Variation in Interferon Production by Polyoma Virus Strains of Differing Oncogenicity. (28040)

ROBERT M. FRIEDMAN, ALAN S. RABSON AND WILLIAM R. KIRKHAM
(Introduced by H. L. Stewart)
Pathologic Anatomy Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Variants of polyoma virus with differences in oncogenic properties have been described (1-3). One pair of variants consisting of a virus grown in 40% human serum (S variant) and a variant obtained by repeated passage of polyoma virus in P388D1 cells grown and maintained in 20% fat-free milk (M variant) show markedly different levels of tumor induction in mice, the S variant inducing tumors in over 80% of test mice, while the M variant in less than 10%. Both variants originated from the same extract of a parotid gland tumor from a C3Hf/Bi mouse (3). Allison has reported interferon production by polyoma virus in mouse embryo cell cultures(4). This report describes greater production of an antiviral substance by the M variant than by the S variant in primary mouse embryo fibroblast cultures (ME), and the characterization of this antiviral substance as interferon. Differing levels of interferon production by the S and the M variants in mouse cells could account for their markedly different oncogenic activity in mice, just as variation in interferon production has been suggested as a possible explanation for attenuation of some virus vaccine strains (5,6).

Methods and materials. Polyoma virus strains. The S variant of polyoma virus used in the present studies had had 10 passages in P388D1 cells in 40% human serum and 60% mixture No. 199 and the M variant, 14 passages in P388D1 cells in 20% autoclaved non-fat milk and 80% mixture No. 199(3). Virus pools were titered by limiting dilution in milk-adapted P388D1 cell cultures and titers reported as 50% tissue culture infectious doses ($TCID_{50}$) in $\log_{10}$. End points were read at 21 days.

Production of interferon. The interferon was produced by infecting ME,* maintained in Medium 199 and 5% horse serum, with approximately $10^5 TCID_{50}$ of polyoma virus from M or S pools. After 11, 19 or 22 days, when cytopathogenic effect by the virus was present, the infected cultures and supernatant solutions were frozen and thawed, treated in the presence of 1/100,000 thymol blue with 1 N HCl until pH 2 was reached, left overnight at 4°C, then brought back to neutrality with 1 N NaOH. The preparations were then centrifuged at 10,000 rpm to remove cellular debris.

Assay for interferon. Interferon was assayed in ME-29 cells, a stable line of mouse embryo fibroblasts originally obtained from Drs. Habel and Glasgow of the National Institute of Allergy and Infectious Diseases, NIH, and grown and maintained in Eagle's Medium and 10% calf serum(7). The cells were incubated overnight at 36°C in 30 ml plastic culture flasks with 4 ml of the dilution to be tested, the supernatants decanted and the cultures challenged with 70 to 100 plaque forming units of an r mutant of encephalomyocarditis virus (EMC)(8). After incubation for one hour at 36° an overlay containing 0.9% Noble Agar, 10% bovine serum and plaque medium 199 was applied (7). A second agar and medium overlay containing 1:30,000 neutral red was made at 20 hours and plaque counts read at 24 and 48 hours. The results of the interferon assays are reported as the reciprocal of the highest serial dilution showing approximately 50% plaque reduction as compared with controls. Positive controls consisting of dilutions of a known pool of interferon were included in all experiments.

Results. The results of one of several experiments on different pools of interferon are shown in Table I. This pool was harvested 19 days after polyoma virus infection. Studies on pools harvested 11 or 22 days after infection produced very similar results.

* Purchased from Microbiological Associates, Inc., Washington, D.C.

TABLE I. Titers of Polyoma Virus and of Interferon Produced by Polyoma Virus Variants Grown in ME and Harvested 19 Days after Polyoma Virus Infection.

Polyoma virus variant	Titer of polyoma virus* ($TCID_{50}$ in $\log_{10}$/0.2 ml)	Titer of interferon†
S	7.0	2
M	2.5	16

* Titration performed on samples obtained before treatment with acid.

† Expressed as reciprocal of highest titer giving approximately 50% plaque reduction as compared to controls.

The S variant was found to grow to much higher virus titers than the M variant. Treatment with acid to pH 2 and ultracentrifugation (105,000 *g* for 2 hours) did not completely eliminate infectious virus from the preparations; however, in all cases the amount of residual virus after treatment was greater in preparations from the S variant than from the M variant. Only small amounts of residual virus remained in the latter. No interference was found when 10^6 to 10^8 $TCID_{50}$ of both M and S polyoma virus grown in P388D1 cells were assayed for such activity in the system employed for titering interferon. Therefore, the presence of some residual polyoma virus in the interferon pools could not have significantly altered the results of the interferon assays.

The results of assay for interferon are also reported in Table I. In contrast to polyoma virus titers, interferon titers were 8 times higher in M variant preparations than in S variant preparations. At least a 4-fold difference was found in all cases. In some S variant preparations no interferon at all could be demonstrated and in no case was the titer greater than 2.

Evidence that the antiviral activity found in these preparations was indeed due to interferon is summarized in Table II. The antiviral activity was stable to acid treatment to pH 2, to 60°C for 1 hour, was not sedimented by 102,000 *g* for 2 hours and was effective against a heterologous virus, EMC. The antiviral activity was destroyed by boiling. The properties are similar to those reported for interferon(9).

