EFFECT OF PROCEDURES DIRECTED TOWARD THE LYMPHOID SYSTEM ON HISTOPHYSIOLOGY OF THE INTESTINAL EPITHELIUM

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With the rapid increase in information on functional diversity of components of the lymphoid population there is an ever-increasing need for re-examination of the principles and mechanisms of lympho-epithelial interaction from new standpoints. Data on the connection of the lymphoid system with the various stages of histogenesis of certain types of epithelium [6, 7], and clinical observations demonstrating morphological and functional parallels between injury to epithelial tissue and disturbance of individual stages of immunogenesis [3-5, 8] have been published. However, the relationship between cause and effect in experimental and clincial situations remains unclear and hypotheses put forward to explain the priority of the lymphoid system in the regulatory mechanisms of lympho-epithelial interactions do not rest on direct proof.

The aim of this investigation was to estimate the contribution of the lymphoid system to mechanisms controlling processes of renewal and functioning of epithelial cells and, in particular, to study the possibility of induction of changes in the epithelial system controlled through immunocompetent tissue. Various techniques based on the principle of disturbance of equilibrium in the immunogenesis system were used on a model of the intestinal epithelium, and various parameters of cellular renewal and of the morphological and functional state of the epithelium of the small intestine were assessed. Procedures directed toward the T component of immunocompetent tissue were used: thymectomy in adult animals, injection of sera against T lymphocytes (ATS) and a mixed population of lymphocytes (ALS), and transplantation of syngeneic thymocytes (ST) and of their hydrocortisone-resistant subpopulation (HRT). Cell populations with different degrees of maturity were tested in normally functioning and functionally destabilized systems of the intestinal epithelium.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL)F_1, (C57BL × CBA)F_1, and (C57BR) mice (males and females) weighing 22-24 g. Thymectomy was performed on the animals at the age of 10-12 weeks under pentobarbital anesthesia (25 mg/kg). Specific ATS and ALS antisera were obtained [1] after immunization of rabbits with thymus cells or a mixture of cells from peripheral and mesenteric lymph nodes and Peyer's patches (5×10^8 cells from each) respectively. Intact and absorbed sera [1] were injected 6 times into mice on alternate days in a dose of 0.25 ml subcutaneously. ST from donors of intact systems or with disturbance of equilibrium in the T and B systems of immunity — as a result of a single injection of 0.2 ml phytohemagglutinin (PHA), 0.5 ml ATS, 200 mg/kg cyclophosphamide (CPA), and 0.5 mmole/100 g 6-mercaptoethanol (ME) subcutaneously, were transplanted intravenously in a dose of 4×10^7 cells into intact and hungry (starved for 3 days) animals. HRT were injected in a dose of 10^7 cells from donors receiving a preliminary injection of hydrocortisone (12.5 mg/100 g). The experimental and control animals were killed 6, 18-20, or 48 h after injection of the cells. The stem-cell, undifferentiated, and mature populations of epithelial cells were evaluated by the microcolonies method (according to the number of regenerating crypts — RC [9], by determination of the mitotic index (MI) in longitudinal median semithin sections (fixation with 2% glutaraldehyde, embedding in Epon) and by histomorphological investigation of sections stained with hematoxylin and eosin. Activity of nonspecific acid phosphatase was determined in frozen sec-

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TABLE 1. Number of RC Around Perimeter of Section Through Duodenum after Transplantation of Thymocytes into Mice with Intact and Functionally Destabilized Intestinal Epithelium (M ± m, n = 10)

