ORGAN-LIKE STRUCTURES FORMED AFTER TRANSPLANTATION OF THE GASTRO-INTESTINAL TRACT

S. N. Zinzar, B. I. Leitina,
B. G. Tumyan, and G. Ya. Svet-Moldavskii

UDC 612.6.02:612.3

The whole embryonic gastrointestinal tract (GIT) or parts of it, when transplanted beneath the skin of syngeneic adult animals, grows progressively and forms organ-like cysts of large size which retain the structure of the corresponding parts of the GIT. Structures of the same type also develop after transplantation of minced tissue from various parts of the GIT subcutaneously into the muscles or into the thoracic cavity. It is suggested that these structures be used as a model for investigating the physiology, immunology, and pathology of the GIT and, in particular, the pathogenesis of infections caused by intestinal viruses and bacteria, and immunity to them.

The absence of an immune response to organ-specific autoantigens, such as the antigens of the lens and brain, is usually attributed to the presence of barriers between the blood stream and these organs. However, other possible mechanisms for the prevention of autoimmune responses may exist. The writers have postulated that some organs can produce substances regulating immunological reactions (immunodepressors or immunostimulators).

To test this hypothesis the effect of grafting tissues of the eye, salivary glands, and various parts of the gastrointestinal tract (GIT), as well as homogenates of these organs, on the survival of allogeneic skin grafts was investigated in mice. An immunodepressant effect was obtained only with salivary gland tissue.

In the course of these experiments the GIT embryos was transplanted either intact or as its various parts subcutaneously into syngeneic adult recipients, and it was found that the GIT can form cysts which may grow to a considerable size. These cysts retained the morphology and some functions of the corresponding parts of the GIT.

The first results of a study of the growth, development, and morphology of these structures are described in this paper.

EXPERIMENTAL METHOD

The GIT of BALB/C or CBA mouse embryos at the stage of 17-20 days was transplanted in its entirety subcutaneously in the dorsal region into syngeneic adult recipients. An incision was made in the dorsal skin wide of the midline. The embryonic intestine was stretched out along the whole length of the spine and introduced into the subcutaneous cellular tissue, after which the skin incision was closed. In some experiments the whole stomach, large intestine, or pieces of the small intestine were transplanted. In addition, pieces of tissue measuring 1-2 mm,obtained from a mixture of all parts of the GIT from embryos

Laboratory of Virology, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Blokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 7, pp. 81-85, July, 1971. Original article submitted December 11, 1970.

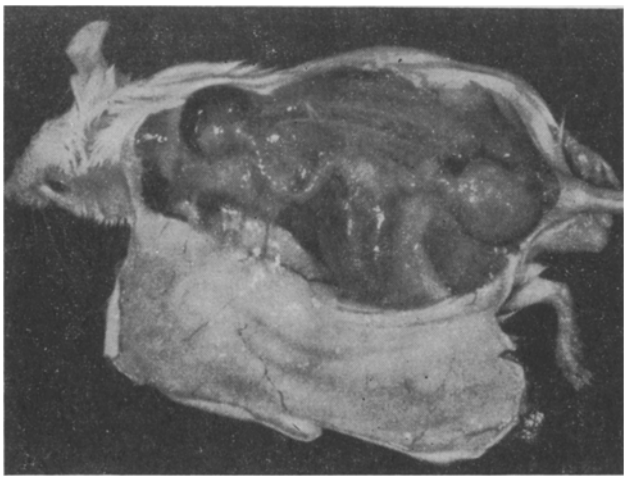

Fig. 1. Cysts formed from graft of whole embryonic gastrointestinal tract.

