carbon-atom deviation of 0.11 Å from the observed structure.

In addition to solving crystal-packing structures, this technique can be adapted to nonbonded interactions between different parts of the same molecule. Such calculations are of considerable interest, for example, in establishing the molecular conformation of proteins (*10*).

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Immunologic Defenses against Metastases: Impairment by Excision of an Allotransplanted Lymphoma

Abstract. *Hamsters grafted with an allotransplantable lymphoma that does not metastasize develop a state of concomitant immunity which renders them refractory to reinoculation with cells of the same tumor. Removal of the tumor 7 days after transplantation rapidly leads to a decrease in immunity, the production of enhancing antibodies, and the appearance of metastatic deposits which are probably derived from preexisting tumor cells in the blood and lymphoid tissues.*

Since the existence of tumor-specific antigens was first demonstrated, much evidence has accumulated to show that cells from a number of different types of cancer are antigenic in genetically similar animals (*1*). The immunity induced by these new antigens has been demonstrated by several methods which share one common feature: in all experiments of this kind, measures have been taken to prevent progressive growth of the immunizing tumor (*1*). The possible immunosuppressive effect which the presence of a primary tumor may exert is emphasized by studies in which no immune response could be detected unless the immunizing tumor was excised (*2*). There are, however, certain genetically incompatible (nonspecific) tumors where antigens other than those that are tumor-specific interact with the host and induce high degrees of immunity even though the primary tumor remains in place and continues to grow—a phenomenon known as concomitant immunity (*3, 4*). This difference in behavior between specific and nonspecific tumors may be explained on a quantitative basis in that the primary tumor may always suppress the immune response to some degree, and only when strong antigenic differences exist, such as those present on a nonspecific tumor, can such immunity be detected. Alternatively, different tumors may evoke qualitatively different responses. One way to distinguish between these interpretations is to study the effect of removal of the tumor in a system where strong immunity is expressed while the primary tumor is present. If the first alternative is correct, excision of the tumor should lead to heightened immunity; evidence suggestive of such a process with nonspecific tumors was produced many years ago by Andervont (*5*). We now describe the opposite situation in which resection of a primary allotransplanted tumor causes a depression of immunity. This change is of particular interest since the decline in immunity coincides with the appearance of metastases from a tumor which does not normally form secondary deposits.

The tumor which constitutes our experimental model has been described elsewhere (*4, 6, 7*). It is a lymphoblastic lymphosarcoma that occurred spontaneously, and it has been maintained by serial transplantation in randomly bred hamsters for several years. It induces a state of concomitant immunity whereby, at its height, a tumor-bearing animal may reject more than 100,000 times the number of tumor cells which is required to produce tumors in normal hamsters. Although viable tumor cells soon appear in the blood and lymph nodes of animals with actively growing primary tumors, metastases do not normally develop (*4, 6, 7*).

To perform cell transfer studies, we used young Syrian golden hamsters (75 to 90 g) from an inbred line (MHA) (*8*). Cell suspensions of the lymphoma were prepared according to a method described previously (*7*), and 1×10^7 cells were injected subcutaneously into the right flank of 16 donor animals. Seven days later, when the tumors weighed 1 to 2 g, the animals were divided into two equal groups. In the first group, the tumor grafts were excised; in the second group, the tumors were left in place, and a segment of skin (3 by 1 cm) was removed from the flank close to the tumor graft. Two days after the operation, peritoneal exudates were raised in all 16 hamsters with 6 ml of 3 percent starch solution by the method of Bennett (*9*). Forty-eight hours later, the animals were exsanguinated, and cell suspensions of the spleens and peritoneal exudates were prepared in a manner previously described (*4*). The cells as well as the serum from these animals were mixed with a suspension of the lymphoma containing 2×10^6 cells per milliliter, and 1 ml of the mixture was inoculated subcutaneously into the flanks of 48 isogenic recipients; this procedure is the Winn cell-transfer test (*10*). Cells and serums from untreated MHA hamsters were used as controls.