Line of mice	Time of observation after injection of cells, h	Irradiation control	Recipients with intact intestinal epithelium injection of thymocytes from donors treated with normal rabbit serum					
			0,14 M NaCl	PHA		ATS	CPA	ME
F_1(CBA×C57Bl)	48	12,5±2,8	13,7±4,4	8,4±2,2	12,0±1,1	16,3±2,4	14,0±3,2	17,4±1,6
F_1(CBA×C57Bl)	48	16,8±3,0	23,3±5,0	20,8±2,1	16,9±1,1	22,2±4,8	15,7±3,1	16,6±7,2
			Recipients with functionally destabilized intestinal epithelium, injection of thymocytes from intact and hydrocortisone-treated donors					
			fat loading	fat loading + thymocytes	hunger	hunger + thymocytes		hunger + HRT
F_1(CBA×C57Bl)	48	10,5±1,7	10,4±1,3	—	21,4±2,8*	—		—
The same	48	28,0±1,4	25,5±1,4	17,2±1,0	—	—		—
» »	48	13,4±2,7	16,1±1,5	13,1±1,4	26,2±1,9*	31,8±1,3		—
		23,0±2,6	---	—	29,9±1,3	36,1±1,8		—
» »	18—20	—	—	—	34,8±2,8	16,4±1,3*		—
» »	18—20	23,3±1,1	—	—	32,0±1,2	24,0±3,9		21,6±1,4*
F_1(C57Bl×CBA)	18—20	8,7±1,5	—	—	17,2±1,8*	11,5±1,2*		—
C57Br	18—20	15,3±3,0	—	—	34,2±5,7*	24,7±8,1		—
C57Br	18—20	27,2±3,7	—	—	47,6±5,0*	27,3±6,0*		—
F_1(CBA×C57Bl)	6	—	—	—	37,2±5,1	21,3±2,4		—
F_1(CBA×C57Bl)	6	—	—	—	33,1±3,2	26,6±3,1		—

Legend. *P < 0.05.

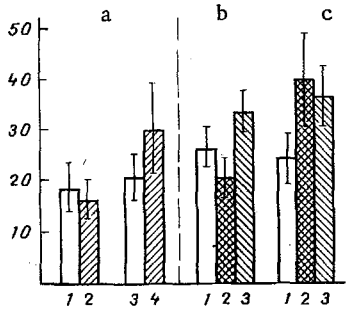

Fig. 1. Effect of thymectomy (a) and injection of ATS (b) and ALS (c) on the stem-cell compartment of the duodenum. Abscissa, time after procedure. a: 1, 3) Control (mock thymectomy), 2) 2 weeks, 4) 6 months; b) after six injections of antiserum, c) 5th day after discontinuation of antisera: 1) control (normal rabbit serum), 2) ATS, 3) ALS. Ordinate, number of RC per transverse section through intestine.

tions stained by Gomori's method over a wide pH range of the incubation medium (4.7-6.7) [2]. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Thymectomy in adult mice caused virtually no significant changes in the stem-cell compartment of the duodenum, in either the early or late period of observation (Fig. 1). Injection of ATS or ALS caused fluctuations of different magnitude in the stem-cell compartment of the intestine: ATS did not change the number of clonogenic cells, or reduce it, whereas ALS increased their number (Fig. 1). The number of mitotically dividing cells showed a small but statistically significant decrease (Fig. 2), but signs of dystrophic changes accompanied by deformation of the villi, a decrease in the number of cells in them, and increased rejection of the epithelial layers not only from the apex, but also from the lateral surface of the villi, took place in the population of mature enterocytes covering the villi. Meanwhile there was a decrease in the intensity and a change in the character of distribution of acid

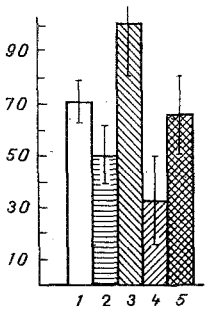

Fig. 2. Effect of ALS on MI of duodenum. Abscissa: 1) control (injection of normal rabbit serum, six injections); 2) injection of ALS (six injections); 3) 5th day after ALS; 4) 11th day after discontinuing ALS; 5) 17th day after discontinuing ALS. Ordinate, MI: number of metaphases per 1000 cells 3 h after injection of colchicine (4 mg/kg).

phosphatase: The pH optimum of cytoplasmic activity shifted toward the acid side, with predominant localization in the apical part of the cell.

Normalization of the epithelium in the intestinal wall, and an increase in MI and the number of RC to exceed their initial level, were observed histologically 5 days after ending injection of ALC, but the phenomena of the previous damage to the epithelium in the form of an altered density of distribution of enterocytes on the villus still remained for 17 days of observation. Considering the specificity of action of the ALS used, which was confirmed histomorphologically in these experiments, it can be postulated that changes observed in the intestinal wall depended on the functional state of the immunocompetent tissue. However, the possibility of a direct nonspecific action of the antisera on epithelial cells and also the injurious factor of the bacterial flora, activated on weakening of the protective barrier, likewise cannot be ruled out.

