

Attempts to Abrogate Immunity to the Brown-Pearce Carcinoma*

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(Received for publication June 25, 1943)

The cells of the reticuloendothelial system are often regarded as barriers against the development and growth of malignant tumors. Experimental substantiation of this has been obtained by attempts to suppress the functions of these cells by so called blockade methods, and by stimulation of these cells for the purpose of increasing their functional activity. The former procedure is considered to decrease, and the latter to increase resistance to tumor growth.

Blockade of the reticuloendothelial system has been accomplished by the administration of substances that are readily taken up by these cells, such as trypan blue, India ink, etc. Foulds (3) found that the treatment of rabbits with trypan blue before inoculation of the Brown-Pearce carcinoma resulted in a definite increase in the percentage of tumors in the lungs, liver, and spleen. Ludford (6) obtained similar results with a mouse tumor.

Stern and Willheim (8) stimulated the reticuloendothelial system in rats by subcutaneous injections of carotin. The treatment was followed by the disappearance of the Flexner-Jobling rat carcinoma and the Jensen rat sarcoma.

The following experiment was designed to determine whether or not interference with reticuloendothelial function could break down acquired resistance to tumor growth. For this purpose, animals that had been rendered immune to the Brown-Pearce carcinoma were used. The Brown-Pearce carcinoma is a malignant tumor of the testicle of the rabbit, which, following intratesticular transplantation, grows and metastasizes rapidly and usually kills the animal within 4 to 6 weeks.

As has been shown on many occasions (1, 2, 7), intradermal transplantation in the rabbit produces a tumor that increases in size for 3 to 4 weeks, attains its maximum dimensions, and then regresses completely. Following regression of the skin tumor the animals

are immune to subsequent transplantation of this neoplasm.

For this experiment we used 12 male, brown-gray rabbits of similar breed. The animals were immunized against the Brown-Pearce carcinoma by intracutaneous transplantation of this tumor as described. In all animals the skin tumor grew well and regressed within 3 to 5 weeks. After its regression, the animals were inoculated intratesticularly with tumor in order to verify the immunity. In none of the animals that had been previously immunized by intracutaneous transplantation was there any tumor in the testicle. In 3 normal control rabbits similarly inoculated, the tumor grew well. Thus the immunity to growth of the Brown-Pearce carcinoma was apparently well established in the 12 treated animals.

A 1 per cent solution of trypan blue in physiological saline solution was used in an attempt to block the reticuloendothelial system. Of this, 3 cc. were injected intravenously every day into 9 of the 12 immune rabbits, the remaining 3 being used as controls. The injections were started 3 days before the injection of the tumor and repeated daily for 2 weeks following the injection of the tumor. It was considered important that the administration of the trypan blue be maintained over this prolonged period because of the unusual capacity of the cells of the reticuloendothelial system to proliferate. By repeated injections we hoped to keep the reticuloendothelial system functionally as inactive as possible, realizing that complete blockade of these cells is practically impossible. After the preliminary 3 day period of treatment with trypan blue a suspension of tumor cells in saline was injected intravenously in all 12 rabbits, including the 3 that did not receive the trypan blue.

None of the 3 control animals that received the intravenous injections of tumor, but did not receive trypan blue, and in which the reticuloendothelial system was thus not interfered with, showed any tumor growth. In 7 of the 9 rabbits that had received intravenous injections with both trypan blue and tumor, growth of the tumor occurred. The distribution of the tumor nodules varied. In 3 of the 7 rabbits there

* Aided by a grant from the Woman's Board and the John D. and Fannie K. Herz Fund.

† This department is in part supported by the Michael Reese Research Foundation.

was widespread dissemination of tumor nodules in the liver, kidneys, and lung; in the remaining 4, tumor was found only in the kidneys. The appearance of the tumor was strikingly different in most instances from that of the usual Brown-Pearce carcinoma. The prominent feature was the extensive necrosis, which suggested that some factor existed in the animal that was tending to inhibit the growth of the tumor. Microscopically, the typical structure of the Brown-Pearce carcinoma was found, but the necrosis was striking, and surrounding groups of tumor cells there were many macrophages.

DISCUSSION

The fact that the cells of the reticuloendothelial system may affect the growth of a tumor is by no means a new observation. Many investigators believe that a relationship exists between neoplastic growth and the reticuloendothelial system. The relative resistance of the spleen to the growth of tumor is probably due to the large numbers of macrophages present in this organ. As mentioned above, so called blockade of the reticuloendothelial system with various dyes and other agents (3, 6) has been found to result in a definite increase in susceptibility to tumor growth. It should be emphasized again, however, that complete blockade of the reticuloendothelial system is almost impossible.

