sulin activity when such preparations were examined by the rat diaphragm tissue assay. After dissociation of insulin from its complex(es) at pH 9.8 insulin-like activity could be demonstrated by this assay. Identical preparations examined by the rat adipose tissue assay showed that this tissue could manifest insulin-like activity in the presence of the insulin complex itself. A difference is thus evident in the mechanism of utilization of the insulin complex(es) by these 2 different tissues.

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Received April 27, 1960. P.S.E.B.M., 1960, v104.

Transplantability Changes of Mouse Mammary Tumors After Passage Through Tolerant Homologous Recipients.* (25857)

CARLOS MARTINEZ, HARVEY KELMAN AND ROBERT A. GOOD
Depts. of Physiology and Pediatrics, University of Minnesota Medical School, Minneapolis

Transplantation of normal and neoplastic tissues in highly inbred strains of mice is ruled by classical principles of inheritance. Tumors taken from animals of one inbred strain can be successfully transplanted into the same individual (autologous graft) or into members of the same strain (isologous grafts). In addition, by crossing mice of 2 different strains, the resulting F_1 hybrids accept tumor transplants taken from individuals of either parent strain. Finally, F_2 hybrids or backcross hybrids to the non-susceptible parent accept the tumor with a frequency which will depend on number of histocompatibility factors required by the particular tumor employed (See review by Little)(1). Barrett and Deringer first reported that after passing a tumor through F_1 hybrids produced by outcrossing the strain of origin times another resistant strain, transplantability of this tumor usually increased when tested in F_2 or backcross hybrids between the same 2 strains used to produce the intermediate F_1 cross(2). The change was demonstrated to be permanent even when the tumor was returned to the original strain(3) and quite specific for the particular genotype employed in the F_1 passage (4). This phenomenon first explained by its discoverers as due to "adaptation" of the tumor, was later thought to be due to "immunoselection" of certain simplified antigenic types of cell populations preexisting in the tumor(5). However, E. and G. Klein(6,7) could not confirm cell selection interpretation. Experiments using preimmunized animals led these investigators to favor the hypothesis that the change in transplantability induced by passage through the F_1 hybrid might be the result of a change in response of the tumor to specific isoantibodies it provokes in the F_1 individual. They also found that the Barrett and Deringer phenomenon could be induced in tumor cells enclosed in cell impermeable diffusion chambers placed into the peritoneal cavity of F_1 hybrid individuals. From these experiments they concluded that the F_1 hybrid effect is probably humoral in nature(8). Using the phenomenon of acquired immunological tolerance we have studied transplantability changes of tumors after passing these tumors through homologous mice previously made tolerant of the strain of animals from which the tumors derived, and compared these results with those

* Aided by grants from Minn. Division, Am. Cancer Soc. and USPHS.

obtained after passage through the corresponding F_1 hybrid. Studies were also conducted to ascertain whether or not similar transplantability changes would be obtained if the intermediate host was either an homologous neonate or an isologous individual made previously tolerant to F_1 hybrid strain tissue. Results of these experiments are herein reported.