Discussion. The present results demonstrate in polyoma virus variants a correlation between interferon production in ME and oncogenicity in mice. Although interferon assays were not performed before 11 days following infection with polyoma virus or after 22 days, it is unlikely that assays on samples taken at these times would have altered the general findings, since maximum interferon production by cells follows maximum virus production in the systems where these have been studied(10,11,12) including the polyoma virus interferon system(4). The maximum production of polyoma virus in ME takes place after the tenth day(13).

Whether the difference in interferon production following infection with S or M variants in ME is causally related to their differing oncogenic properties in mice is currently under investigation. Preliminary studies of interferon production caused by the S variant in ME made from C57 black, a strain resistant to the oncogenic effects of polyoma virus infection, showed very low interferon titers. These results indicate that among mouse strains, differences in the oncogenic effects of polyoma virus are probably not due to differences in interferon production in response to infection by the agent. These results are similar to those of Vanio *et al.* who found that the basis for genetic resistance to West Nile virus in mice was probably not interferon(14).

The variation in interferon production could, however, account for the larger plaque size of oncogenic variants when compared to that of non-oncogenic variants(3), and also, for the higher virus titers seen in S variant cultures grown in ME (Table I). In this regard it is of interest that M and S variants grow to equally high titers in P388D1 cells.

TABLE II. Properties of Interferon from Polyoma Cultures.

Treatment	Antiviral activity following treatment
Acid (pH-2)	+*
60°C for 1 hr	+
Boiling, ½ hr	0
Sedimentation (105,000 *g* × 2 hr)	+
Effect on heterologous virus (EMC)	+

* +, activity retained; 0, activity destroyed.

No interferon has been found in pools of M and S variants made in these cells.

Summary. Interferon production in mouse embryo fibroblast cultures infected with the highly oncogenic S variant and the poorly oncogenic M variant of polyoma virus has been studied. Infection of cultures by the M variant was associated with a 4-fold or greater production of interferon by the infected cells. Cultures infected with the S variant, however, produced higher titers of virus. These results may explain several findings such as the differing plaque size of polyoma virus variants of high and low oncogenicity, and suggest a possible relationship between the differing oncogenicity in mice of these virus strains and the variation in interferon production caused by infection of mouse embryo fibroblast cultures by them.

1. Sachs, L., Medina, D., *Nature,* 1960, v187, 715.
2. Gotlieb-Stematsky, T., Leventon, S., *Brit. J. Exp. Path.,* 1960, v41, 507.
3. Law, L. W., Rabson, A. S., Dawe, C. J., *Nature,* 1961, v190, 97.
4. Allison, A. C., *Virology,* 1961, v15, 47.
5. Enders, J. F., *Trans. Coll. Phys.,* Philadelphia, 1960, v28, 68.
6. DeMaeyer, E., Enders, J. F., unpublished data.
7. Glasgow, L. A., Habel, K., *J. Exp. Med.,* 1962, v115, 503.
8. Takemoto, K. K., Liebhaber, H., *Virology,* 1961, v14, 156.
9. Isaacs, A., *Symp. Soc. Gen. Microbiol.,* 1959, v9, 102.
10. Wagner, R. R., *Bact. Rev.,* 1960, v24, 151.
11. Isaacs, A., Hitchcock, G., *Lancet,* 1960, v2, 69.
12. Hitchcock, G., Porterfield, J. S., *Virology,* 1961, v13, 363.
13. Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., Grubbs, G. E., *ibid.,* 1957, v3, 380.
14. Vanio, T., Gwatkin, R., Koprowski, H., *ibid.,* 1961, v14, 385.

Received October 25, 1962. P.S.E.B.M., 1963, v112.

Hyperreactivity to Endotoxin in BCG Treated Guinea Pigs and Its Relationship to Delayed Hypersensitivity.* (28041)

ANTHONY TRAKATELLIS, WARREN R. STINEBRING AND A. E. AXELROD
Biochemistry and Microbiology Departments, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Hyperreactivity to endotoxins of Gram negative bacteria has been studied by many investigators(1,2,3). Recently, Suter(4) described the increased reactivity to endotoxin following BCG immunization of mice. The present investigation was undertaken to clarify the relationship of delayed hypersensitivity to such hyperreactivity in BCG vaccinated guinea pigs, animals more amenable to the study of delayed hypersensitivity.

Materials and methods. Male guinea pigs of the Hartley strain, weighing between 450 and 550 g, maintained on a commercial chow diet† were used.

Immunization. Ten- or 11-day-old cultures of BCG in albumin growth medium were injected intraperitoneally to produce hyperreactivity and sensitivity. Human gamma globulin‡ (HGG) was denatured by heating at 60°C for 60 minutes. For immunization, equal parts of denatured human gamma globulin (DHGG) in saline and incomplete adjuvant§ were mixed. 0.1 ml of the mixture was injected into each of the 4 footpads of the guinea pigs. At times 0.4 ml was injected intraperitoneally. Total dose was 20 μg per guinea pig. With such a course of immunization, good delayed type skin reactivity to DHGG has been observed as early as 5 days after the initial injections.

Test substances. Escherichia coli 0111:

* This investigation was supported in part by grants from Nat. Inst. of Arthritis and Metab. Dis., and Nat. Inst. of Allergy and Inf. Dis., N.I.H., U.S.P.H.S.

† Purina Chow, Ralston Purina Co., St. Louis.

‡ Pentex, Inc., Kankakee, Ill.

§ Difco Laboratories, Inc., Detroit, Mich.