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LIGHT MICROSCOPIC AND IMMUNOHISTOCHEMICAL INVESTIGATION OF PEYERS PATCHES IN CALVES EXPERIMENTALLY INFECTED WITH ROTAVIRUS

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Pathomorphological and immunohistochemical examinations were carried out on tissue sections of the jejunum and ileum from calves experimentally infected with rota virus immediately after birth. Rota virus particles were present in the gut lumen in absorptive enterocytes next to, and in the direct vicinity of dome epithelial cells, but were never seen inside these cells. Thus, dome epithelial cells did not reveal any participation in rota virus infection. In this work, we demonstrate that a human antiserum recognizing a phylogenetically conserved part of the CD3 epsilon cytoplasmatic tail detects T cells of calves in formalid-fixed paraffin-embedded tissue sections. This antiserum enables the morphological characterization of normal and pathological lymphoid tissues and lymphoid infiltrations in experimental work and in animal disease.

Key words: rota virus antigen, calf intestine, CD3 peroxidasa-antiperoxidasa

INTRODUCTION

The number and structure of Peyers patches (PP) depend on the species, the age of the individual and the gut region. It has been shown (Parsons et al., 1989) that in 40-week-old calfes fetuses there are 76 discrete Peyers patches (DPP) in the duodenum and one continual Peyers patch in the proximal colon during the fetal period. In older calves the number of PP is decreasing. The depletion of B lymphocytes after the artificial removal of CPP and the impossibility to increase lyphopoesis after inducing antigens in isolated segments of the gut led to the hypothesis that PP is the primmary lymphatic organ in calves equivalent to the Bursa of Fabricius in birds (Lieber et al., 1995).

At the base of each Peyers patch there is a lymphoid follicle with a germinative center lying in the submucosa and containing small, medium and large lymphocytes. In calves the majority of lymphocytes belong to the IgM B subpopulation (Lieber et al., 1988a; Lieber at al., 1988b). Besides them, in the

center of lymphoid follicles there are also IgA plasma cells, T lymphocytes, macrophages and dendritic cells. The part of the lymphoid follicle towards the lumen of the gut is called the dome and also contains different subsets of T and B lymphocytes. The dome region is covered with an epithelium composed of equal numbers of M cells, absorptive enterocytes and lymphocytes (Pospischil et al., 1989).

The phenotype of the intraepithelial lymphocytes (IEL) of young calves was initially described as a very heterogenous cell population localized at the site where antigens enter the gut. There are data that the majority of these lymphocytes express gamma/delta receptors on their surface (Diacovo et al. 1996., Waters et al., 1995; Wilson et al., 1996). It is also known that the cell receptor - TCR (alpha/beta or gamma/delta) combines with CD3 gamma, delta, epsilon and zeta chains, thus forming a TCR-CD3 complex, which is very important for signal transmission in the cell (Kereszetes et al., 1996).

The structure and enzyme histohemistry of Peyers patches have been studied in naturally and experimentally infected calves (Knežević et. al., 1992; Pospischil et al., 1986).

The present study was conducted to explore the behaviour of rotavirus and some T lymphocyte subpopulations in gut-associated lymphoid tissue in calves experimentally infected with rotavirus.

MATERIALS AND METHODS

Animals. The investigations were carried out on five calves experimentally infected with rota virus, immediately before colostrum intake. This infection was carried out by oral application of 5 ml viral suspension in phosphate buffer (pH 7,7). All calves were bacteriologically sterile before inoculation, as determined by cultures of rectal swabs on blood agar plates. After death or sacrifice of the animal, sections of jejunum and ileum were taken for histopathological and immunohistochemical investigations. Sections of the jejunum and ileum from five healthy calves were taken for comparison.

Sample preparation. The gut samples were fixedin 10% neutral formalin for not langer than 24 hours) and absolute methanol. After fixation processing was completed using an automatic tissue processor involving graded alcohols, xylol, and paraffin wax. Finally, 6 mm thick paraffin sections were stained by HE (hematoxylin and eosin), PAS (periodic acid Schiff), PAP (Peroxidase-anti peroxidase) and DP (direct peroxidase) methods.

Immunohistochemistry. To test for rotavirus antigens by the PAP (peroxidase-antiperoxidase) method, primary rabbit-anti rota (human) antibodies (1:80, Dako), secondary swine-anti rabbit Ig (1:100, Dako), and PAP rabbit complex (PAP-Rabbit 1:500, Dako) were used. To detected IgM by the PAP method we used primary rabbit-anti human IgM antibodies (1:300, Dako) secondary swine-anti rabbit Ig (1:100, Dako) and PAP-rabbit complex (PAP Rabbit 1:500, Dako). Gut sections from infected calves incubated with normal rabbit serum (NRS) as antibodies in the PAP procedure, were used as the negative

control in the PAP method, while for CD3 we used "DAKO EPOS Negative control". The formalin fixed tissue sections went through the process of proteolytic demasking of antigene by trypsinization prior to incubation using commercial lyophilised trypsin (Dako) from bovine pancreas.