Method. Two experiments were performed. In one the tumor used was a mammary adenocarcinoma appearing spontaneously in a breeder female mouse of the A strain. This tumor was first transplanted into small groups of mice of the A, AZ F_1 hybrids, Z mice previously made tolerant of AZ F_1 hybrid tissue by injection with AZ F_1 cells in the neonatal period and A animals previously made tolerant of AZ F_1 hybrid tissue. Approximately 40 days after first tumor inoculation a newly developed tumor was removed from one mouse of each preceding group and retransplanted into $(AZF_1 \times AZF_1)$ F_2. $(AZF_1 \times A)$ BC and $(AZF_1 \times Z)$ BC. In these groups, incidence of tumor takes ending in death of the host was determined. Tolerant animals were prepared by the method recommended by Billingham & Brent(9) consisting of intravenous injection of prospective recipient animals with viable spleen cells taken from individuals of the prospective donor strain during neonatal period (0-24 hrs after delivery). Before tumor inoculation the existence of a state of tolerance was established by demonstrating survival of a skin homograft taken from a homologous donor of the same strain which had contributed the spleen cells. In all instances tumors were transplanted subcutaneously into the right groin by injecting .2 cc of a 10% tumor cell suspension prepared by cutting the tumor into small pieces with scissors and grinding these pieces in a glass homogenizer. In the second experiment the tumor used was a mammary adenocarcinoma which originally appeared in an A breeder mouse. The tumor was subsequently maintained in the laboratory for 3 successive transfers carried out in mice of the strain of origin. The intermediate hosts were: A, $(A \times Z)F_1$ hybrids, A mice previously made tolerant of $(A \times Z)F_1$ hybrid tissue and Z newborn mice. The latter group received the tumor transplant as neonates (0-24 hr after birth) by introducing a bit of tumor tissue subcutaneously through a small incision in the skin of the back, followed by closure of the wound with a single silk suture. When this procedure was used most of the animals grew the transplanted homologous tumor(10). Here again approximately 40 days after inoculation a newly developed tumor was removed from one mouse of each group and retransplanted by subcutaneous inoculation of a 10% tumor cell suspension into groups of $(AZF_1 \times AZF_1)$ F_2 hybrids. As in the first experiment, incidence of tumor takes ending in death of the host was determined in each group of F_2 hybrids.

Results. Results are recorded in Tables I and II. In the first experiment (Table I) when intermediate hosts were mice of the same strain as that contributing the tumor, incidence of tumor takes in AZ F_2 hybrids was only 2% (1/44); in back-cross hybrids to the susceptible parent (ABC) incidence of tumor takes was 90% (20/22) and when the secondary hosts were back-cross hybrids to the non-susceptible parent (ZBC) no takes were observed (0/44). When intermediate hosts had been AZ F_1 hybrids, incidence of takes in AZ F_2 increased to 23% (11/48); all ABC hybrids accepted this tumor and 10% (4/40) of the ZBC hybrids were susceptible.

When intermediate hosts had been homologous Z strain mice previously made tolerant

TABLE I. Barrett-Deringer Effect on Spontaneous Mammary Adenocarcinoma.

Original tumor	1st host	2nd host	# of takes in 2nd host*	%
A Spont. #1	A	AZ F_2	1/44	2
Idem	"	ABC	20/22	90
"	"	ZBC	0/44	0
"	AZ F_1	AZ F_2	11/48	23
"	"	ABC	23/23	100
"	"	ZBC	4/40	10
"	Z tol. AZ F_1	AZ F_2	15/55	27
"	"	ABC	30/32	94
"	"	ZBC	2/42	5
"	A tol. AZ F_1	AZ F_2	7/49	14
"	"	ABC	19/19	100
"	"	ZBC	2/19	11

* No. +/No. of grafted.

TABLE II. Barrett-Deringer Effect in F_1 Tolerant and Newborn Mice.

Original tumor	1st host	2nd host	# of takes in 2nd host*	%
A (3rd gen.) #2	A	AZ F_2	1/20	5
Idem	AZ F_1	"	6/27	22
"	A tol. AZ F_1	"	8/30	27
"	Z newborn	"	14/27	52

* No. +/No. of grafted.

of AZ F_1 hybrid tissue, incidence of tumor takes was 27% (15/55) in AZ F_2 hybrids, 94% in ABC hybrids and 5% in ZBC hybrids. Finally, when intermediate hosts were A strain isologous mice made tolerant of AZ F_1 tissue, incidence of tumor takes in the AZ F_2 was 14% (7/49); in ABC mice, 100% (19/19), and in ZBC mice, 11% (2/19).

In the second experiment in which the tumor was a mammary adenocarcinoma which had been passed 3 times (Table II) the results were as follows: When intermediate hosts had been of same strain as the tumor, incidence of takes obtained in AZ F_2 hybrids was 5% (1/20). Incidence of tumor takes increased to 22% (6/27), when intermediate recipients had been AZ F_1 hybrids, to 27% (8/30) when the tumor had first been transferred to isologous A mice tolerant of AZ F_1 tissues and, finally increased to 52% (14/27) when intermediate hosts were homologous Z strain mice which tolerated the tumor by virtue of the fact that transplantation was accomplished at birth.