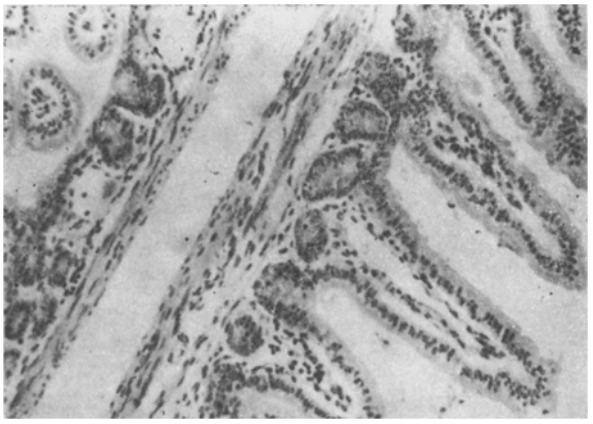

Fig. 2. Graft of entire gastrointestinal tract; segment of small intestine. Hematoxylin-eosin, 108×.

or separately from the stomach and large or small intestine, were transplanted. A suspension of the pieces was injected subcutaneously, intramuscularly, or into the thoracic cavity of syngeneic male or female mice through a trocar, in a volume of 0.2-0.25 ml. Each mouse received about 0.05-0.1-0.2 g tissue in the various experiments.

EXPERIMENTAL RESULTS

Transplantation of the Whole Intestine or Its Parts. In all 7 BALB/C males and 15 CBA males receiving grafts of the whole embryonic gut, structures which at first resembled a thick band (measuring 0.5×7 cm) lying along the whole length of the recipient's spine were found between the 8th and 13th day at the site of injection. Later, some of these structures still remained in the form of a band, while others formed circular cysts filled with secretion (Fig. 1). The cysts increased in size rapidly, and between the 30th and 40th days they reached their largest size (3×9 cm). These large cysts persisted for a long time (longest period of observation 106 days). Cysts were formed in all (9) cases of transplantation of the whole stomach, large intestine (5), and segments of the small intestine (12). The first cysts from the stomach were found on the 14th day, and some appeared on the 29th day. Both types measured 0.3×0.2 cm, gradually increasing in size to 1.2×0.5 cm and 1×1 cm (by about the 90th day after transplantation). Cysts were formed earlier from the large intestine and segments of the small intestine, and they reached a large size more rapidly. By the 10th-15th day after transplantation, some cysts had attained a size of 1.6×1, or even 2×2.5 cm.

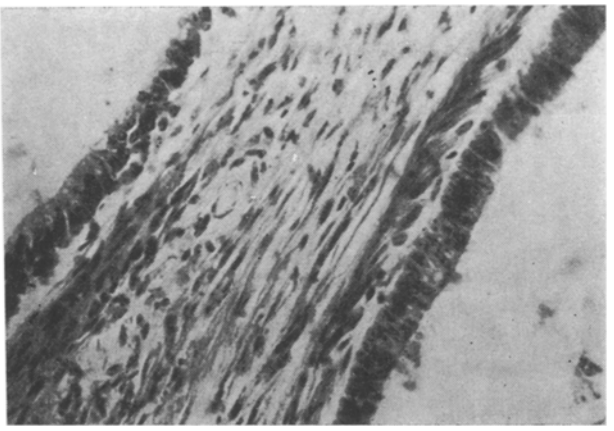

Fig. 3. Graft of minced large intestine: atypical mucous membrane. Hematoxylin-eosin, 200×.

Transplantation of the Minced GIT. In all 25 cases in which pieces of a mixture of different parts of the intestine were transplanted subcutaneously, in 67 of the 72 cases of transplantation of the stomach, in 71 of 75 cases of transplantation of the large intestine, and 88 of 90 cases of transplantation of the small intestine, numerous small cysts (0.5×0.3 or 0.5×0.5 cm) were found on the 5th-8th day at the site of injection, and they increased rapidly in size. The cysts could grow freely under the skin, and for this reason, when grafted in this manner they were able to grow particularly large (9.5×6 cm).

The cysts growing in the thorax were usually small, but some of them grew large enough to occupy a considerable part of the thoracic cavity and to cause death of the animals.

After injection of material into the muscle, cysts also were found, and they were able to grow for a long time (longest period of observation 3 months), but they never grew as large as those transplanted subcutaneously.

