ness, bronchitis, bronchiolitis, bronchopneumonia and, rarely, croup.

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Resistance of Polyoma Virus Immune Animals to Transplanted Polyoma Tumors. (26453)

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Previous reports(1,2), described establishment of transplantable lines of subcutaneous fibrosarcomas from tumors produced by inoculation of newborn animals with polyoma virus. Transplantable tumors in the hamster line and in one of the 2 mouse lines (C57-695) have never had demonstrable virus associated with them. The second mouse line (C57-1923) had virus associated with tumor for the first 10 transplant passages, but not in the subsequent 8 passages. Virus-negative tumors in tissue culture were susceptible to challenge with polyoma virus, and virus production could not be induced in them by irradiation. From this and the lack of demonstrable polyoma virus antigens in tumors, it was concluded that once virus had transformed normal cells to tumor cells it was no longer necessary for maintenance of the tumor.

As an additional piece of evidence for the above conclusion in studies of the hamster tumor(1), it was shown that adult hamsters made immune to virus and having demonstrable antiviral antibodies were still capable of supporting the transplantable tumor. However, this result was based on challenge with a large inoculum of minced tumor. The purpose of this report is to present evidence indicating that adult hamsters and mice made immune to polyoma virus do develop a resistance to transplantable tumors provided the challenge with tumor cells is made on a quantitative basis. Evidence is also presented that this resistance is specific and not due to serum antibodies.

Materials and methods. Animals. General purpose (G.P.) mice were random-bred Swiss, C_3H were C_3H/Hen and C57 were C57/Bl. Hamsters were random-bred Syrian hamsters. All animals were reared in the NIH animal colony. *Tumors.* The transplantable polyoma hamster tumor and C57-695 and C57-1923 transplantable polyoma mouse tumors have been described(1,2). The SW tumor was a fibrosarcoma originating in a C57/Bl mouse that had been inoculated with radioactive thorium and was kindly supplied by Dr. Richard Swarm of Nat. Cancer Inst. in its 16th transplant passage. The S180 tumor was maintained in the ascites form in G.P. mice and was originally obtained from Dr. Morris Belkin of the Cancer Institute. *Virus.* Polyoma virus grown in mouse embryo tissue culture had a titer of 10^7 ID_{50}/ml. *Hyperimmune rabbit serum.* Rabbits

TABLE I. Adult Hamsters Immunized with Polyoma Virus and Challenged with Hamster Tumor Cells 5 Weeks Later.

Cells transplanted	Normal hamsters		Immune hamsters	
	Tumors	Tumor size*	Tumors	Tumor size*
2.5×10^6	2/2	50 × 60 35 × 40	2/2	15 × 15 25 × 50
5×10^5	2/2	45 × 45 35 × 65	1/2	30 × 30
1×10^5	2/2	35 × 50 50 × 70	1/2	25 × 25
2×10^4	1/1	30 × 40	2/2	3 × 3 20 × 20

* Size of tumor in mm at 1 mo.

were immunized by an intramuscular dose of polyoma virus mixed with Freund's incomplete adjuvant and 8 intradermal doses in saline. It had a hemagglutinin inhibition (HI) titer of 1/10,000 and a neutralization titer of 1/25 against 100 $TCID_{50}$ of virus. *Immunization.* Mice referred to as "polyoma immune" had received 0.2 ml intraperitoneally (IP) of a 10^{-2} dilution of standard virus 4 to 10 weeks prior to a test. *Transplantation.* Tumors were maintained by subcutaneous (SC) inoculation of a heavy mince of tumor but quantitative tests used single cell suspensions of trypsinized tumors containing the indicated numbers of viable cells. All transplants were made into the interscapular subcutaneous area.

Results. Immunity to hamster tumor. Adult hamsters were inoculated IP with 0.5 ml of standard polyoma virus. Five weeks later the 24th transplant passage tumor was trypsinized and a single cell suspension counted. Groups of virus-immunized and control hamsters were inoculated SC with 0.1 ml of dilutions of this cell suspension (Table I). At time of tumor cell inoculation the immunized hamsters had polyoma HI antibodies to a titer of 1/1600 in their serum. Animals were held for 2 months at which time 2 in the immune group still had no evidence of tumors, whereas all the controls were dead, with large tumors. In Table I and Fig. 1 can be seen the marked delay in tumor development in those virus-immune animals which did get tumors. *Immunity to mouse tumors.* Adult C57 mice were immunized by a single IP dose of 0.2 ml of 10^{-2} dilution of standard polyoma virus. Three weeks later they and a similar group of unimmunized mice received a heavy mince of transplant passage 12 of the 1923 tumor. After 4 months' observation, 5 of 5 normal mice had large tumors, whereas only 1 of 5 virus-immune mice had a much smaller tumor. A repetition of this experiment produced 2 of 5 controls with tumors and 0 of 5 immunes. (This tumor line has been difficult to transplant successfully in recent passages.)

