Histol Histopath (1992) 7: 137-142

Histology and Histopathology

Regional variation in ontogeny of class II antigens in enterocytes of mouse small intestine

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Summary. The ontogeny of major histocompatibility class II antigens in small intestine enterocytes of postnatal C3H/He mice was investigated. Cryosections of duodenal, jejunal, and ileal segments from 7-, 14-, 16-, 20-, 21-, 23-, 25-, 27-, 28-day-old and 7-week-old mice were stained for the class II antigens with MRC OX6 monoclonal antibodies by peroxidase-antiperoxidase labelling. In adults, the duodenum exhibited least expression of class II antigens that increased progressively towards the ileum. The expression in the villous epithelium was first seen in the duodenum and jejunum 21 days after birth but the ileal enterocytes did not exhibit any class II antigens. The earliest appearance (21 days postnatal) of class II antigens in the enterocytes coincides with the age of weaning which suggests that immunologic stimulation by ingested antigens after weaning may influence expression of these antigens. At day 28 after birth, the duodenum and jejunum expressed levels comparable to those in the adults. The first expression of the antigens seen in the ileum was at day 28 postpartum. Crypt epithelium of the three regions of the small intestine showed expression similar to that of corresponding regional villous enterocytes. We conclude that there is an age-dependent regional variation in the expression of class II antigens in enterocytes, and the expression increases with age. The variation in expression of the class II antigens in enterocytes of postnatal mice is attributed to the developmental status of the tissue. The nature of postnatal expression of the antigens is important since an early appearance of these antigens may have implications in autoimmunity.

Key words: Class II antigens, Enterocytes, Small intestine, Antigen presenting cell

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Introduction

The gastrointestinal tract of mammals is generally considered a secondary lymphoid organ that has an important role in the immune response to the luminal antigens. Enterocytes produced during late fetal and early postnatal life are reported to be structurally different from those found in the adult (Smith and Peacock, 1980). Expression of major histocompatibility complex (MHC) class II antigens in small intestinal enterocytes has been demonstrated in guinea-pig (Wiman et al., 1978), mouse (Parr and McKenzie, 1979), man (Scott et al., 1980) and rat (Mayrhofer et al., 1983). Class II antigens are integral membrane glycoproteins generally present on antigen presenting cells (APC), and certain parenchymal cells.

In humans and rodents, the extent of class II antigen expression in parenchymal cells depends on the age (Natali et al., 1982; Mayrhofer et al., 1983); for example, during fetal development, the intestinal epithelium of mice is negative for the class II antigens (Natali et al., 1981a,b). Immunofluorescent studies have shown that gastrointestinal epithelium expresses class II antigens after birth (Natali et al., 1981b). In rats, the initial expression of these antigens begins at approximately 3-4 weeks postnatally; this age coincides with weaning time (Mayrhofer et al., 1983). The expression levels in adults are reached at approximately one month of age (Natali et al., 1981b). There is abundant evidence from the work done on rats and rabbits that the postnatal development of small intestine mucosa is in a cranio-caudal direction (Buts and De Meyer, 1981; Toofanian and Targowski, 1982). The regional differences in enzymatic activities along the luminal border of small intestine during fetal, postnatal and in adult mice are well documented (Moog, 1961; Moog et al., 1973; Kendall et al., 1979; Calvert et al., 1981). These studies suggest that the functions of enterocytes depend on age of the individual and the region of the organ. The aim of this

investigation was to determine whether class II antigens are expressed concurrently in all regions of the developing small intestine.

Materials and methods

Animals and tissue processing

Adult, C3H/He brown inbred mice of k-haplotype were obtained from Charles River (Montreal, Quebec). The mice were bred at the Atlantic Veterinary College to obtain pups of different age groups. All mice were fed *ad libitum* and had free access to water.

Conception was determined by the appearance of a vaginal plug the morning after a male mouse was placed with four or five females the previous evening. Presence of a vaginal plug the following morning was designated as day 0 of pregnancy for the particular animal.

