animals inoculated are given in Column 6, and Column 7 lists the percentage of animals with tumors for each group. Tumor rates significantly differing ( $p < 0.05$ ) from that of the control experiments where only polyoma virus was given are in *italics*.

The arithmetic means of the logarithms of hemagglutination inhibition antibody titers against polyoma virus and of ACU antibody titers against A/PR 8/34 virus are given in Columns 8 and 9. Values differing significantly (*t* test,  $p < 0.05$ ) from that of control groups are also italicized. Note that vaccination with active and heat-inactivated influenza virus on Day 1 resulted in significant decrease of tumor rates. In contrast, administration of influenza virus on Days 3 and 10 failed to influence the oncogenic activity of polyoma virus, but resulted in increased influenza antibody response.

The antibody response to influenza virus was, in all groups, significantly enhanced by inoculation with polyoma virus. In contrast, influenza virus vaccination decreased significantly, in a number of experiments, the antibody response to polyoma virus.

The means of kidney weights of animals with tumors in groups inoculated both with influenza virus and polyoma virus and in the corresponding control group were tested

Table 1  
Influence of vaccination with A/PR 8/34 influenza virus on the oncogenic activity of polyoma virus in newborn Wistar rats

Experiment (1)	Dose of polyoma virus (hemagglutinin units given/animal) (2)	Influenza virus		Given on Day (5)	No. of animals with tumors/no. of animals inoculated <sup>a</sup> (6)	% of animals with tumors <sup>a</sup> (7)	Logarithms of hemagglutination inhibition antibodies against polyoma virus <sup>a</sup> (8)	Logarithms of ACU titers against A/PR 8 virus <sup>a</sup> (9)
		Dose (HCU × ml/animal) (3)	Remarks (4)					
A1	150	0			12/22	54.4	2.6187 ± 0.4549 <sup>b</sup>	0
A2	150	100	Active	1	4/34	11.8	2.3537 ± 0.4677	2.5716 ± 0.2894 <sup>b</sup>
A3	0	100	Active	1	0/28	0	<1.6232 ± 0	2.2003 ± 0.3093
B1	600	0			23/28	82.1	2.2943 ± 0.3259	0
B2	600	100	Active	1	10/22	45.5	1.9995 ± 0.3784	2.1514 ± 0.4258
B3	0	100	Active	1	0/34	0	<1.6232 ± 0	1.8642 ± 0.3947
B4	600	100	Inactivated	1	13/24	54.2	2.1227 ± 0.3091	2.2754 ± 0.5870
B5	600	100	Active	3	14/19	73.3	2.2755 ± 0.4246	2.4833 ± 0.3993
B6	600	100	Active	10	19/21	90.5	2.4115 ± 0.4189	3.1172 ± 0.3521
C1	576	0			22/66	33.3	2.5462 ± 0.3833	0
C2	576	100	Active	1	3/34	8.8	2.5108 ± 0.3511	2.0032 ± 0.4346
C3	0	100	Active	1	0/51	0	<1.6232 ± 0	1.7941 ± 0.2754

<sup>a</sup> *Italicized values* differed significantly ( $p < 0.05$ ) from controls (Experiments A1, B1, C1).

<sup>b</sup> *Italicized values* differed significantly ( $p < 0.05$ ) from controls (Experiments A3, B3, C3).

<sup>c</sup> Mean ± S.D.

for significant differences by means of a *t* test. The means of the kidney weights of animals with tumors in Groups B2 and B4 were significantly (Group B2,  $p < 0.03$ ; Group B4,  $p < 0.1$ ) smaller than the corresponding value recorded for Group B1. No significant differences were found for Groups B5 and B6. The number of animals with tumors in Groups A2 and C2 was too small to permit a statistical analysis.

These findings suggest that simultaneous inoculation with influenza virus and polyoma virus did reduce the tumor growth, and that vaccination with influenza virus 3 or 10 days after inoculation with polyoma virus did not influence the tumor growth.

Table 2 gives the results of Series D experiments. With the exception of Experiment D4, vaccination with influenza virus failed to reduce the tumor rates. However, with the exception of Experiment D8, where a very small dose (20 HCU's) of influenza virus was given, the average kidney weights of animals with tumors were significantly smaller in groups vaccinated with influenza virus than in the corresponding control groups. This finding indicates that vaccination with influenza virus did significantly reduce the tumor growth in this series of experiments. Note that influenza virus antibodies were significantly increased by inoculation with polyoma virus in groups vaccinated with 20 HCU's of influenza virus, only.

Not shown for convenience in presentation is that, in all series, the influenza antibody titers of animals with and without tumors failed to differ significantly from each other and that animals with tumors had significantly higher polyoma virus antibody titers than did animals without tumors in some groups, only. Furthermore, no influence of the sex of animals on tumor rates and antibody titers was found.

## DISCUSSION

The results presented in this paper warrant the conclusion that simultaneous inoculation with influenza virus and polyoma virus can significantly reduce the oncogenic activity of polyoma virus in newborn rats.

In 3 series of experiments, vaccination with influenza virus resulted in significant reduction of tumor rates while, in most experiments of a 4th series, reduction of tumor growth, only, was observed.

