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UDC 616.34-008.953.2-031.84-053.1-091.8

KEY WORDS: Peyer's patches; lymphocyte; human fetus

Peyer's patches (PP) are concentrations of lymphoid follicles (LF). They are found mainly in the ileum and undergo age-related changes [5]. The epithelium of PP contains M cells [12], which take part in the transmission of antigenic information from the intestinal contents to the lymphocytes for the formation of local and general immunologic responses [7]. According to some observations [6], PP are laid down in the 4th or 5th month of intrauterine development and they increase in complexity with the age of the fetus [1]. Some authorities [10, 11, 14] consider that PP are the analog of the bursa of Fabricius in birds. Others consider that they are an organ for the synthesis predominantly of IgA by plasma cells, which have traveled along a complex path of migration during maturation [8, 13], associated with cooperation of T and B cells. There is no information on immunologic parameters of the cell systems of PP in the human fetus.

The aim of this investigation was to study the character of differentiation of lymphocytes of PP in the ileum among the surface membrane receptors of the cells and to examine the development of the tissues composing the necessary microenvironment for this process during human embryogenesis.

EXPERIMENTAL METHOD

We studied PP from 110 human fetuses aged from 8 to 29 weeks of gestation, obtained from clinically healthy mothers in Moscow maternity homes. The age of the fetus was deduced from the crown-heel length and weight of the fetus. Sections through the organ were stained with hematoxylin and eosin, for glycogen and mucoproteins by McManus' method with amylase control, and for reticular fibers with silver nitrate by Gordon's method [3]. T lymphocytes (E-RFC) and their auto-RFC subpopulation were determined by the spontaneous lymphocyte rosette formation test with sheep's red blood cells and with autologous red blood cells by the method described in [9]. Among the B lymphocytes we determined EAC-RFC by the lymphocyte

TABLE 1. T and B Lymphocytes in PP of Human Fetal Ileum ($M \pm m$, %)

Age of fetus, weeks	T-lymphocytes		B lymphocytes		
	E-RFC	auto-RFC	EAC-RFC	IgM-positive cells	IgG-positive cells
8-9	$1,9 \pm 0,3$ (6)	n/d	$0,1 \pm 0,1$ (7)	n/d	n/d
15-19	$3,0 \pm 0,8$ (11)	$0,7 \pm 0,2$ (10)	$0,9 \pm 0,4$ (12)	$0,9 \pm 0,5$ (4)	$1,5 \pm 0,3$ (3)
20-23	$3,9 \pm 0,7$ (20)	$0,6 \pm 0,2$ (22)	$1,1 \pm 0,3$ (21)	$2,6 \pm 0,6$ (18)	$1,5 \pm 0,4$ (15)
24-27	$5,0 \pm 0,9$ (20)	$0,6 \pm 0,2$ (21)	$1,4 \pm 0,5$ (19)	$1,1 \pm 0,4$ (11)	$2,0 \pm 0,8$ (10)
28-29	$7,4 \pm 3,1$ (4)	$0,3 \pm 0,1$ (4)	$4,0 \pm 2,5$ (3)	$0,9 \pm 0,7$ (5)	$3,2 \pm 1,0$ (5)

Legend. Number of fetuses investigated shown in parentheses. n/d) Not determined.

Laboratory of Embryonic Histogenesis, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 105, No. 5, pp. 621-623, May, 1988. Original article submitted May 18, 1987.

