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EFFECT OF SYNGENEIC THYMOCYTES ON PROLIFERATION OF THE SMALL INTESTINAL EPITHELIUM IN MICE

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KEY WORDS: intestinal epithelium; proliferation; thymocytes.

The principal function of the lymphoid system is controlling antigen-structural homeostasis [3, 7]. Data have been obtained in recent years to show that lymphocytes can not only eliminate genetically defective cells, but they can also regulate proliferation and differentiation of unchanged somatic cells [1, 2, 6, 12]. The study of the role of the lymphoid system in regulation of physiological regeneration, a process determining the dynamic constancy of the cellular composition of the tissues, is undoubtedly important.

The aim of the present investigation was to study the action of syngeneic thymocytes on proliferation of the epithelium of the mouse small intestine.

EXPERIMENTAL METHOD

Male CBA mice aged 3 months were used. The animals were divided into 4 groups. The mice of group 1 were given an intravenous injection of thymocytes from intact blood donors in a dose of $4 \cdot 10^7$ cells 18-20 h before an injection of ^{3}H-thymidine. Animals of group 2 were injected with hydrocortisone-resistant thymocytes in a dose of 10^7 cells. Two days before sacrifice the cell donors were given an injection of hydrocortisone in a dose of 2.5 mg per mouse. Animals of group 3 were injected with 0.5 ml of medium 199. The mice of group 4 remained intact. All animals were given an intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g body weight at 9 a.m. and killed 1 h later. A segment of jejunum 2 cm long, taken 1 cm distally to Treitz' ligament, was fixed in Carnoy's fluid. Paraffin sections were coated with type M photographic emulsion, exposed for 2 weeks at 4°C, and developed in amidol developer. Histoautoradiographs were stained with Mayer's hemalum. To study the zone of proliferation, the crypts were selected and divided along their longitudinal axis. The role of enterocytes from the midpoint on the floor of the crypt to the base of the villus was described as a "cryptal column" (CC). The total number of enterocytes and the number of labeled cells in CC was counted and a curve of the distribution of CC plotted on the basis of the number of DNA-synthesizing cells. The index of labeled cells (labeling index — LI) was determined both for the total number of enterocytes in CC and for each cell position. Curves of distribution of LI based on cellular positions of CC were plotted. Intraepithelial leukocytes were excluded from analysis.

The numerical data were subjected to statistical analysis by Student's test at a level of significance of $P \leq 0.02$.

EXPERIMENTAL RESULTS

The animals receiving an injection of medium 199 were indistinguishable from intact mice with respect to all parameters studied. The procedure of intravenous injection evidently does not affect the state of the intestinal epithelium, and these two groups can be regarded as an

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TABLE 1. Parameters of Proliferating Portion of Jejunal Epithelium of CBA Mice (M ± m)

Group of animals	Number of animals	Number of CC	Number of cells in CC	Number of labeled cells in CC	LI, %
Intact	8	260	23,4±0,14	7,71±0,13	33,0±0,52
Receiving 199	9	290	23,9±0,53	7,75±0,15	32,4±0,57
Recipients of intact thymocytes	9	300	23,0±0,18	5,90±0,20	25,6±0,80
Recipients of hydrocortisone-resistant thymocytes	7	150	22,2±0,16	5,60±0,16	25,2±0,81

equivalent control. The total number of enterocytes in CC of the mice receiving thymocytes from intact blood donors did not differ from their number in the control animals (Table 1). However, a significant decrease in the number of labeled cells was found in CC from recipients of intact thymocytes. On average it was reduced by 24%. The decrease in the number of DNA-synthesizing cells when the total number of enterocytes in the crypts was unchanged indicates a decrease in the intensity of proliferation. This is well reflected also by the reduction in LI (by 22%).

The incidence of DNA-synthesizing cells in CC in the experimental and control animals corresponded to the normal distribution, evidence of the uniformity of response of the proliferating portion of the epithelium to the experimental procedure.