Growth of lymphoma cells in isogenic recipients was suppressed when the tumor cells were inoculated with splenic or peritoneal cells (but not serum) from sham-operated animals in which the immunizing tumor was preserved (Fig. 1 and Table 1). Similar cells from hamsters in which the immunizing tumor was previously resected were considerably less effective; serum from hamsters in which the original tumor had been resected potentiated tumor growth (Fig. 1).

Further investigations were carried out to answer the following questions: (i) Is the decrease in immunity produced by resection of the primary tumor (and demonstrated by the Winn test) accompanied by increased susceptibility to regrafting with the same tumor? (ii) If such susceptibility is demonstrable, can it be correlated with the appearance of metastases? (iii) Is the potentiating effect of serum from animals whose tumors have been excised due to enhancing antibody?

Thirty-two young, randomly bred

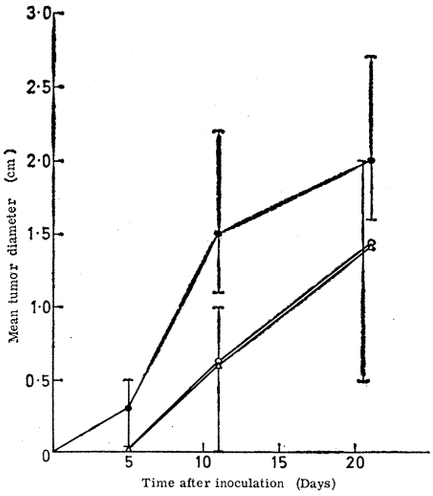

Fig. 1. Growth rate of tumors produced in MHA hamsters inoculated with 1×10^6 lymphoma cells incubated with various test serums (diluted to 1:2). ●, Serum from hamsters in which the immunizing tumor had been excised; △, serum from animals in which the tumor was left in place; ○, serum from normal hamsters.

hamsters were grafted in the right flank with 1×10^7 tumor cells. Seven days later, 20 of the tumors were excised: 12 of these hamsters were inoculated in the left flank with 2×10^6 tumor cells, and eight of them received no further treatment. The remaining 12 hamsters had a segment of skin removed from the right flank, but the immunizing tumor was left in place. These animals were then inoculated in the left flank with tumor cells as above. As an additional control, eight normal hamsters were grafted with 2×10^6 tumor cells in the left flank only. The animals were exsanguinated 12 days later, and material was removed at autopsy for histology and for immunofluorescent studies (*11*). Serums were preserved at 4°C and tested for cytotoxic antibodies by the method of Gorer and O'Gorman (*12*).

If the primary tumor was resected, a second (similar) tumor graft was commonly accepted, that is, in 9 out of 12 animals. If the primary graft was left intact, the animal was refractory to rechallenge with the same dose of tumor cells, and none of the 12 animals developed tumors. This indicates that the decreased immunity previously demonstrated by the Winn cell-transfer test is indeed accompanied by increased susceptibility to rechallenge by a second graft of the same tumor.

No metastases were seen at autopsy in immune hamsters when the tumors in the right flank were intact. By contrast, secondary deposits were observed in about half the animals in which the immunizing tumor had been excised, regardless of whether the hamster had been reinoculated with tumor. Two conclusions may be drawn. First, the appearance of metastases can be correlated with a decrease in tumor immunity. Second, the observation that metastases appear after resection of the primary tumor, independent of the presence of a new graft in the opposite flank, suggests that such deposits are derived from the original tumor; viable cells are known to occur in the blood and lymph nodes soon after the primary tumor graft is established, even though metastases are not formed.

Immunofluorescent studies, in which a rabbit antiserum to hamster γ-globulin conjugated with fluorescein isothiocyanate (*11*) was used, showed the presence of fixed γ-globulin on eight of eight tumors from animals whose original tumor was excised and on none of four of the others. These observations were supplemented by studies of the distribution of cytotoxic antibodies. No cytotoxic activity was demonstrable in serums from animals with an immunizing tumor left in place, but cytotoxic antibodies were found after removal of the primary tumor. It thus appears that serums from hamsters in which the immunizing tumor has been resected contain antibodies, production of which seems to coincide with the decrease in immunity.