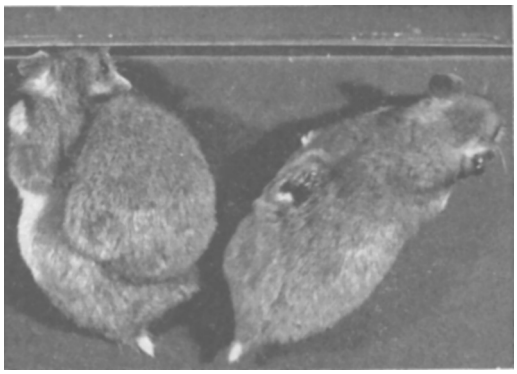

FIG. 1. Normal hamster (left) and polyoma immunized (right) challenged with 2.5×10^6 hamster tumor cells 34 days before photograph.

TABLE II. Adult C57 Mice Immunized with Polyoma Virus and Challenged with 695 Tumor Cells 4 Weeks Later.

Cells transplanted	Exp. 1		Exp. 2	
	Normal	Immune	Normal	Immune
10^6	5/5*	5/5	5/5	3/5
10^5	5/5	2/5	5/5	0/5
10^4	3/5	0/5	0/5	0/5

* No. of mice developing tumors/No. inoculated.

With the 695 tumor, it was possible to quantitate susceptibility to the transplanted tumor. C57 mice were immunized as above and challenged 4 weeks later with 20th passage of the trypsinized 695 tumor. Table II shows that it required 10 times as many cells to produce a successful transplant in virus-immune mice as in controls. The same results were obtained in a repetition of this experiment (Table II). *Specificity of resistance.* To determine whether the demonstrated resistance produced by virus immunity was specific for tumor originally produced by polyoma virus or was a broad resistance to all

TABLE III. Adult Mice Immunized with Polyoma Virus and Challenged 6 Weeks Later with Indicated Tumor Cells.

Cells transplanted	S180 tumor		SW tumor	
	Normal	Immune	Normal	Immune
10^6	5/5*	5/5	5/5	5/5
10^5	4/5	3/5	5/5	5/5
10^4	0/5	1/5	1/5	1/5

* No. of mice developing tumor/No. inoculated.

transplantable fibrosarcomas, 2 non-polyoma tumors were used. C_3H mice were immunized in the usual way with virus and challenged subcutaneously by serial dilutions of trypsinized S180 tumor cells obtained from an ascites passage of this tumor in GP mice. In a second experiment the same was done with dilutions of the SW tumor in C57 mice. Results given in Table III show equal susceptibility to these tumors by virus-immune and normal mice. *Effect of anti-polyoma serum antibody.* Groups of C57 mice were inoculated with serial dilutions of trypsinized 23rd passage of 695 tumor cells. One set of mice received 0.2 ml IP of polyoma hyperimmune rabbit serum, one set normal rabbit serum and one set no serum. The serum inoculation was repeated at 2 day intervals for 3 doses. Table IV shows no protective effect with the antiviral serum. *Anti-tumor complement fixation and cytotoxic antibodies.* Using serum from mice and hamsters made immune to virus and from virus-immune animals that had resisted transplantation of tumors, complement fixation and cytotoxic tests were run against the hamster and 695 mouse tumor. The complement fixation test was carried out with 2 full units of complement and overnight 4°C fixation. The cytotoxic test involved fully grown tube cultures of the tumor cells to which was added the serum to be tested, either alone or with fresh guinea pig serum, and observation for clumping or lysis of the tissue culture cells on incubation. With neither type of serum was there any evidence of complement-fixing or cytotoxic antibodies against the tumors.