The study of the topography of the zone of proliferation in the crypt wall showed the typical distribution of labeled cells in the crypt of the control animals with a maximum in its middle part, corresponding to the pattern observed previously in mice and rats [8, 9, 11]. The zone of proliferation in recipients of syngeneic thymocytes also was bounded by the middle and lower portions of the crypt, but whereas the mucosa was microscopically unchanged, and the crypts preserved their general features of the normal crypt, narrowing of the zone of proliferation and enlargement of the zone of differentiation of enterocytes were found. The distribution of DNA-synthesizing cells among the cell positions of CC shows that the change in the ratio between the zones in the crypt was due to a shift of the upper boundary of the zone of proliferation toward the base of the crypt by 10% of the length of the crypt. Narrowing of the zone of proliferation was unequivocally shown by displacement of the abscissa of the point corresponding to the 50% level of the maximum of the curve of distribution of LI among cell positions of CC, and displacement of the cell position where LI did not differ significantly from zero. It must be noted that with a relatively uniform decrease in the fraction of labeled cells throughout the zone of proliferation, more marked changes were observed in its upper parts. After injection of hydrocortisone-resistant thymocytes into the mice in a dose of 10^7 cells, changes analogous to those observed after injection of intact thymocytes in a dose of $4 \cdot 10^7$ cells developed in the epithelium. Furthermore, hydrocortisone-resistant thymocytes induced a significant decrease in the total number of enterocytes in CC.

Thus under these experimental conditions syngeneic thymocytes can reduce the number of DNA-synthesizing cells in the intestinal epithelium, causing narrowing of the zone of proliferation and enlargement of the zone of differentiation of the enterocytes. The homogeneity of the response of the proliferating part of the epithelium to the experimental procedure may be evidence that the action of the thymus cells, when injected intravenously, is uniform in direction. It is evident that more mature thymocytes have an inhibitor action, since treatment with hydrocortisone *in vivo* selectively eliminates young cell forms [2].

Discovery of the inhibitory action of thymus cells on proliferation of the intestinal epithelium in the adult animal is, in our opinion, important evidence of the regulating role of the immune system with respect to cell reproduction. Investigations have shown that lymphocytes stimulate proliferation of cells of nonlymphoid tissues under reparative regeneration conditions [2, 12]. However, under conditions of physiological regeneration, the relationship may be different. There is evidence that splenocytes of animals exposed to stress cause a reduction of proliferative activity in the liver of intact recipients [5]. In view of the results now obtained it can be tentatively suggested that the immune system, depending on the concrete conditions of functioning of the epithelium, can act in two ways on the process of cell multiplication: it may stimulate or inhibit it, and thus may take part in the maintenance of tissue homeostasis.

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EFFECT OF PERFUSION WITH NUTRIENT MEDIUM ON SECRETORY ACTIVITY OF RAT HEPATOCYTES AND PANCREATIC ISLET CELLS

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Controlled cell culture has attracted ever-increasing attention of research workers in recent years. Both the systems of continuous-flow culture and the various semipermeable synthetic materials suitable for culture of specialized cells, including hormone-secreting cells, have been improved [1, 2, 5, 7, 10, 12].

The writers showed previously that under the conditions of a continuous-flow system of culture cells of the adenohypophysis and the pancreatic islets of Langerhans, when placed on porous membranes, become attached, grow, and secrete their specific hormones [1, 5]. Of the many synthetic semipermeable membranes studied, membranes of sodium borosilicate glass with an effective pore radius of 5-10 nm and a thickness of 1 mm were selected. Pituitary cells placed on such a membrane secreted hormones which, depending on their molecular weight, penetrated selectively into the culture medium.

The present investigation was conducted on an apparatus for continuous-flow cultures by an improved system. Hepatocytes, secreting serum albumin, and pancreatic islet cells, actively producing the hormone insulin, were chosen as biological models. The aim of the investigation was to study the viability and functional activity of these cellular structures during perfusion with nutrient medium.

EXPERIMENTAL METHOD

The basic scheme of the apparatus for cell culture in a chamber with a porous membrane was fully described previously [1]. In this system the gas mixture, containing air and suf-

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