Discussion. These results are of interest from several points of view. First, they confirm the observations of Barrett and Deringer(2), who discovered that changes occurred in transplantability of repeatedly transplanted tumors following passage through F_1 hybrid mice produced by outcrossing of the tumor bearing strain with another resistant strain. In our experiments a similar effect of passage through F_1 hybrids was observed in spontaneous mammary adenocarcinoma which had not previously been transplanted and in tumors which had previously been subjected to only 3 successive transfers within strain of origin. In the first experiment, transfer of an A strain tumor first to isologous A mice and later into AZ F_2 hybrids gave a 2% incidence of takes in the latter animals indicating the expression of approximately 13 histocompatibility factors ($0.75^{13} = 0.02$). However, when the tumor was first transplanted to AZ F_1 hybrids, incidence of takes obtained in AZ F_2 hybrids was 23% indicating that number of histocompatibility factors being expressed had dropped considerably from approximately 13 to approximately 5 ($0.76^5 = 0.23$).

Similar results were obtained in the second experiment although in this instance the histocompatibility factors operating decreased from approximately 10 ($0.75^{10} = 0.06$) to 5 ($0.75^5 = 0.23$) after F_1 hybrid passage.

Of particular interest are the findings regarding previous passage of the tumor through animals of the Z homologous strain rendered tolerant of the tumor either by injection of AZ F_1 spleen cells (first experiment) or by transplanting the tumor during neonatal period (second experiment).

In both instances incidence of takes of these tumors when tested in AZ F_2 hybrids increased as compared to that observed after previous residence in isologous hosts, indicating that the foreign environment to which either tumor was subjected induced changes in transplantability which are the same as those induced after the tumor had been grown in the F_1 environment.

Klein has pointed out(8) that the F_1 hybrid effect as well as the effect on tumors by passing the tumor through homologous individuals in which the homograft reaction is not effective, *i.e.*, by direct transfer of the tumor to embryos of a homologous strain(11) or by transfer to previously irradiated animals (12) may have analogous mechanisms. Our results indicate that homologous mice made immunologically tolerant of the tumor by spleen injection at birth also provide an environment which acts upon tumors transplanted to them to induce the Barrett and Deringer phenomenon.

In the second experiment the magnitude of the change in transplantability of the tumor when the latter had been implanted in newborn homologous Z strain mice was greater than that obtained after AZ F_1 passage. This

might possibly be fortuitous or may be due to the genetic difference between these 2 types of recipients. It is conceivable that the Z newborn individual which tolerates the homologous tumor by virtue of its immunological nullness provides an environment more conducive to adaptation of the tumor because the foreign Z environment differs more markedly from the A tumor than does the AZ F_1 hybrid environment.

Changes in transplantability of the A strain tumor were also induced by passage through A strain mice previously made tolerant of homologous AZ F_1 hybrid tissue. These results are even more difficult of interpretation. However, if one assumes that immunological tolerance induced by intravenous injection of homologous spleen cells or by other methods depends upon constant presence of foreign antigens in residence and circulating in the blood of the tolerant host, perhaps in the form of intact cells(13), then it follows that under the conditions of these experiments growth of the tumor takes place in an individual having circulating reticulo-endothelial cells of its own as well as those of the AZ F_1 hybrid. Therefore, in the tolerant isologous animal the tumor is being constantly subjected to a humoral or cellular environment which might be quite similar to that offered to the tumor by the F_1 hybrid itself.

Summary. 1. Modification of transplantability of tumors by passage through F_1 hybrid as originally reported by Barrett and Deringer has been confirmed. 2. Modification of transplantability of tumors induced by foreign environment holds for spontaneous mammary adenocarcinoma and for mammary adenocarcinoma, passaged a few times. 3. A similar modification of transplantability occurs when A strain tumor has been passed through homologous host made tolerant of A strain tissue by intravenous injection of spleen cells at birth. 4. Modification of transplantability of tumors is also induced by passage of tumor through isologous hosts made tolerant of F_1 hybrid homologous strain by injection of homologous spleen cells in neonatal period. 5. The most striking modification of transplantability of mammary adenocarcinoma was obtained by passage through homologous (foreign) hosts at birth. The physiological and genetic basis for alteration of transplantable tumors in these and previous studies remains obscure.

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