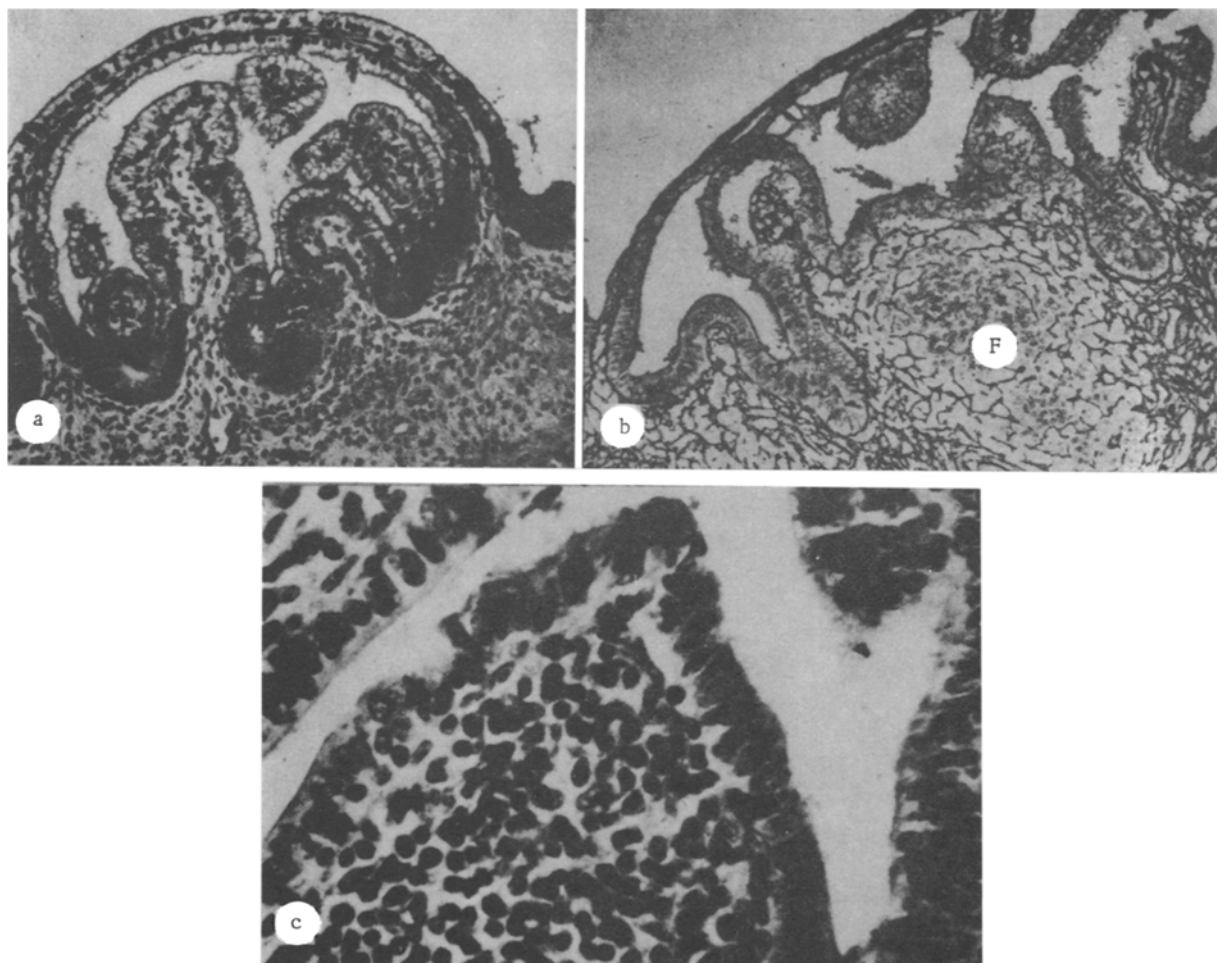


Fig. 1. Stages of development of PP in ileum of human fetus. a) PP of 12-week fetus; b) PP of 19-week fetus — lymphoid follicle (F); c) PP of 20-week fetus — cupola. a, c) Stained with Schiff's reagent; b) impregnated with silver nitrate by Gordon's method. Magnification: a, b) 160; c) 650.

rosette formation test with bovine red blood cells loaded with first response antibodies and complement, and IgM- and IgG-positive cells by the antiglobulin rosette test, using Kudryavtseva's method [2]. The percentage of lymphocytes investigated was counted among 200 mononuclear cells in a Goryaev's chamber. The number of PP, the area occupied by them in the ileum, and the number of LF in one PP were determined in two-dimensional macroscopic preparations of the human fetal ileum stained by Hellmann's method [4].

