CHANGES IN THE IMMUNOGENIC PROPERTIES OF THREE TRANSPLANTED TUMORS AFTER PROLONGED HOMOTRANSPLANTATION IN INBRED MICE

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In a previous communication [1, 2] we showed that the antigenic composition of different types of transplanted tumor, if inoculated into one inbred animal, possesses both common and distinguishable features. The object of the present investigation was to study immunogenic properties and their relationship to the antigenic individuality of transplanted tumors. We also were interested to discover whether changes took place in the immunological properties of these tumors under the influence of prolonged transplantation in inbred mice.

The most intensive immunity is observed when tumors are absorbed [3, 5]. In our experiments we inoculated tumors subcutaneously in the tail of mice by Andervont's method. Inoculation of the tumor into the tail and its subsequent absorption led to the development of a lasting immunity, the presence of which was verified by reinoculating this animal with the tumor subcutaneously in the trunk. By varying the primary inoculations of various transplanted and nontransplanted tumors into the tail, after reinoculation we were able to study the development of immunity both to the tumor with which the animal was immunized and to the alternative (transplanted and nontransplanted) tumors, i.e, to ascertain the state of crossed immunity. We called the inoculation of the tumors into the tail primary, or immunizing, and into the trunk secondary, or demonstrating immunity.

EXPERIMENTAL METHOD

Three primary mouse tumors were studied—Ehrlich's adenocarcinoma, Crocker's sarcoma, and acridine sarcoma. The scheme of passage through line A mice was described in our first report [1, 2]. In our experiments we used both nontransplanted (original) tumors and tumors of the 30th-35th passage.

The tumor to be transplanted was excised from the donor, cut into pieces of uniform size, and introduced beneath the skin of the recipient mouse's tail by means of a trocar, taking care not to injure the blood vessels. The most suitable place for transplantation was one-third of the way along the tail from its base. After 25-30 days the tumor attained its maximal size, after which it gradually began to undergo ulceration, necrosis, and absorption.

Secondary inoculation of these mice was performed subcutaneously in the animal's trunk, using a 5% suspension of tumor cells in a dose of 0.2-0.1 ml 35-40 days after the primary inoculation. Each experiment consisted of three groups, for it was important to obtain reproducible results. Altogether 45-50 mice were used in each experiment: there were 36 experiments and 1674 inbred mice were treated.

EXPERIMENTAL RESULTS

Examination of the results (see table) shows clearly that a lasting immunity developed after injection of the tumor tissue subcutaneously into the tail. For example, if we compare the immunogenic properties of the nontransplanted tumors, immunity to the identical tumor was observed in 100% of cases in relation to the acridine sarcoma and 98.1 and 92.7%, respectively in relation to Crocker's sarcoma and Ehrlich's carcinoma.

It is also clear that the immunizing effect against homologous tumors was superior to that against heterologous. For example, when an original Ehrlich's carcinoma was implanted into the tail, 92.7% of the recipient mice became immune to this tumor, compared with 75.4% to original Crocker's sarcoma and 74% to original acridine sarcoma.

Immunogenic Properties of the Original and Transplanted Tumors after Crossed Inoculation of Mice Subcutaneously in the Tail

Immunizing inoculation in tail with		Inoculation of tumor detecting immunity: nontransplanted — Ehrlich's carcinoma: no. of mice in expt.	Ehrlich's carcinoma: no. of mice dying from tumor	Ehrlich's carcinoma: immune mice (% of no. of mice in expt.)	Crocker's sarcoma: no. of mice in expt.	Crocker's sarcoma: no. of mice dying from tumor	Crocker's sarcoma: immune mice (% of no. of mice in expt.)	Acridine sarcoma: no. of mice in expt.	Acridine sarcoma: no. of mice dying from tumor	Acridine sarcoma: immune mice (% of no. of mice in expt.)	transplanted — Ehrlich's carcinoma: no. of mice in expt.	Ehrlich's carcinoma: no. of mice dying from tumor	Ehrlich's carcinoma: immune mice (% of no. of mice in expt.)	Crocker's sarcoma: no. of mice in expt.	Crocker's sarcoma: no. of mice dying from tumor	Crocker's sarcoma: immune mice (% of no. of mice in expt.)	Acridine sarcoma: no. of mice in expt.	Acridine sarcoma: no. of mice dying from tumor	Acridine sarcoma: immune mice (% of no. of mice in expt.)
Non-transplanted	Ehrlich's carcinoma	45	3	92.7	45	11	75.4	46	12	74.0	45	13	71.1	45	17	62.6	46	19	58.7
	Crocker's sarcoma	45	9	79.8	45	1	98.1	46	9	80.8	45	12	72.5	45	6	86.6	46	10	78.0
	Acridine sarcoma	45	7	84.5	47	5	89.3	46	0	100.0	45	14	68.8	47	10	78.7	46	7	84.5
Transplanted	Ehrlich's carcinoma	46	29	37.1	46	33	28.1	48	35	27.1	46	20	56.4	46	27	41.3	48	27	43.6
	Crocker's sarcoma	47	29	38.1	48	22	54.2	46	25	46.2	47	20	57.3	48	12	75.9	46	17	62.5
	Acridine sarcoma	48	30	37.5	48	27	43.7	50	22	56.6	48	19	60.9	48	15	68.6	50	12	76.8

The superior immunogenic effect against the homonymous tumor by comparison with the other tumors was also observed. If, for example, a nontransplanted Ehrlich's carcinoma were inoculated primarily, reinoculation of the same tumor revealed immunity in 92.7% of mice, but after transplantation this tumor produced immunity in only 56.4% of mice. Similar results were also obtained with the other tumors (bottom left of the table). The lowest percentage of immune mice was observed in the experiment in which vaccination was carried out with transplanted tumors and a nontransplanted tumor was used for secondary inoculation (bottom left of the table). For example, in the experiment in which a transplanted Ehrlich's carcinoma was used for primary inoculation, tests of the immunity to the same nontransplanted tumor showed its presence in only 37.1% of mice. In analogous experiments, a transplanted Crocker's sarcoma and acridine sarcoma produced a state of immunity to the identical, nontransplanted tumors in 54.2 and 56.6% of animals, respectively.