During later phases of this study three pregnant mice of the same strain were purchased to obtain the pups (Charles River, Boston, Massachusetts, USA). The pups were kept with their mothers at all times and they had access to solid food approximately by day 15 postnatally. Three animals were used for each age group. All pups were raised in similar conventional environmental conditions.

Mice were killed by cervical dislocation. Specimens from the duodenum, jejunum and ileum were obtained from 7-, 14-, 16-, 18-, 19-, 20-, 21-, 23-, 25-, 27- and 28-day-old and 7-week-old animals, postpartum. Small intestine was flushed with 0.01 M phosphate buffered saline solution (PBS), pH 7.4. To keep the lumen patent, a solution containing equal amounts of O.C.T., an embedding material (Canlab, Toronto, Ontario), and PBS were introduced into the intestine and both ends of the organ were clamped. The intestine was immediately frozen with Cytocool, a tissue freezing aerosol (Canlab). Samples of duodenum represented areas 1.5 cm posterior to the pylorus, jejunal samples were collected from the mid-region of the small intestine and samples of ileum were obtained from areas 1.5 cm anterior to the ileocecal junction. Duodenal, jejunal and ileal segments of 3 mm in length were embedded in O.C.T. compound, snap frozen in liquid nitrogen and were stored at –80° C until prepared for staining. Transverse cryostat sections of 6 μm thickness were affixed to subbed slides, air dried for 1 h at –20° C and post-fixed in absolute ethanol for 10 min at –20° C.

Peroxidase-antiperoxidase Labelling

Sections were incubated with 20% normal goat serum for 20 min at room temperature (RT), after rinsing the sections in PBS for 15 min. Excess normal goat serum was drained off and removed. Each experimental trial constituted three sets of adjacent sections from the small intestine of a particular age group. Each set was represented by two slides. Samples in the first set were incubated with 50 μl of anti-mouse class II antigen monoclonal antibodies (Daymar Labs, Toronto, Ontario) at 1:100 dilution. Samples in the second set received unrelated monoclonal antibody at a dilution similar to that in the preceding group. Samples in the third set were incubated with PBS alone. All groups were incubated in a humid atmosphere for 24 hr at 4° C.

Samples in all the sets were brought to RT, rinsed and washed in PBS for 20 min. The tissue sections were incubated for 30 min at RT with 50 μl of affinity purified goat anti-mouse IgG (Bio/Can, Toronto, Ontario) at a 1:50 dilution. The sections were rinsed and washed in PBS for 20 min at RT. The mouse peroxidase-antiperoxidase (PAP) soluble complex (Sigma, St. Louis, Missouri, USA) diluted at 1:100 was added to the tissue sections and incubated for 30 min at RT. After washing the tissue in 0.05M Tris-HCl (Sigma), pH 7.6, the tissue bound PAP complexes were revealed by addition of 0.05% 3,3'-diaminobenzidine-tetrahydrochloride (DAB) (Polysciences, Barrington, Pennsylvania, USA) and 0.02% H_2O_2 in 0.05 M Tris-HCl buffer for 20 min at RT. After having been rinsed and washed in Tris-HCl buffer for 20 min, the tissue was counter-stained with Harris' haematoxylin, dehydrated and mounted in Entallen (BDH Chemicals, Dartmouth, Nova Scotia). To determine non-specific binding of any of the reagents, the following controls were used: a) the primary antibody, ie., MRC-OX6 was eliminated from the above protocol, however, concentration, time and dilutions of the remaining reagents were the same as described above; b) the secondary antibody was eliminated from the above protocol; c) incubation with PAP was deleted as a step and; d) some sections were incubated only with DAB and H_2O_2, with deletion of all other steps. Irreversible inhibition of endogenous peroxidase was performed prior to the immunostaining in some control sections and sections stained for class II antigens. Blocking of endogenous peroxidase was achieved by immersing the slides in a bath of 100 ml of absolute ethanol containing 0.2 ml of concentrated HCl for 15 min.