Hoch-Ligeti (4) found that after the injection of 1,2,5,6-dibenzanthracene the titer of antibodies formed in response to the administration of foreign proteins was much reduced. She believed that this is due to the effect of 1,2,5,6-dibenzanthracene on the cells of the reticuloendothelial system. If it is accepted that the cells of the reticuloendothelial system are responsible for the production of antibodies, one may be justified in assuming that blockade of these cells in immune animals by the administration of trypan blue diminishes their capacity to produce antibodies against the growth of this tumor. Production of antibodies to the growth of the Brown-Pearce carcinoma has been shown recently by Kidd (5), who demonstrated the appearance of complement-fixing antibodies in the blood of rabbits bearing Brown-Pearce carcinoma, and also in the blood of animals that had been immunized to the growth of this tumor. Although there is no definite experimental evidence to bear this out, it seems likely that the cells of the reticuloendothelial system are responsible for the formation of antibodies, and that blockade of these cells by trypan blue suppresses their ability to form antibodies against the Brown-Pearce carcinoma, and so permits the tumor to grow in animals that had been previously immunized. However, one must consider that the phagocytic activity of the reticuloendothelial cells may also be an

important factor in controlling the growth and development of a tumor.

The extensive necrosis in the tumor is interesting, for it suggests that despite the administration of trypan blue, proliferation of the cells of the reticuloendothelial system and continued production of antibodies against the growth of this tumor continued, and that this formation of antibodies was sufficient to cause some necrosis of the tumor but was not sufficient completely to suppress its growth in the immune animals treated with trypan blue as it did in immune animals that were not so treated.

In conclusion, it should be emphasized again that complete blockade of the reticuloendothelial system is impossible. This was borne out by preliminary experiments in which we had tried to break down immunity to the Brown-Pearce carcinoma by this method and in which we had been uniformly unsuccessful. The reason for our failures in these preliminary experiments apparently was due to the fact that we did not continue the administration of trypan blue after the animals had received their intravenous injections of tumor cells. Thus after the tumor cells had been injected the cells of the reticuloendothelial system continued to proliferate sufficiently to prevent growth of the tumor. In the present experiment we prevented this from occurring by continuing the injections of trypan blue for a period of 2 weeks after the transplantation of the tumor, thus trying to obtain as much suppression of the reticuloendothelial system as possible.

SUMMARY

Prolonged intravenous administration of trypan blue in rabbits that had been rendered immune to the Brown-Pearce carcinoma by intracutaneous transplantation, resulted in some animals in abrogation of this immunity. In 7 of 9 tumor-immune rabbits so treated, tumor growth followed intravenous injection with a suspension of tumor cells.

REFERENCES

1. BESREDKA, A., and GROSS, L. Vaccination du lapin contre l'épithélioma sous-cutané. *Compt. rend. Acad. d. sc.*, **202**:1217-1219. 1936.
2. BESREDKA, A., MAGAT, I., LAVAL, P., and BESNARD, P. L'épithélioma intracutané du lapin et son pouvoir immunisant. *Ann. Inst. Pasteur*, **56**:125-136. 1936.
3. FOULDS, L. The Effect of Vital Staining on Distribution of Brown-Pearce Rabbit Tumour. *Scient. Rep. Invest. Imp. Cancer Research Fund*, **10**:21-31. 1932.
4. HOCH-LIGETI, C. Studies on the Effect of Carcinogenic Hydrocarbons on Immunity Reactions. *Brit. J. Exper. Path.*, **22**:233-240. 1941.
5. KIDD, J. G. A Distinctive Substance Associated with the

- Brown-Pearce Rabbit Carcinoma. II. Properties of the Substance: Discussion. *J. Exper. Med.*, **71**:351-371. 1940.
6. LUDFORD, R. J. The Influence of Vital Dyestuffs and of Metallic Colloids on Resistance to Transplantable New Growths. *Scient. Rep. Invest. Imp. Cancer Research Fund*, **10**:1-20. 1932.
 7. PEARCE, L., and BROWN, W. H. Studies Based on a Malignant Tumor of the Rabbit. IV. The Results of Miscellaneous Methods of Transplantation, with a Discussion of Factors Influencing Transplantation in General. *J. Exper. Med.*, **37**:811-828. 1923.
 8. STERN, K., and WILLHEIM, R. Beeinflussung des reticulo-endothelialen Systems durch Carotin: nebst Versuchsung über Einwirkung des Carotins auf das Tumorwachstum. *Ztschr. f. d. ges. exper. Med.*, **97**:354-361. 1935.