The Nonspecific Nature of Induced Resistance to Tumors

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Some forty years ago, when the systematic investigation of transplantable new growths was begun, it was noticed that animals which had been unsuccessfully inoculated with a tumor of their own species, or in which such a neoplasm had receded, generally resisted a second inoculation. Soon afterward it was shown that a similar immunity could be elicited with homologous normal tissues. Although the difference between a spontaneous new growth and one making its way in the alien soil of another animal were almost immediately pointed out, the hope of discovering an effective serum or tissue extract led to an enormous amount of labor and a flow of contradictory publications that has only just begun to slacken. As this early work has been reviewed by one of us (12) it need not be discussed here; in any case its interest is now mainly historical.

The confusing nature of the problem and the prominence which it attained are revealed in the detailed study by Russell (11), who in 1912 reviewed the concepts of his time and resolved some of the apparent contradictions that were then rife. Others could not be explained until new developments in the science of genetics had made it clear that neoplasms propagated in mixed stocks such as those formerly used elicit antibodies that are directed against them not as tumors, but merely as cells originally derived from genetically different individuals. The chief experimental evidence on this point is summarized in the following brief review of recent papers.

In 1936 Bittner (3) showed that resistance could not be produced in mice of pure strains against carcinomas originating within their own strains. Comparable results have been described recently by Barrett (1), who found that the degree of resistance induced varied with the genetic interrelationship of the host, the tumor, and the donor of the blood used for immunization. With the two pure-line neoplasms employed, no significant resistance could be aroused in inbred mice against growths originating within their own strains. The work of Lewis (8), too, on the transplantability of dibenzanthracene sarcomas produced in various inbred strains gives ample evidence that genetic differences between a tumor and the animal into which it is introduced are alone responsible for acquired immunity. Thus a sarcoma from one strain, when inoculated into mice of another, made them refractory to a second graft of the same tumor, but a transplant in mice of the line in which a new growth originated produced no resistance.

Still further proof is furnished by the work of Gorer (6) on genetically pure stocks and by that of Phelps (10), who demonstrated that the serum antibodies investigated by Lumsden (9), and ascribed to the presence of neoplasms that were from unrelated stocks, are not specifically directed against malignant cells. On the contrary, they appear to be isoantibodies formed in response to the injection of foreign homologous cells.

Despite this array of evidence to indicate that resistance is a nonspecific reaction depending upon differences in the genetic constitution of an animal in which a neoplasm originates and the animals that maintain its existence in successive generations, the Brown-Pearce carcinoma of the rabbit has gained favor as a test object in a renewed attempt to establish the specific nature of tumor immunity. Although this growth is undoubtedly of some value, because of the comparatively large size of the animal in which it is propagated and the profusion of its metastases, serious disadvantages accompany investigations based solely upon its use. It must generally be grown in nonuniform stocks which certainly are not related to the original bearer, and the percentage of successful inoculations is subject to considerable variation since partially or totally resistant animals are not infrequently encountered.

Basing their assertion upon experiments with this neoplasm, Besredka and Gross (2) stated that a preliminary intracutaneous implantation which gave rise to a receding tumor caused permanent resistance to intratesticular inoculation. This observation has been confirmed recently by Cheever and Janeway (4), but these authors regarded the immunity as not wholly specific since it could be elicited with embryo skin also. Furthermore, Jacobs and Houghton (7) found that the complement-fixing reactions described by previous investigators between saline extracts of the Brown-Pearce carcinoma and the serum of some rabbits bearing it were weak and variable, and suggested that genetic differences between neoplasm and host might be responsible for the existence of these antibodies.

Experimental

The experiments to be described in the present paper bring additional evidence that acquired resistance to propagable neoplasms is nonspecific in its nature. Advantage was taken of an ideal situation. The growth employed (R 2426) is a transplantable mammary adenocarcinoma of the rat, described pre-

viously by one of us (5), which originated spontaneously in a female of the 27th brother-by-sister generation of the August line. The high degree of homozygosity of the members of this, and the succeeding generations employed for transplantation, is revealed by the pronounced specificity of the tumor and the regularity with which its transplants proliferate. Not a single instance of failure in rats of the strain in which it arose has been recorded during the 2½ years, and 18 generations, of its propagation. On the other hand, it rarely grows in unrelated stocks.

The August strain originated from a cross between rats of an earlier August strain (line 990) and line 1561. Line 990 has since been maintained as a distinct family by continued inbreeding; line 1561, however, no longer exists.

A fair degree of success was achieved with transplants in rats of line 990, clearly indicating the maintenance of a genetic relationship between this and the August line, despite the fact that they have been inbred separately for many years. Thus of 19 rats of line 990 previously tested, 11 grew the tumor, though its proliferation was slower than in animals of the August line, in which it originated, and necrosis more pronounced. Nevertheless, the results were sufficiently different from those following transplantation into wholly unrelated stocks to suggest that this carcinoma would be useful for the experiment now about to be described.

The immunizing effect of embryo skin, a highly efficient agent when both it and the tumor against which it is employed are derived from heterozygous sources, was investigated in rats of the August line, in which the tumor originated, and in related animals of line 990. Embryos were removed aseptically toward the end of pregnancy, the skin was removed, clipped with scissors until it could be drawn with ease into a Bashford syringe, and injected subcutaneously on one side of the body in doses of 0.3 cc. Here it consistently produced a foreign body reaction which did not vary significantly with the source of the material or the strain into which it was introduced. Grafts of the tumor weighing approximately 0.003 gm. were inoculated subcutaneously into the opposite side of the body 13 days later. Both sexes were employed, and the animals were from 2 to 5 months of age when inoculated. The viability of the tumor was always tested by simultaneous transplantation into August rats.

The following combinations were employed:

1. August strain embryo skin into August strain rats; 2. Line 990 embryo skin into line 990 rats; 3. August strain embryo skin into line 990 rats.

The results are set forth in the accompanying table. It is clear that embryo skin from the August line, in which carcinoma R 2426 originated, had no power to induce resistance in rats of this strain, nor was any significant amount elicited in line 990 animals with embryo skin from line 990. But when rats of line 990 were injected with embryo skin from August rats, a high degree of resistance ensued.

In other words, embryo skin that was genetically identical with the tumor possessed the capacity to immunize against it only in rats of a line partially alien as regards both the embryo skin and the corresponding tumor.

TABLE I: EFFECT OF PREVIOUS TREATMENT WITH EMBRYO SKIN ON GRAFTS OF A CARCINOMA IN RATS OF THE STRAIN IN WHICH IT ORIGINATED AND OF A RELATED STRAIN

Number of rats	Strain of inoculated animals	Source of embryo skin	Number with tumors	Percentage of resistance
10	August	August	10	0
22	Line 990	Line 990	14 (3 R)	36.3
40 *	Line 990	August	3	92.5
41	Line 990	Untreated Controls	32 (10 R)	21.9

R = Tumors which proliferated for a time but eventually regressed.
* Because of inadequate material 6 rats of this group received 0.3 c.c. embryo liver mash, and were among those that did not grow the tumor.

SUMMARY

Rats of a highly inbred line, which are uniformly susceptible to inoculation with a mammary carcinoma that arose spontaneously in a female of this line, could not be immunized against the tumor with embryo skin from the same line, though this material was highly effective in a partially related line.

When the experiment was repeated in this partially related line with embryo skin derived from within it, no significant degree of immunity was achieved.

Thus acquired resistance to transplantable tumors depends upon genetic differences between the animal inoculated and the one that originally produced the neoplasm, and is not specifically directed against the malignant cell.

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