Results

Duodenum, Jejunum, Ileum (21-day-old mice)

Ontogenic studies of MHC class II antigens in the small intestine demonstrated that when mice pups were reared in conventional environment, the epithelial lining of all regions at age groups preceding day 21 of postnatal life did not stain for the antigens. The first expression of these antigens was seen in the villi (Table 1) and crypt epithelium of duodenum and jejunum at 21 days after birth. However, there was a variation in degree of expression of the antigens in these regions of the small intestine with age.

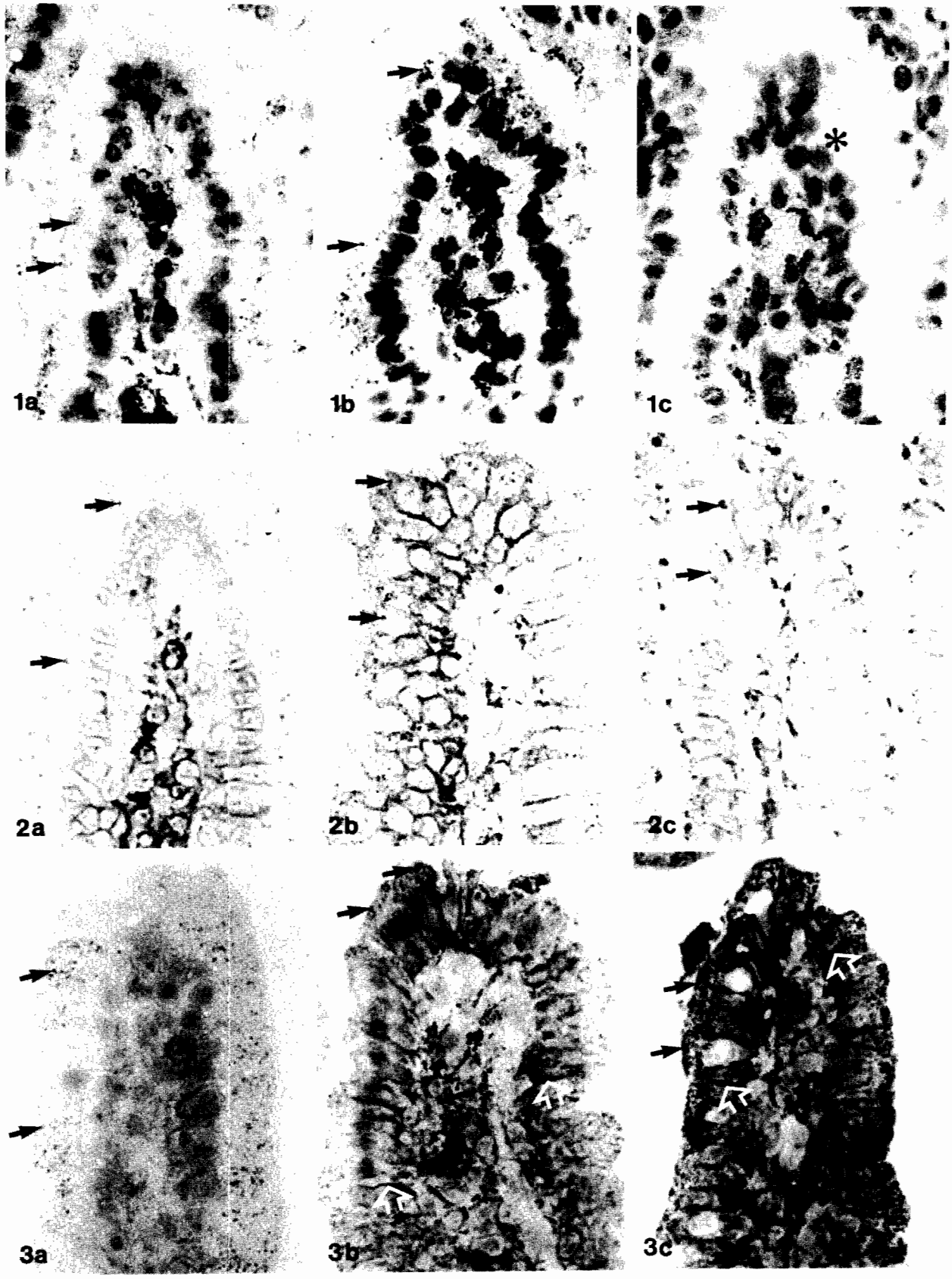

Fig. 1 a-c. Photomicrographs of cryosections from small intestine of 21-day-old C3H/He mice stained by the PAP method and counterstained with Harris' hematoxylin. × 550. **1a.** Section of duodenum showing class II antigen staining in apical cytoplasm of some enterocytes (black arrows). **1b.** Some jejunal enterocytes exhibited immunostained in apical cytoplasm (black arrows). **1c.** No enterocytes of the ileum show staining (asterisk).

Fig. 2a-c. Micrographs of cryosections from small intestine of 28-day-old mice stained by PAP method and counterstained with Harris' hematoxylin. × 550. **2a.** Many enterocytes (black arrows) of the duodenal villus expressed class II antigens in apical cytoplasm. **2b.** All enterocytes covering the jejunal villus showed immunoreaction (black arrows) in apical and basal cytoplasm. Some cells exhibited lateral cell surface (outlined arrows) staining. **2c.** Ileal enterocytes expressed class II antigens in the apical cytoplasm (black arrows).