In the opposite order of the experiment, when nontransplanted tumors were used for primary and transplanted tumors for secondary inoculation, the efficacy of immunization was much higher, and was exceeded only in the experiments in which immunity was produced by and tested with nontransplanted tumors.

It should be noted that immunizing power of the tissues of Ehrlich's carcinoma was much less than that of Crocker's sarcoma or the acridine sarcoma. The experiments with transplanted tumors were particularly demonstrative in this respect. We have already pointed out that as a result of passage all three tumors lost their immunogenic properties, but this was most conspicuous in the case of Ehrlich's carcinoma. It may be seen from that part of the table giving the results of immunization with transplanted tumors in relation to nontransplanted, that the transplanted Ehrlich's carcinoma caused immunity to the nontransplanted Ehrlich's carcinoma in only 37.1% of cases, and to Crocker's sarcoma and the acridine sarcoma still less frequently—in 28.1 and 27.1% of cases, respectively. The other two tumors, although giving a low percentage of immune animals, were nevertheless more highly immunogenic.

After analysis of the findings relating to the crossed immunization with the three tumors (transplanted and nontransplanted), some conclusions may be drawn.

If we use the state of absolute immunity in all the comparable groups as our criterion, we may note delicate changes in the antigenic structure of the tumors, arising as a result of prolonged passage through the inbred animals. Moreover, in the biological experiment we found the same changes in the composition of the antigens as in our previous experiments [1, 2] using the complement fixation reaction with specific absorption and anaphylaxis with desensitization.

In fact we observed a decrease in the immunogenic properties of the transplanted tumors by comparison with the nontransplanted. This fact was evidently due primarily to the closer approximation of the antigenic composition of the tumors, for they were transplanted for a long time through inbred mice, antigenically very closely related. In each passage, all three tumors grew in one organism, assimilating proteins from the same host, which was bound to be reflected in a gradual decrease in the dissimilarity of their antigens and, consequently, in a weakening of their immunogenicity.

It would be wrong, however, to consider that these changes affected only the nonspecific components of the tumor. Had this been so, we should have expected that the transplanted tumors, having lost certain nonspecific antigens and possessing an equal degree of dissimilarity, would produce roughly the same immunizing effect in relation to both transplanted and original tumors. However, this was not observed. By comparing the results obtained during the study of the immunity caused by vaccination with transplanted tumors against the transplanted and original tumors, we saw that in the first case immunity was manifested more strongly than in the second. Changes evidently had taken place in the specific proteins of the transplanted tumors, which had acquired some qualitative difference from the original tumor proteins.

We reach the same conclusion if we examine the relative percentages of immune mice after vaccination with nontransplanted tumors. The antigenic composition of the nontransplanted tumors remained unchanged and was characterized by the widest assortment of proteins and, consequently, by greater dissimilarity. If certain antigens had merely been lost during transplantation, immunization with nontransplanted tumors would have led to the development of immunity in an equal percentage of cases to both the original and the transplanted tumors. However, in the experiments in which the secondary inoculation consisted of transplanted tumors, the percentage of immune animals was lower. Here too, it seems, the factor of specificity and the qualitative change in the tumor antigens as a result of transplantation were significant.

In the experiments to study crossed immunity, our ideas on the similarity and dissimilarity of different tumors were confirmed. We constantly observed that primary inoculation with a given tumor in the tail gave rise to a more intensive immunity to the secondary inoculation of the same than a different tumor. In this case the factor of specificity assumed first place, and although during primary inoculation, all the tumors were equally foreign to the recipient, the immune reaction bore a strictly specific character. The biological experiment thus showed that the specific component distinguishing one tumor from another plays a decisive role in the development of the immunological reactions of the organism.

We must consider further an observation made during analysis of the results of crossed immunization with the two sarcomas (Crocker's and acridine). When the animals were vaccinated with Crocker's sarcoma against acridine sarcoma, or vice versa, the relative percentage of immune mice was higher than in the experiments in which the immunity produced by Ehrlich's carcinoma against these two sarcomas was tested. In other words, despite the significant differences in the antigenic structure of the three varieties of tumors, it could be seen that the antigenic structure of the two sarcomas had slightly more in common with each other's than with that of the Ehrlich's carcinoma, so that their immunogenic properties were displayed to a different degree.

Hence the prolonged transplantation of three varieties of nonstrain-specific tumors through inbred mice caused changes in the immunological properties of these tumors not only on account of the nonspecific components, but also on account of the tumor antigens themselves.

SUMMARY

A study was made of the immuno-biological properties of three transplantable tumors inoculated into one animal and during prolonged passage through inbred mice.

In studying the cross-immunity caused by immunization into tail (Andervont's method) there occurred in these tumors changes of immunobiological properties which took place not only at the expense of nonspecific components, but also at the expense of the tumor antigens themselves.

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