# Thymus: Role in Resistance to Polyoma Virus Oncogenesis

Abstract. *Mice of the C57BL strain are highly resistant to the oncogenic property of LID-1 polyoma virus, but complete thymectomy within 24 hours of birth rendered these mice susceptible. In thymectomized mice inoculated with adult syngeneic spleen cells, the capacity to resist oncogenesis was restored. The results add further weight to the concept that induction and progression of tumors by polyoma virus has an immunologic basis.*

Polyoma virus will induce neoplasms in most strains of mice if they are injected in the immediate postnatal period. Mice older than 2 weeks at the time of virus injection usually do not develop neoplasms (*1, 2*). These polyoma-induced neoplasms usually arise at multiple sites and establish themselves from epithelial tissue. The C57BL/KaLw mice, however, are not susceptible to the oncogenic property of the virus (*2*). In this laboratory five different "strains" of polyoma virus introduced immediately after birth have induced less than 2 percent neoplasms, and these were restricted to the salivary glands. This resistance of C57BL mice has a genetic basis: Data on the $F_2$ generation and on backcrossing indicate that the resistance is attributable to a single autosomal gene with incomplete dominance (*3*). Organ cultures of the salivary gland rudiments of C57BL mice show the same proliferative response and transformation to the neoplastic state as the susceptible tissues of C3H mice show to polyoma virus infection (*4*). The process of control of the neoplastic conversion, therefore, appears to rest within the intact animal.

There is evidence that neoplasms arising after infection with polyoma virus have specific antigenicity (*5, 6*). This "foreign" antigen apparently is similar in mice from tumor to tumor and from strain to strain. Adult mice that have been given virus in an immunizing dose, which does not produce infection, are subsequently resistant to transplantation of syngeneic polyoma-type tumors, provided the number of cells constituting the challenge dose is regulated. This resistance is not dependent upon circulating viral antibodies and can apparently be transferred to normal recipients by lymphoid cells from immunized mice.

The obvious reason for the susceptibility of most neonatal mice to polyoma virus is the inadequacy of the immune response and the readiness with which tolerance to foreign antigens can be established at this time. The C57BL mice become immunologically competent soon after birth and are indeed relatively resistant to the induction of tolerance (*7*). Sjögren *et al.* (*5*) and Habel (*6*) have interpreted their findings of resistance to challenge with syngeneic transplantable polyoma tumors with the concept that polyoma virus transforms normal cells to neoplastic cells in the newborn and the adult; the transformed cells, containing a new foreign cell-histocompatability antigen, provoke an immune response in adults but not in newborns. Neoplastic cells, therefore, arise after virus infection in the early postnatal period, and they are tolerated and grow progressively. Adult animals are capable of rejecting these tumor cells containing foreign antigen.

This concept is supported by the finding that thymectomy in the neonatal period abrogates the resistance of mice to syngeneic transplants of tumors containing polyoma antigen (*8*). Thymectomy also renders highly resistant C57BL mice susceptible to the oncogenic action of polyoma virus (*9, 10*). In our study (*9*), C57BL mice thymectomized at 3 days of age were unaffected insofar as bodily growth, number of lymphocytes in the blood, and ability to form polyoma hemagglutination-inhibiting (HI) antibodies were concerned, yet 90 percent of the animals developed polyoma-type tumors at an average age of 2 months.

Additional evidence is now reported that interference with the immune mechanism is a necessary prerequisite for successful oncogenesis with polyoma virus in C57BL mice.

Litters from polyoma HI-negative C57BL mothers were divided into three groups that were (i) thymectomized within 24 hours of birth, (ii) thymectomized and received an intravenous inoculation of 6 to 12 $\times$ $10^6$ spleen cells from adult C57BL female donors 24 hours later, that is, at 48 hours after birth, and (iii) either given a sham-operation or kept as intact controls. Disassociated spleen cells were prepared according to the method of Billingham (*11*) except that Hanks solution was

Table 1. Body weights, peripheral leukocytes, and antibody response in C57BL mice. The number of leukocytes was determined when the mice were 2 to 3 months of age; hemolysins, at 2 months. Serums were collected 7 days after intraperitoneal inoculation of 0.1 ml of 10 percent washed sheep red blood cells (RBC). T, Thymectomized mice; C, sham-operated or intact controls; and T + S, thymectomized mice inoculated with adult syngeneic spleen cells.

| Group | No. of mice | Body weights (g) | | Peripheral leukocytes (per mm³) | | Hemolysins to sheep RBC (reciprocals of mean titers) |
|---|---|---|---|---|---|---|
| | | 2 mo | 4 mo | Total | No. of lymphocytes | |
| T | 10 | 14.0 | Dead | 10,400 | 3,430 | 5.0 (0–40) |
| C | 15 | 21.2 | 25.7 | 16,410 | 12,970 | 125 (40–320) |
| T + S | 18 | 19.7 | 26.1 | 13,700 | 10,830 | 120 (40–320) |

Table 2. Effect of neonatal thymectomy and spleen cell restoration on induction of neoplasms by polyoma virus in C57BL and (BL $\times$ C3H)$F_1$ mice. T, Thymectomized mice; C, sham-operated or intact controls; T + S, thymectomized and inoculated with spleen cells; and T + G, thymectomized and the recipients of thymic grafts.

| Group | No. of mice | No. and percent with neoplasms | Mean latent period (mo) | No. tumor-free | Time observed (mo)* |
|---|---|---|---|---|---|
| | | *C57BL mice* | | | |
| T | 20 | 16 (80%) | $2\frac{1}{2}$ ($1\frac{1}{2}$–3) | Dead† | |
| C | 37 | 1 (2.7%) | $3\frac{1}{2}$ | 36 | 8 |
| T + S | 41 | 15 (36.6%) | $3\frac{1}{2}$ ($2\frac{1}{2}$–6) | 25 | 8 |
| | | *(BL $\times$ C3H)$F_1$ mice* | | | |
| T | 13 | 10 (77%) | $3\frac{1}{2}$ ($2\frac{1}{2}$–$5\frac{1}{2}$) | Dead‡ | |
| C | 15 | 0 | | 15 | 8 |
| T + G | 7 | 1 (15.7%) | $6\frac{1}{2}$ | 6 | 8 |

* Final time of observation expressed in months. † All mice dead at 3 months. ‡ All mice dead at 5 months.

used instead of Ringer solution. They were inoculated intravenously through the orbital branch of the anterior facial vein. The absence of mediastinal thymic tissue was verified microscopically at necropsy.