EXPERIMENTAL RESULTS

PP in the human fetal ileum develop in a definite order from the cranial toward the caudal end of the organ. At sites of development of PP the mucous membrane acquires a distinctive structure. In features at 9-10 weeks the entire surface of the mucous membrane has high villi, which have an atypical structure at the sites of PP. Examination of a transverse section through the oval PP of a 10-week fetus (Fig. 1a) shows that its peripheral villi are lengthened and make contact above to form a unique kind of chamber, in which new, short villi proliferate. At this time there are no LF, only small, diffuse concentrations of lymphocytes in the stroma, composed of a uniform network of reticular fibers. The epithelium covering the anlagen of PP differs from that in the remaining parts of the ileum by its small number of goblet cells.

By the 18th-19th week of gestation LF of various sizes can be clearly identified in the region of PP in the lamina propria of the mucous membrane (Fig. 1b). In these LF the network of reticular fibers becomes sparse and contains a large number of lymphocytes. With an increase in the size of PP during fetal development, the outermost adherent villi bound-

ing the chamber become thinner and rupture, to release the short atypical villi growing in it. This process begins at one end of PP and gradually spreads to the opposite end, but does not reach the final point for a long time. After the 21st week of gestation and until birth the number of PP in the ileum increases from 31 to 46, their area increases from 8.5 to 49 mm², the number of LF in one PP increases from 29 to 68, and they have no pale centers.

In the fetus at 8-9 weeks (Table 1) it is possible for the first time to extract a suspension of lymphocytes from the mucous membrane of the ileum and to identify T cells (E-RFC) and B cells (EAC-RFC) in them — 1.9 and 0.1%, respectively. Starting with the 15th-19th week, coinciding with the appearance of LF, the percentage of E-RFC and EAC-RFC in the organ rise sharply, and by birth they account for up to 7.4 and 4% by weight, respectively. Among the total T lymphocyte population a subpopulation of auto-RFC can be identified, and among the B lymphocytes IgM- and IgG-positive lymphocytes can be detected, mainly the latter. Toward the end of gestation, although the number of B lymphocytes has increased, it remains below the number of T lymphocytes. The predominant cells in the suspension are still O cells.

The newly appearing LF form wide cupola-like eminences of the mucous membrane (Fig. 1c). The base of the cupola is formed by a widely looped network of reticular fibers, packed with lymphocytes, but above it is covered by epithelium which does not contain goblet cells or mucoproteins. This epithelium becomes easily permeable for lymphocytes, which accumulate in it on the apex of the cupole, and some of them pass through it into the lumen of the ileum. By the 28th week the efferent lymphatic vessels are packed with lymphocytes, indicating outflow of lymphocytes from PP into the general lymphatic circulation.

Thus the anlagen of PP appear in the 9th-10th week of gestation as concentrations of atypical villi, forming closed chambers in the region of which distinctive cupolas develop; these are covered by epithelium which contains no neutral mucoproteins. LF arise in the lamina propria of the mucous membrane of the ileum toward the 18th-19th week. Transformation of the stroma from loose into reticular tissue is associated with this event. Enlargement of LF and the increase in their number are accompanied by a decrease in thickness, followed by rupture of the wall of the chamber and release of atypical villi and cupolas.

Heterogeneity of the lymphocytes in PP is observed in the fetus after the 8th-9th week. Among the lymphocytes T- and B-cell systems of immunity and some of their subpopulations and O cells can be distinguished. At all times of development the highest percentage is accounted for by O cells, followed by E-RFC. Among B lymphocytes higher percentages are accounted for by EAC-RFC and IgG-positive cells. By the 20th week migration of lymphocytes into the epithelium of the cupola is observed, and into the efferent lymphatic vessels by the 28th week. The human fetus is a germfree, but not an antigen-free, organism. The intestinal contents evidently act as a stimulus to accelerate differentiation of the lymphocytes and their migration from PP in the two directions indicated above. On the basis of these results PP can be regarded as local organs of defense which, however, even in the fetal period, are involved in the general system of immunogenesis as its peripheral components.

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