Fig. 3a-c. Micrographs of cryosections from small intestine of adult (7-week-old) C3H/He mice by PAP labelling and counterstained with Harris' hematoxylin. **3a.** The apical cytoplasm of some duodenal enterocytes showed expression of class II antigen (black arrows). **3b.** Increased expression was seen in apical and basal cytoplasm of jejunal enterocytes (black arrows). Some cells exhibited lateral cell surface staining (outlined arrows). **3c.** An intense apical (arrows) and basal cytoplasmic and baso-lateral surface (outlined arrows) staining was exhibited in ileal enterocytes (black arrows).

Compare **Figs. 1-3** where expression of class II antigens increase progressively in enterocytes with age and along the length of the small intestine.

Table 1. Ontogeny of expression of MHC class II antigens in small intestine of C3H/He strain of mice.

Days Postpartum	Location	Region of Gut		
		Duodenum	Jejunum	Ileum
7	Enterocytes	–	–	–
	Lamina propria	+	+	+
14	Enterocytes	–	–	–
	Lamina propria	+	+	+
21	Enterocytes	+	+	–
	Lamina propria	+	+	+
28	Enterocytes	+	+	+
	Lamina propria	+	+	+
49	Enterocytes	+	+	+
	Lamina propria	+	+	+

+ Presence of class II positive cells
– Absence of class II antigens

Table 2. Distribution of MHC class II antigens in enterocytes of small intestine of postnatal C3H/He mice.

Region of Gut			
Days Postpartum	Duodenum	Jejunum	Ileum
7	–	–	–
14	–	–	–
21	+	+	–
28	+	++	++
49	+	++	++

++Entire villus epithelium was positive for class II antigens.
+ Some cells demostrate class II antigens
– Immunoreaction absent

Duodenum

The earliest expression of class II antigens was observed at day 21 of postnatal life of the mice. A weak immunoreaction was observed in the form of granules in the apical cytoplasm of some villi enterocytes (Fig. 1a). The majority of the enterocytes did not show any immunoreactivity (Table 2). The crypt epithelial cells showed a staining pattern similar to that of the villous epithelium. Lamina propria contained numerous cells that exhibited positive immunoreaction to class II antigens. The corresponding enterocytes from the control groups were negative for the antigen.