At present, no statement can be made as to whether other influenza virus strains have the same antioncogenic activity as found for A/PR 8 influenza virus. Furthermore, it is unknown whether this activity is due to intact virus or whether it can also be found when isolated hemagglutinins and/or neuraminidases are used, and no statement can be made as to whether the use of virus doses differing from those used in this paper would also yield measurable influence of vaccination with influenza virus on the oncogenic activity of polyoma virus.

The mechanisms responsible for the antioncogenic activity of influenza virus observed are unknown. However, the following hypothetical explanations could be considered.

1. Influenza virus vaccination could stimulate interferon production, resulting in a decrease of polyoma virus multiplication with reduced oncogenicity. However, it is reasonable to assume that a decrease of polyoma virus multiplication would reduce both the oncogenicity and antigenicity of virus. In contrast, the results presented in this paper showed that the oncogenicity was much more decreased than was the antigenicity and, in a number of experiments, vaccination with influenza virus failed to reduce the anti-

Table 2

Influence of vaccination with A/PR 8/34 influenza virus on the oncogenic activity of polyoma virus in newborn Wistar rats

Experiment (1)	Dose of polyoma virus (hemagglutinin units given/animal) (2)	Influenza virus dose (HCU × ml/animal) <sup>a</sup> (3)	No. of animals with tumors/ no. of animals inoculated <sup>b</sup> (4)	% of animals with tumors <sup>b</sup> (5)	Av. kidney wt. (g) for animals		Logarithms of hemagglutination inhibition polyoma antibody titers <sup>c</sup> (8)	Logarithms of ACU titers against A/PR 8 <sup>c</sup> (9)
					With tumors <sup>d</sup> (6)	Without tumors <sup>d</sup> (7)		
D1	960	0	22/27	81.4	1.229 ± 0.912 <sup>d</sup>	0.56 ± 0.044 <sup>d</sup>	2.3757 ± 0.4782 <sup>d</sup>	0
D2	960	500	20/30	66.6	0.755 ± 0.383	0.568 ± 0.077	2.0918 ± 0.4365	1.6045 ± 0.5034 <sup>d</sup>
D3	960	100	18/23	78.2	0.794 ± 0.476	0.537 ± 0.094	2.3248 ± 0.5257	1.5207 ± 0.3711
D4	960	20	7/16	43.7	0.705 ± 0.214	0.535 ± 0.07	2.0372 ± 0.4172	1.5588 ± 0.3723
D5	288	0	16/31	51.6	0.785 ± 0.311	0.575 ± 0.063	2.1501 ± 0.4870	0
D6	288	500	14/21	66.6	0.598 ± 0.10	0.520 ± 0.094	2.1371 ± 0.3929	1.5427 ± 0.4073
D7	288	100	6/21	28.6	0.459 ± 0.134	0.522 ± 0.077	2.2398 ± 0.3461	1.7771 ± 0.4568
D8	288	20	12/21	57.1	0.830 ± 0.421	0.530 ± 0.130	2.2044 ± 0.5403	1.9080 ± 0.3836
D9	0	500	0/15	0	0	0.516 ± 0.083	<1.6232 ± 0	1.5815 ± 0.3721
D10	0	100	0/20	0	0	0.555 ± 0.089	<1.6232 ± 0	1.6875 ± 0.4885
D11	0	20	0/17	0	0	0.553 ± 0.054	<1.6232 ± 0	1.3474 ± 0.2316

<sup>a</sup> Animals were vaccinated on Day 1 with active influenza virus.

<sup>b</sup> *Italicized values*, those differed significantly ( $p < 0.05$ ) from values obtained in controls inoculated with the same dose of polyoma virus without vaccination with influenza virus.

<sup>c</sup> *Italicized values*, those differed significantly ( $p < 0.05$ ) from values obtained in controls vaccinated with the same dose of influenza virus without inoculation with polyoma virus.

<sup>d</sup> Mean ± S.D.

genicity of polyomavirus. In a preceding publication (2), the dose-response curves relating the oncogenicity and antigenicity of polyoma virus to the virus doses used were established. Applying these relationships to the data presented in this paper, the conclusion was reached that the observed reduction of polyoma virus antigenicity in animals vaccinated with influenza virus was too small to account for the reduction of oncogenicity. Thus, it seems unlikely that the antioncogenic activity of influenza virus was due to inhibition of polyoma virus multiplication.

2. The decreased rate of tumor growth observed after BCG vaccination was explained as resulting from a stimulation of antibody response oriented to tumor antigens (10). However, since only influenza virus vaccination was effective, if inoculations with both viruses were done simultaneously, it seems rather unlikely that this mechanism could be responsible for the antioncogenic activity of influenza virus.

3. Since it is known that influenza virus and polyoma virus share receptors on the cell surface (7), it could be assumed that influenza virus combines with such receptors on target cells and thereby prevents adsorption of polyoma virus and subsequent transformation of cells. This assumption would also account for the finding that both active and inactivated influenza virus were antioncogenic.

At present, experiments are in progress which were designed in order to elucidate the mechanism responsible for the antioncogenic activity of influenza virus.

#### ADDENDUM

Evidence now available suggests that both the antigenicity and antioncogenic activity of influenza virus can decrease if suspensions are stored at 4° for more than 4 months prior to vaccination.

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