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Mediation of Immunity to Tumor Isografts in Mice by Heterologous Ribonucleic Acid

Abstract. *The growth of tumor isografts in inbred mice is inhibited by intraperitoneal injections of syngeneic spleen incubated, in vitro, with ribonucleic acid extracted from guinea pigs immunized with the same mouse tumor. This inhibition is partially tumor-specific. Treatment with ribonuclease abolishes the response.*

Ribonucleic acid (RNA) extracted from lymphoid tissues of immunized animals can transform normal lymphoid cells to immunoreactive cells (*1*). Mannick and Egdahl (*2*) and Sabbadini and Sehon (*3*) transferred allograft immunity with RNA extracted from the regional lymph nodes or spleens (or both) of animals in which skin allografts were being actively rejected. Recipients of spleen cells that had been incubated with this RNA rejected specific skin grafts in an accelerated fashion. Similar results have been obtained in our laboratory (*4*). Rigby (*5*) has prolonged the survival of mice bearing Ehrlich ascites tumors by administering syngeneic spleen cells previously incubated with RNA from the spleens of mice immunized with this tumor. Cohen *et al.* (*6*) suggested that the conversion of normal lymphoid cells to immunoreactive cells by RNA from lymphoid organs of immunized animals ("immune" RNA) was at least partially strain-specific within a single species. We have observed a decreased incidence in the growth of tumor isografts in inbred mice after administration of syngeneic spleen cells incubated with heterologous RNA preparations—that is, RNA extracted from the lymphoid tissues of guinea pigs immunized with the mouse tumor to be treated.

A benzpyrene-induced fibrosarcoma, designated BP-4, carried in C3H/FB (mammary tumor agent free) mice was used to immunize Hartley guinea pigs. Each pig received 0.5 ml of a concentrated tumor cell suspension in complete Freund's adjuvant in each foot pad. An intraperitoneal injection, without adjuvant, was also given. After 10 to 14 days, the spleens and the axillary, popliteal, and inguinal lymph nodes (sites of antigen processing) were excised and immediately frozen in Dry Ice. RNA was extracted (*4*), washed, dissolved in Earle's balanced salt solution (BSS) to a concentration of 400 to 1000 μg/ml, and made 0.7*M* with respect to sucrose. Such preparations contained, per milliliter, 75 to 150 μg of DNA and 65 to 100 μg of protein (*7*).

Cell suspensions were prepared from the spleens of normal C3H/HeN mice by passage through No. 40 stainless steel mesh in medium 199 and filtration through No. 80 stainless steel mesh. The cells (10^7 to 10^8 per milliliter) were then incubated in the RNA solutions at 37°C for 20 minutes in a shaking water bath. They were washed in BSS and counted, and the concentration was adjusted to 1 to 2×10^8 viable cells (by trypan blue exclusion) per milliliter. Normal spleen cells were also incubated with RNA extracted from guinea pigs immunized with a mixture of normal C3H lung, liver, kidney, and spleen cells. As a control, RNA was prepared from the lymphoid tissues of pigs immunized with Freund's adjuvant only, and RNA extracted from pigs immunized with BP-4 was treated with ribonuclease (20 μg/ml) (*8*) for 15 minutes at 37°C prior to incubation with spleen cells.

Normal C3H mice were divided into groups of approximately 30. Each mouse was given, on two successive days, intraperitoneal injections of 5 to 10×10^7 spleen cells that had been

Table 1. Development of BP-4 tumor isografts in C3H mice after subcutaneous injection of 10^4 tumor cells. Experimental mice also received intraperitoneal injections of syngeneic spleen cells incubated with indicated RNA preparations. Results are combined from three separate experiments.

Groups	No. developing tumors/ total No. in group
Recipients of spleen cells incubated with RNA from guinea pigs immunized with:	
BP-4 tumor cells	19/58 (32.7%) $P < .01$*
Normal C3H lung, liver, kidney, and spleen	26/49 (53%) $.2 < P < .3$*
BP-4 tumor cells (ribonuclease)†	35/54 (65%)
Freund's adjuvant only	36/46 (78%)
Controls	
Challenge controls (no spleen cells)	89/150 (59.3%)
Spleen cells not incubated with RNA	16/27 (59%)

* P value by χ^2 with Yates's correction for this group when compared to control groups. † These RNA preparations were treated with ribonuclease prior to incubation with spleen cells.

incubated in the RNA preparation designated for its group. In addition, one group of mice received normal C3H spleen cells that had not been incubated with RNA, and one group received no spleen cells at all. Mice in all groups received a subcutaneous inoculum of 10^4 BP-4 cells in the flank, simultaneously with the first injection of spleen cells. All animals were observed for tumor development.