In the tumor system under investigation, excision of the immunizing graft may abolish or greatly diminish immunity, stimulate the production of enhancing antibodies, and cause the emergence of metastases. It is not clear whether the enhancing antibodies are directly responsible for the loss of immunity or are merely associated with it. Furthermore, it is not known why resection of the primary tumor is accompanied by such striking changes, but these and other experiments (*6, 13*) suggest that it is the removal of tumor antigen which is important rather than nonspecific factors such as operative shock. Of interest in this context is the demonstration that the administration of large amounts of antigen to animals in which a state of "pure" delayed hypersensitivity has been induced may lead to a rapid loss of 24-hour skin-test reactivity and to the production of circulating antibody (*14*). It would thus appear that the immune response is delicately balanced and that sudden changes in antigenic input, either a decrease or an increase, can affect its qualitative expression.

The experimental system described here needs extensive study before the relevant mechanisms can be adequately discussed. Our results indicate, however, that increased immunity is not an invariable accompaniment of tumor removal, and they reemphasize the extraordinary complexity of the immune response. They also indicate that excision of a primary transplanted tumor may, in certain instances, lead to the appearance of metastases; the implications of this observation must be considered with caution because it is not yet known whether a primary autochthonous tumor can act antigenically or possess any of the other properties of a nonspecific tumor maintained for many transplant generations.

Table 1. Effects of grafting normal MHA hamsters with mixtures of tumor cells and lymphoid cells or serums obtained from hamsters inoculated 12 days previously with 1×10^7 tumor cells. In all cases 3 ml of the test material was mixed with 3 ml of a tumor-cell suspension containing 2×10^6 cells per milliliter, and 1 ml of the final mixture was inoculated subcutaneously. The growth rate of the tumors incubated with the various test serums is shown in Fig. 1.

Source of cells or serum	Number of cells per milliliter or volume of serum	Number of animals with tumors per number of animals inoculated
Spleen cells		
Tumor *in situ*	2×10^7	0/6
	2×10^6	1/6
Tumor removed	2×10^7	0/6
	2×10^6	6/6
Normal (uninoculated)	2×10^7	6/6
Peritoneal exudate cells		
Tumor *in situ*	6×10^6	0/6
	2×10^6	0/6
Tumor removed	6×10^6	0/6
	2×10^6	5/6
Normal	6×10^6	6/6
Serum		
Tumor *in situ*	3 ml	6/6
Tumor removed	3 ml	6/6
Normal	3 ml	6/6

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Somatic Reduction in Cycads

Abstract. *Recurrent somatic reduction is a normal ontogenetic process in apogeotropic roots of cycads, which develop into dichotomously branching coralloid masses. The reduced cells make up part of a ring of differentiated cortical tissue lying midway between the pericycle and the epidermis; they serve as fillers among the large cells and become charged with slime. The differentiated tissue is colonized by a species of blue-green algae.*

Reduction in numbers of chromosomes by dividing somatic cells is a cytological occurrence found occasionally in vascular plants; it is a naturally recurring process in *Allium cepa* L. and *Rhoeo discolor* L. (*1, 2*), and among hybrids of *Rubus* species (*3*); it can be induced and accelerated by treatments with chemicals (*2, 4*). In *Rubus*, somatically reduced cells give rise to phenotypically dissimilar primocanes and to mosaic patterns and checks in leaves (*3*); in sorghum they give rise to haploid mutants (*5*).

Somatic reduction seemed to play no significant role in cell differentiation and function; consequently it ceased to evoke interest among plant scientists as a phenomenon warranting continuing study. Cycads provide an exception to the concept of an insignificant role for natural, recurrent somatic reduction, which is found in certain roots that develop into massive coralloid structures. Chamberlain (*6*) described these roots as follows:

> All cycads have remarkable apogeotropic roots. These grow up instead of down, branch dichotomously and profusely, forming coralloid masses above the ground. The vascular structure is about the same as normal roots, but bacteria, or "bacteriodes," get in very near the tip and cause some distortion, which seems to prepare the way for the entrance of a blue-green alga, *Anabaena*. The alga multiplies rapidly, so that there is a blue-green zone midway between the vascular cylinder and the epidermis. While the zone is usually only one cell wide, the cells are so enlarged radially that the zone is readily visible to the naked eye. The tubercles are almost universal in seedlings and are much more prevalent in the greenhouse than in the field.