In these methods the tissue is first treated with a hydrogen peroxide solution (3%) to suppress endogenous peroxidase activity. This is followed by incubation with normal swine serum (NSS, 10 min.) to quench nonspecific protein binding to certain tissue elements. Tris-buffer (TBS, 0,1M Tris-HCl, 0,9 NaCl, pH 7,8) was used for all washings and dilutions during the immunohistochemical reaction. Visualization of PAP and DP reactions was achieved with diaminobenzidine (DAB/0,1M imidazole-HCl, pH 7,1) for 10 minutes. The samples were then counterstained with hematoxylin and coverslipped.

RESULTS

After peroral infection all experimental calves developed diarrhea. Two animals succumbed the second day and the remaining 3 were sacrificed 18. 24. and 72 hours after the first signs ofdiarrhea. The control healthy animals were sacrificed at the same time intervals.

Macroscopic examination in the experimental calves did not reveal any characteristic changes. Only the jejunum and the ileum were somewhat enlarged and filled with watery of mashlike content, grey-white or light-yellow in appearance.

Moderately atrophied intestinal villi, rounded or fingerlike, were seen in paraffin sections of the jejunum and the ileum. Epithelial cells of the villi were wholly desquamated in same places. In addition to the villi with exposed basal membranes,some villi lined with flattened epithelium were also observed. Some intestinal glands were enlarged and filled with detritus. Monouclear cells infiltrating substantially the lamina propria were seen. A great number of these cells predominantly expressed CD3 antigen. Moreover, the PAP stained preparations showed viral particles in epithelial cells of the jejunum and ileum. The greatest accumulation of rota virus antigens occurred in lamina epithelialis of the ileum from calves dying two days after outbreak of diarrhea. (Figure 1)

Immunohistochemically positive brown particles of the virus could also be seen in the detached desquamated cells lying freely in the intestinal lumen. All of these changes were seen in the absorptive enterocytes and in the direct vicinity of dome epithelial cells, but never seen inside these cells. (Figure 2)

Lymphoid follicles of Peyer's patches showed no morphological changes. Their main cell population consists of IgM B lymphocytes. The distribution of gamma/delta T cells in lymphatic tissue of calves is somewhat different from that in other species. Bovine gama/delta T lymphocytes are concentrated in lymphoepithelial and subepithelial zones of villi intestinales and are rarely found in T

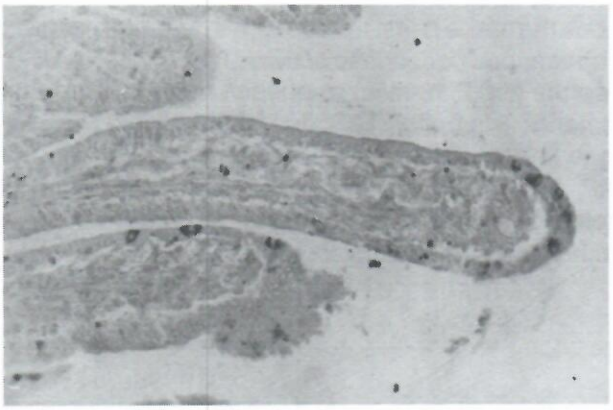

Figure 1. Calf ileum, accumulation of rota virus in absorptive eneterocytes, PAP, 400x

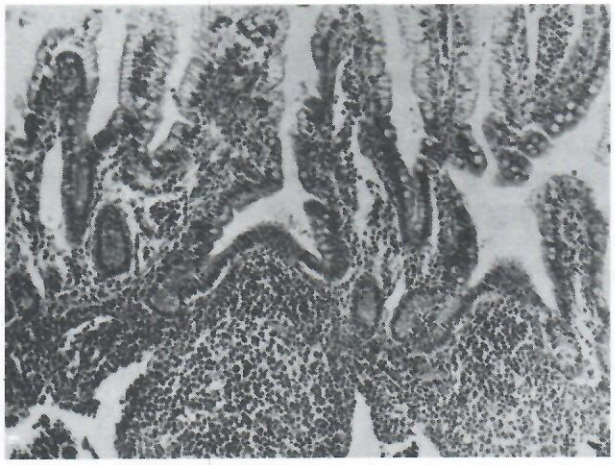

Figure 2 Calf ileum, dome epithelial cells of Peyers patches (PP), HE, 160x

and B zones of lymphatic follicles. The distribution of CD3 T lymphocytes in lamina epithelialis and lamina propria in sick and helthy calves was very similar (Figure 3)