Recipients of intraperitoneal injections of spleen cells incubated with RNA from guinea pigs immunized with BP-4 evidenced a statistically significant inhibition of tumor development when compared with untreated mice or with mice receiving spleen cells that had not been incubated with RNA (Table 1). When RNA from pigs immunized with BP-4 was treated with ribonuclease, no inhibition of tumor development resulted. Groups of mice receiving spleen cells incubated with RNA from pigs immunized with Freund's adjuvant alone also exhibited no inhibition of tumor growth.

This inhibition of tumor development may represent the transfer of immunity to transplantation antigens with RNA extracted from lymphoid organs of heterologous, immunized animals. Mouse tumor cells, when injected into a guinea pig, constitute a xenograft. We had, therefore, not expected to note any tumor specificity in these responses, since, in the guinea pig, the contributions of tumor-specific transplantation antigens was expected to be inconsequential in the presence of strong, mouse transplantation antigens. However, RNA extracted from pigs immunized with tumor was significantly more effective in mediating responses than was RNA from pigs immunized with normal mouse tissues. This suggests that some degree of tumor specificity is present.

Alexander *et al.* have reported the regression of rat sarcomas after the direct injection of nucleic acids from lymphocytes of sheep immunized with the sarcoma (*9*). However, we believe our results represent the first reported instance of the transfer of immunity with spleen cells incubated with heterologous, rather than homologous, "immune" RNA. Cohen has suggested that genetic differences between lymphocytes and the RNA in which they are incubated might diminish or abrogate these types of responses (*6*). However, on theoretical grounds, the remarkable structural uniformity of nucleic acids from bacteria to man should not preclude the possibility of immune responses mediated by heterologous RNA. Molecular structures of nucleic acids are relatively constant throughout phylogeny, differences existing primarily in the number and sequence of their bases. Animals within the same order show the highest degrees of correspondence in the base compositions of their nucleic acids (*10*). Thus, it seems logical to assume that, within the rodent system herein described, immunity can be transferred with heterologous RNA.

It is possible that incorporation of informational "immune" RNA by the spleen cells during incubation is responsible for the conversion of these normal lymphoid cells to immunoreactive cells. However, Gottlieb and others have submitted convincing evidence that there is persistence of antigen (probably processed antigen) bound to RNA extracted from immune lymphoid cells (*11*). An RNA-antigen complex might sensitize lymphoid cells in a more efficient fashion than antigen not bound to RNA. This indeed was demonstrated with the antigen hemocyanin (*12*). Such RNA-antigen complexes of markedly increased immunogenicity have been termed "super antigens" by Friedman, and by Fishman and Adler (*13*). If immunologic specificity resides within this processed antigen, it would seem that, as long as the RNA does not alter antigenic specificity when binding to antigen, the species of origin of the RNA is not of prime importance. The role of RNA in such RNA-antigen complexes may be analogous to that of the protein in a hapten-protein conjugate, where the nature of the protein carrier in no way alters the antigenic specificity of the haptenic moiety. Whatever the mechanism of action of "immune" RNA, the fact that treatment of our RNA extracts with ribonuclease inactivated the preparations indicates that RNA is essential in this system of transferring immunity.

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DNA Synthesis during Yeast Sporulation: Genetic Control of an Early Developmental Event

Abstract. *In yeast the mating type alleles* a *and* α *also control sporulation; heterozygous strains* (a/α) *sporulate while homozygous strains* (α/α *or* a/a) *do not. Net DNA synthesis, an early event preceding sporulation, occurred normally in the heterozygous strains but did not occur in the homozygous strains. Therefore, the* α/a *alleles also influence early development well before spore formation begins.*

Sporulation in *Saccharomyces cerevisiae* is governed by the mating type alleles *a* and α located on chromosome III (*1*). This was demonstrated by the observation that diploid strains heterozygous for these alleles (that is, α/a) could sporulate, while strains homozygous for either one of these alleles (that is, α/α or a/a) failed to sporulate (*1, 2*). However, it is not known when during sporulation these alleles first begin to affect development. Since net DNA synthesis is one of the earliest events of the sporulation cycle (*3*), we examined the effects of the mating type alleles on this process. Our results show that the α/a alleles exert a profound influence early in development, well before the terminal steps of spore formation begin.

The strains of *S. cerevisiae* used and