For this reason a different technical approach was used: transplantation of ST into mice with an intact intestinal epithelial system and mice with an intestinal epithelial system destabilized by an acute change in feeding schedule. Fat loading was found not to change the stem-cell compartment, whereas starvation for 3 days led to an increase in the number and size of RC and their degree of basophilia, to a decrease in MI of the epithelium (Table 1), and also to a decrease in the cell population in the stroma of the villi and to the development of dystrophic changes in the epithelium of the intestinal mucosa. Transplantation of cells from the thymus, spleen, and Peyer's patches of intact syngeneic donors and animals treated with PHA, ATS, CPA, and ME, caused no statistically significant changes in the number of RC in receipients with a functionally stable digestive system. After injection of thymocytes into hungry animals 18-20 h after transplantation a decrease in the number of RC and their level of basophilia, a decrease in MI of the proliferating population, and aggravation of the dystrophic changes in the compartment of mature epithelial cells and, in some cases, partial atropy of the villi were observed. Considering the functional heterogeneity of the thymus cell population, it was separated with the aid of hydrocortisone. Injection of HRT, in a dose equal to one-third of that of the undivided thymocyte population, was found to bring about similar changes (Table 1), accompanied by a considerable decrease in the cell population in the stromal layer of the muscosa and by degenerative changes in the epithelial cells of the hungry animals. The results thus suggest that changes in the intestinal epithelium, the heterogeneity of which evidently depends on the character of the procedure and the initial state of the epithelial cells, are mianly inhibitory in character, they are induced by fluctuations in the lymphoid system, and are probably ascribable not so much to T-B relations as to imbalance between subpopulations in the T system, including lymphocytes with different functional activity. The question of which population of lymphoid cells or one of its subpopulations is responsible for changes in the proliferative-differential potential of the intestinal epithelium, and what are the mechanisms of lympho-epithelial interaction, remains unclear and requires further study.

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NEW ASPECTS OF CONSERVATION OF HUMAN FETAL PANCREATIC β-CELLS BY THE REGULAR PASSAGE METHOD

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Much progress in recent years has been made in the development of methods of culture in insulin-secreting human pancreatic β-cells with a view to their transplantation into diabetic patients. However, the problem of accumulation and long-term preservation of functionally active pancreatic β-cells has not been solved and remains an urgent task in conservation.

In recent research some Western investigators have shown that long-term culture of isolated islets of Langerhans of experimental animals [12, 13] and of human adults [10] and fetuses [8] is possible, with preservation of insulin-secreting capacity, for between 75 and 97 days, but in only a few publications have cases of long-term preservation of pancreatic β-cells in tissue culture for periods ranging from a few weeks up to 2 months been described [5, 7, 9]. Previously the present writers studied long-term culture of pancreatic β-cells from guinea pigs [2]. The positive results of this work laid the foundations for a similar study of human fetal pancreatic β-cells in culture.

This paper gives data on the possibility of using a technique of cell subculture *in vitro* as an approach to the accumulation and preservation of functionally active human fetal pancreatic β-cells.

EXPERIMENTAL METHOD

A monolayer of primary trypsinized cultures obtained from the pancreas of human fetuses aged from 16 weeks to 9 months of intrauterine development, served as the original material for subculture. Primary trypsinized cultures were obtained by the method described previously [1, 4]. Disaggregation of the monolayer of primary trypsinized and subcultured human fetal pancreatic β-cells, seeding, and culture of the cells were carried out by the method described by the writers previously [2], and devised for guinea pig pancreatic β-cells. A mixture of equal volumes of medium 199, lactalbumin hydrolysate, 20% bovine serum, and 30% of conditioned medium obtained from a monolayer of β-cells was used as the growth medium for the subcultures of human fetal islet cells. The seeding dose and the intervals between passages were determined depending on the age of the experiments. For cytologic study of islet cells a culture of different passages of β-cells was seeded in penicillin flasks with coverslips. Preparations fixed with Bouin's fluid were stained with hematoxylin and eosin and with aldehyde-fuschsine. The insulin content was determined in washings of the cultures of different passages by radioimmunoassay.

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