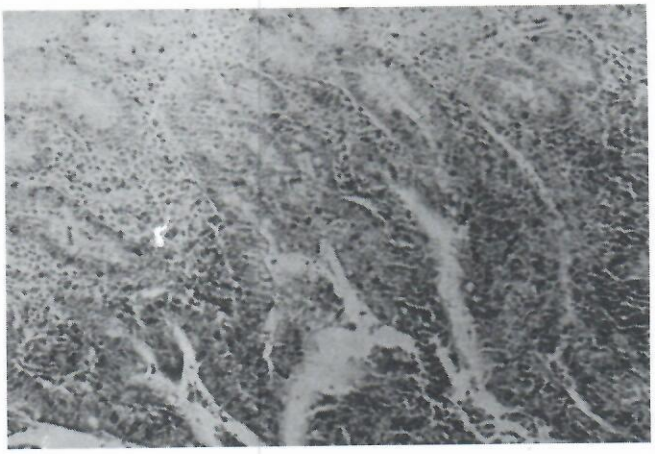

Figure 3 Calf ileum, distribution of CD3 in lamina epithelials and lamina propria, DP, 160x

DISCUSSION

The close contact between epithelial cells and lymphocytes enables Peyers patches to play an important role in defence mechanisms against intestinal infections. Pathogens use different routes to enter lamina epithelialis of the gut.

Several enteric pathogens have a preference for dome epithelial cells. Some, including chlamydia in calves, and rabbit dysentery Escherichia coli type I, are closely associated with the brush border and do not enter dome epithelial cells. Others, however, including salmonella and poliovirus enter dome epithelial cells and subsequently disseminate to other host organs. Another group of enteric pathogens, known to remain restricted to the gastrointestinal tract, does not have any preference for dome epithelial cells. These are: coronavirus, E. coli, mycobacteria and Shigela. Rotavirus is thought to be part of the latter group, but recent experiments indicate that rotaviruses occur in dome epithelial cells, and are an important feature in the pathogenesis of rotavirus disease (Lieber et al., 1995; Pospischil et al., 1986;).

Histopathological and immunohistochemical investigations in calves experimentally infected with rota virus in our case showed no participation of epithelia of lymphoid parts of the small intestine in the infection. Particles of rota virus antigens were detected in the villus epithelia in the vicinity of and beside Peyers patch domes. In the gut regions with lymphoepithelium no necrosis and desquamation of cells like those seen on villi covered with enterocytes were detected. Rota viruses probably do not replicate in dome epithelial cells and therefore there was no immunohistochemical detection of virus antigens in these cells. This is one of the differences between rota virus infection and astro and

breda virus infections (Pospischil et al., 1986). We suppose that the initial reaction for rota viruses is connected with their interaction with the receptors located on the glycocalyx. Considering that the glycocalyx of the epithelium in lymphoid areas of calfes gut is poorly developed, rota viruses remain more or less limited to the regions with absorptive enterocytes (Pospischil, 1989). Our results indicate that the rota virus is limited to the gut lumen and has no affinity for epithelial cells of Peyers patch domes and therefore causes no general infection in the host. The CD3 lymphocytes in calf intestine are predominantly intraepithelially accumulated, indicating that this is the site where they probably function.

The in situ identification of T cells has been difficult and restricted to frozen tissue samples of a limited range of species. In this work, we demonstrate that a human antiserum recognizing a phylogenetically conserved part of the CD3 epsilon cytoplasmatic domain detects T cells of calves in formalin-fixed paraffin-embedded tissue sections. This antiserum enables the morphological characterization of normal and pathological lymphoid tissues and lymphoid infiltrations in experimental work and in animal disease (Keresztes et al., 1996).

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PATOHISTOLOŠKA I IMUNOHISTOHEMIJSKA ISPITIVANJA PAJEROVIH PLOČA TELADI EKSPERIMENTALNO INFICIRANIH ROTA VIRUSOM

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SADRŽAJ

Patomorfološka i iimunohistohemijska ispitivanja tkivnih isečaka jejunuma i ileuma urađena su kod teladi koja su neposredno posle rođenja inficirana rota virusom. Rotavirusne partikule prisutne su u crevnom lumenu, absorptivnim enterocitima i u neposrednoj blizini ćelija limfoepitela mada se nikada ne nalaze u ovim ćelijama. Ovi rezultati jasno ukazuju da ćelije limfoepitela nemaju učešća u rota virusnoj infekciji. U ovome radu pokazali smo da humani antiserum prepoznaje filogenetski konzervisani deo CD3 epsilon T limfocita u teladi na parafinskim presecima tkiva fiksiranih u formalinu. Ovaj antiserum omogućava morfološku karakterizaciju normalnog i patološkog limfatičnog tkiva i limfocitnog infiltrata u eksperimentalnom radu i kod obolelih životinja.