TABLE IV. Serum Protection Test. C57 mice received 0.2 ml IP of normal or polyoma immune rabbit serum on day 1, 3 and 5. Day 1 indicated number of 695 tumor cells subcutaneously.

Cells transplanted	Normal serum	Immune serum	No serum
10^6	5/5*	5/5	5/5
10^5	5/5	5/5	5/5
10^4	2/5	5/5	4/5

* No. of mice developing tumors/No. inoculated.

Discussion and Summary. By producing an inapparent infection in adult hamsters or C57 mice with a single dose of diluted polyoma virus these animals subsequently manifest a relative resistance to transplantation of isologous polyoma fibrosarcomas. These sarcomas, although originally induced by virus inoculated into newborn animals, no longer contain any demonstrable virus or viral antigens. This resistance can be overcome if a large enough number of tumor cells are given in the challenge transplant; it is apparently specific for tumors of polyoma origin and would appear to be cell mediated since hyperimmune polyoma antiserum is not capable of transferring such resistance to normal mice.

The most logical explanation of this phenomenon is based on the hypothesis that polyoma induced tumors contain a cell antigen which is not present in normal isologous cells and the degree of this antigenic difference must be small. This new antigen would be produced by the normal cell after it has been transformed as the result of polyoma virus infection, both in the infected newborn and adult animal. The newborn being immunologically non-reactive would tolerate the foreign antigen and allow the transformed cells to multiply and produce a tumor. The adult animal being immunologically mature would reject the transformed cell with its foreign antigen and in so doing become hyper-reactive or resistant to the tumor when challenged with the transplant. This hypothesis is consistent with the findings of Law and Dawe(3) that adult mice given polyoma virus after whole body X-radiation do develop tumors.

If this hypothesis can be proven by immunological experiments, it would suggest that the genome of the virus-transformed cell either now contains viral genome or has been genetically altered by a non-lytic virus infec-

tion. In either case the result of this genotypic alteration would be the development in the transformed cell of an antigen immunologically slightly different from the corresponding antigen in the normal cell.

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An Erythematous Disease of Adult Guinea Pigs Following Transplantation of Homologous Lymphoid Cells.* (26454)

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During studies on the transfer of delayed hypersensitivity to simple chemicals from highly sensitized to normal adult guinea pigs (1), occasional recipients of lymphoid cells developed an illness characterized by intermittent fever, squealing on handling, progressive weight loss, and generalized erythema. Evidence has now been obtained that this disorder is entirely independent of any artificial sensitization of the donor and may be produced with unprecedented high frequency by lymphoid tissue from a specific category of normal animals.

Following the discovery that certain guinea pigs possess natural, delayed type iso-hypersensitivity toward a heritable factor present in sera of other guinea pigs(2), an investigation was undertaken to determine whether a relationship might exist between this state of iso-hypersensitiveness and the illness following lymphoid cell transfer. As the following experiments show, the lymphoid tissue from guinea pigs lacking serum factor but possessing delayed iso-hypersensitivity toward it regularly induced the illness in adult recipients, while lymphoid tissue from guinea pigs possessing serum factor but lacking iso-hypersensitivity was incapable of inducing the disease.

The experimentally produced illness, which superficially resembles "runt", "homologous" and "secondary disease"(3-7), but which differs from them by its induction in normal non-inbred adults and by its characteristic erythema, has been termed "erythematous disease"(8,9). Certain features of erythematous disease recall and allow comparison with aspects of "parabiotic intoxication" or "parabiotic disease"(10,11).

Methods. Experiments concerned with inducing erythematous disease consisted of transplanting washed lymphoid cells from one or more donor guinea pigs into one or more recipients after testing each animal for serum factor and for iso-hypersensitivity to the factor.† Guinea pigs bred from Rockefeller Institute stock, weighing more than 400 g and kept in temperature controlled quarters, were used throughout. Presence or absence of serum factor was determined by injecting 0.1 ml of each animal's serum intracutaneously into separate "assay" guinea pigs of known dermal reactivity(2); an erythematous response greater than 10 mm in diameter at 24 hours was deemed positive for serum factor. Possession or lack of iso-hypersensitivity was ascertained on day of transplantation by observing an animal's cutaneous reaction to sera, known to contain serum factor(2), inoculated intradermally

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† Such tests, done to provide information for the matching of animals, are not necessary for induction of erythematous disease as the illness can be produced in animals that have not received any prior injection.