A large-plaque strain of polyoma virus, derived from the LID-1 strain and maintained on C3H mouse embryo cells, was injected subcutaneously into 4- to 6-day-old mice. Each mouse received $2 \times 10^6$ plaque-forming units of the virus.

The thymectomized mice failed to gain weight normally, showed a reduction in the number of lymphocytes in the peripheral blood and lymphoid organs, and had a reduced capacity to form hemolysins to sheep erythrocytes. Most of these thymectomized mice developed tumors of the parotid gland, unilateral or bilateral, and all were dead 3 months after operation. When syngeneic spleen cells were administered, all except 3 of 44 thymectomized mice were restored and 25 of these remained tumor-free and in good condition at 8 months (Table 1). The lymphoid system of the tumorous mice in the group treated with syngeneic cells showed at necropsy no depletion of lymphoid elements, whereas there was a depletion of lymphocytes in the blood and lymphoid organs of their neonatally thymectomized littermates. The presence of parotid gland tumors in 36.6 percent of these treated mice probably results from the early introduction of virus (at 4 to 6 days of age) before adequate restoration of immunologic faculty. More recent data indicate that induction of tumors by polyoma virus is almost completely prevented in C57BL mice treated with spleen cells when the virus is inoculated at a later time (10 to 12 days).

Among the 37 animals that received a sham operation for thymus removal or among the control littermates only one developed a parotid gland tumor; this tumor was unilateral (Table 2). The primary neoplasm in all of the tumorous mice in this study appeared to arise in the parotid gland(s); other salivary glands showed varying degrees of neoplastic foci. Rarely were sites outside of the salivary gland area involved in neoplastic transformation.

The response of a small group of (C57BL × C3H) $F_1$ mice to the LID-1 strain of polyoma virus was similar to that of parental C57BL mice. Intact mice completely resisted the oncogenic effects of virus while those $F_1$ mice thymectomized within the first 24 hours were strikingly susceptible. Mice in these groups were 6 to 8 days old when inoculated with the polyoma virus. In one group, grafted at 1 to 3 days of age, thymuses from 1-day-old C57BL donors reestablished the capacity to resist oncogenesis (Table 2).

These data show that thymectomy within the first 24 hours of life of C57BL (and $F_1$) mice leads to abrogation of resistance to the oncogenic activity of polyoma virus. Though wasting, lymphoid depletion, and impairment of immunologic competence resulted in the mice that were thymectomized shortly after birth, it is known from previous work (*9*) that nonspecific factors affecting the health of the animal and inadequate antibody response to polyoma virus do not contribute to this susceptibility. Treatment of neonatally thymectomized C57BL mice by spleen cell inoculations, which ensured normal growth and immunologic development, also leads to a restoration of resistance. Such supplementation by lymphoid cells is known to result from "seeding out" of these immunologically competent adult cells which have benefited from the presence of thymic tissue. Thymic tissue per se is therefore a necessary antecedent of resistance to polyoma virus to the extent that it provides maturation of the immunologic faculty and consequently prevents the progression of neoplastic clones induced by polyoma virus.

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12. Acknowledgment is made to Britton Smith for expert technical assistance.

14 October 1964

## Spore Discharge in Basidiomycetes: A Unified Theory

Abstract. *Olive's demonstration that a bubble bursting at the basidiospore apiculus breaks the connection between spore and sterigma convincingly explains the mechanism of severance; but it does not explain spore discharge, because the bursting force is not applied in the appropriate direction. The form of the developing spore and sterigma shows that the spore is forcibly abstricted while the membrane is still fluid. If the abstricting force persists until the fracture of the sterigma, it provides a mechanism whereby the spore is reliably discharged.*

The demonstration by Olive (*1*) that the "droplet" formed at the apiculus of a basidiospore, just before its discharge, is in fact a bubble indicates that the tip of the sterigma is fractured by the blow transmitted through the spore when the bubble bursts. Thus, after 75 years of speculation, this structure can finally be assigned a functional role. However, the bubble seems to deliver its blow transversely or diagonally, and it is difficult to believe that it supplies the main propulsive force, which acts along the axis of the sterigma. What is this force?

Theories of jet discharge are invalidated by the fact that the sterigmata preserve their turgor. Buller (*2*) suggested that surface forces on the droplet provide the impetus, but this was no explanation, for no one could suggest how such forces might operate. Prince (*3*) noted a double septum in the sterigma tip of *Gymnosporangium* and proposed that, as the intervening sterigma wall ruptures, these septa evaginate against each other and flip the spore away. However, the combined travel of the two septa could at most be about 0.2 $\mu$, too short to allow them to reach a useful velocity. A stronger objection to Prince's explanation is that the mechanism demands absolutely synchronous fracturing of the full circumference of the apiculus wall; for, if one part held for an instant longer than the rest, the spore would discharge laterally or tip sideways without discharging, whereas almost all spores are discharged directly away from the hymenium (along the axis of the sterigmata) with impressive regularity. The