Usually numerous small and from 2 to 4 large cysts (from 1 to 4 cm in diameter) were formed from the transplanted fragments; the large cysts were evidently formed by the fusion of several small cysts. Some cysts had a very thin and soft wall, others a thicker and firmer wall; both types were filled with secretion, which was always sterile. Sometimes the cyst walls ruptured under the pressure of their contained secretion, which was expelled; this led to the formation of a sinus at the site of rupture, which took a long time to heal. The cyst walls were penetrated by numerous blood vessels.

The longest period of observation was 5 months. Histological investigation of the organ-like structures formed after transplantation of the whole GIT showed that for a long time they preserved the structure of the mucous membrane and of its glandular components as typically found in the corresponding part of the GIT (Fig. 2). However, intensively proliferating connective tissue and sometimes a layer of smooth-muscle fibers, of considerable thickness, were found in the bed of the graft.

In the cysts formed from minced tissue of the stomach or large or small intestine, the cysts lined with epithelium of a mucous membrane typical for each segment were mixed at random with layers of smooth-muscle fibers, areas of proliferating loose, fibrous connective tissue, and epithelium of the serous membrane. A large quantity of proliferating connective tissue was found in the bed of all the grafts, just as in the bed of the grafts of the entire GIT, and its whole mass was penetrated by numerous blood vessels, frequently dilated and distended with blood. Besides the typical mucous membrane of the stomach and intestine, these cysts also contained bands of epithelium without the characteristic folds, villi, crypts, and glands; in these cases the epithelium was simple, cylindrical (Fig. 3). Structures growing from the entire GIT and its parts, as well as the cysts growing from minced tissue, always contained numerous lymphocytes which were distributed as diffuse foci or as follicles in the connective tissue of the submucosa, and as typical lymph glands. Lymphocytes, together with polymorphs, infiltrated in large numbers into the connective-tissue capsule of the foci of inflammation and necrosis which, in some cases, were found in the grafts.

These experiments thus showed that transplantation of the whole embryonic GIT or of its parts, as well as transplantation of minced segments of the GIT, in a syngeneic system lead to the formation of cysts capable of prolonged and progressive growth, with the preservation of differentiation and of glandulo-epithelial, lymphoid, and connective-tissue structures.

Lazarenko [1] showed previously in a nonsyngeneic system that different types of epithelium grow intensively around the connective tissue in a focus of aseptic inflammation and form microcysts, which subsequently degenerate and are absorbed. Toolan [2] later showed that minced embryonic stomach and intestinal tissue, if transplanted into nonsyngeneic rabbits receiving cortisone or into irradiated rats, forms cysts which largely retain a characteristically intestinal structure. However, her experiments did not receive their due attention.

The structures obtained in the present investigation, and especially those arising from grafts of the whole GIT, can be used as a convenient model for studying tissue interaction during growth and organogenesis. This model also offers fresh opportunities for the study of the physiology of the GIT and, in particular, the secretory activity of its various parts and cells, of digestion, of the nervous and humoral regulation of the functions of the GIT, and of hormone production by its various divisions. The system is also convenient for studying intestinal bacterial and virus infections. It can also be used for the induction of intestinal tumors by a carcinogen. In the present authors' experiments, injection of dimethylbenzanthracene into cysts formed from various parts of the GIT led after 4-5 months to the formation of tumors, and these are still being studied.

The presence of a large quantity of lymphoid tissue in the intestinal grafts suggests that this model can be used to study immunological problems: the physiology of the intestinal lymphocytes, synthesis of IgA, regulation of the serum protein level, and the induction of tolerance.

The authors are grateful to Academician N. A. Kraevskii, and to Drs. M. S. Didukh and L. I. Ravkin for help with the analysis of the hitological sections, and to E. N. Al'tshuller for technical assistance.

LITERATURE CITED

1. F. M. Lazarenko, Principles of Growth and Conversion of Tissues and Organs in Cultivation (Implantation) in Vivo [in Russian], Moscow (1959).
2. H. Toolan, Cancer Res., 17, 707 (1957).