Meyer (*7*) reported that the endophytes of cycads nourish themselves at least partly on slime that is secreted by cortical cells into the intercellular spaces in the region of differentiation.

I first chanced upon somatic reduction in *Cycas revoluta* Thunb. ($2n = 22$). The discovery led me to investigate *C. circinalis* L. ($2n = 22$), *Ceratozamia mexicana* Brongn. ($2n = 16$), *Dioon spinulosum* Dyer ($2n = 18$), *Encephalartos horridus* Lehm. ($2n = 18$), *Macrozamia moorei* F. Muell. ($2n = 16$), and *Zamia floridana* A. DC. ($2n = 16$); recurrent somatic reduction is an ontogenetic characteristic common to all.

In view of Chamberlain's statement that all cycads have coralloid apogeotropic roots inhabited by algae, and of my finding that somatic reduction occurs in the several species examined cytologically, one may rationalize that the cytological features seen in these species probably occur in all members of the Cycadaceae.

The cytological phenomena described herein were seen during studies of excised root tips collected and squashed in aceto-orcein by use of normal procedures. In order to make certain that the phenomena seen really do exist and are not artifacts induced by treatment or squashing, sectioned roots were stained with crystal violet by Randolph's procedure (*8*). This material was not amenable to photography, however, because seldom can all chromosomes be found on the same plane of focus. The fact that the walls of reduced cells are fragile, tending to rupture under the pressure of squashing, accounts for the appearance of chromosomes in fragments of cytoplasm in some of the photographs (see Fig. 1).

All the photographs (Fig. 1) used for illustrating various features of somatic reduction are of *C. revoluta*, but identical features occur in the other species of cycads examined. Tubercles developing on roots of a young offset of *C. revoluta* are shown in Fig. 1A. The midcortical zone of cells, which is inhabited by the blue-green alga *Anabaena cycadeae* Reinke, is clearly evident in Fig. 1B. Cells in this zone, undergoing reduction, as well as those remaining diploid but becoming large and thin-walled, divide largely along longitudinal lines. There are, however, enough divisions along radial and lateral lines to maintain the zone's width at one or two cells and its position at mid-cortex. Cells interior and exterior to it follow the usual pattern of mitosis and differentiate into typical cortical tissue.

The chromosome complement, a mitotic metaphase, and a large cell in the differentiated zone are shown in Fig. 1, C–E. Somatic reduction begins with a typical metaphase in a diploid initial, like the one in Fig. 1D. Instead of dividing equationally, however, the chromosomes assort into two haploid groups (Fig. 1F). After cytokinesis, the reduced groups may organize nuclei of metabolic cells, or, after a brief interphase, may undergo further reduction (Fig. 1, G–I). Again, metabolic nuclei may be organized (Fig. 1J), or further reduction may occur (Fig. 1K). Reduction can continue to the point where nuclei are organized containing only two chromosomes (Fig. 1L) and sometimes only a single chromosome. The end products are groups of small linear cells interspersed among the larger diploid cells (Fig. 1, M–O).

Cycas revoluta and *C. circinalis* have 22 somatic chromosomes, so the first reduction distributes 11 chromosomes to the two daughter cells. Since 11 is an odd number, one daughter cell receives five chromosomes, the other six chromosomes coming from the second reduction (Fig. 1I). The third reduction results in 3:2 and 3:3 distributions, respectively, and final reduction results in nuclei having only one or two chromosomes. In species having 18 chromosomes, such as *D. spinulosum* and *E. horridus,* the first reduction results in daughter cells having nine chromosomes each; assortment thereafter, is uneven because of the odd number, just as it is in *C. revoluta* and *C. circinalis*. In species having 16 chromosomes, such as *Ceratozamia*