Jejunum

On day 21 of postnatal life, the enterocytes showed a weak immunoreactivity to class II antigens (Fig. 1b). The reaction product was seen mostly in the apical cytoplasm of a few epithelial cells on the villi (Table 2). Basal cell cytoplasm and cell surfaces demonstrated no immunostaining for the antigens. Immunoreactivity did not follow a distinct pattern along the villi. Many crypt epithelial cells were positive for class II antigens. Expression of the antigens in lamina propria cells was comparatively less in intensity than that in the duodenum, although the cell types that stained positive for these antigens appeared similar in both regions.

Ileum

At 21 days postpartum, the ileum was not developed as much as the proximal part of the small intestine. No immunostaining was seen in the cytoplasm and cell surfaces of the enterocytes for class II antigens (Fig. 1c; Table 2). Crypt epithelium did not show immunoreactivity. Immunoreaction, however, was observed in cells of the lamina propria.

Duodenum, Jejunum, Ileum (23-, 25-, 27-, 28-day-old mice)

Animals of age groups of day 23, 25 and 27 showed immunoreaction in duodenal and jejunal epithelium. The extent of the staining increased with age. At day 28 of postnatal life, the villous and crypt epithelium of duodenum (Fig. 2a) and jejunum (Fig. 2b) demonstrated levels of class II antigen expression comparable to those in the adults whereas enterocytes of 28-day-old ileum villi (Fig. 2c) and crypts exhibited a weaker expression of class II antigens than those in the ileum from the adult animals. Lamina propria showed considerable immunoreaction for class II antigens.

Duodenum, Jejunum, Ileum (7-week-old mice)

The enterocytes of duodenum showed immunoreaction in apices of many cells (Fig. 3a). In the jejunum enhanced expression was exhibited in the apical and basal cytoplasm and in the lateral surfaces of most villi epithelia (Fig. 3b). All ileal enterocytes demonstrated intense cytoplasmic and baso-lateral cell surface immunoreaction (Fig. 3c).

Discussion

This study demonstrated that expression of MHC class II antigens varies in different regions of the developing mouse small intestine. The antigens were first detected in enterocytes of duodenum and jejunum at 21 days and in the ileum at 28 days postpartum. The intensity and distribution of the antigens in immature enterocytes was different from that in the enterocytes of adult mice. The expression of class II antigens increased with age (Compare Figs. 1, 2 and 3). The age at which these antigens were first observed in duodenum and jejunum coincides with the age of weaning in mice, which is at approximately 17-21 days postnatal. Hence, it appears that when the intestinal mucosa first comes in contact with environmental antigens, the enterocytes begin to express class II antigens.

The expression also appears to relate to the development of small intestine. The postnatal development of mucosa of the small intestine is in a cranio-caudal direction (Buts and DeMeyer, 1981). Our study has shown that the development of class II antigens follows the same direction. It has been observed that the expression of class II antigens increases with the maturity of the cells expressing them (Spencer et al., 1987). The immature enterocytes are either incapable of expressing these antigens because they are differentiating (Natali et al., 1984), or the antigens may still be in a developmental state. Alternatively, the enterocytes during fetal and neonatal life may express a level of class II antigens which is below the sensitivity of the assay used. An increased exposure to antigenic stimuli after birth might enhance synthesis of class II antigens (Natali et al., 1981b). However, in mice of C3H/FeJ strain, expression of the antigens in the enterocytes of the duodenum and jejunum were detectable at approximately seven days after birth (Natali et al., 1981b). This early expression may be due to exposure of animals to different antigenic environment or a strain to strain variation in level of expression of class II antigens.

The antigen presenting function of class II positive cells of the spleen reaches levels similar to those in adults by three weeks of age; this period coincides with age of weaning (Lu et al., 1980). These workers speculated that a maternal factor present in the milk which is subsequently transmitted to the neonate, might be blocking T-lymphocyte and macrophage maturation. After weaning, APC are allowed to differentiate because the postulated blocking factor is removed. Since enterocytes might serve as APC (Bland and Warren, 1986), they may also be regulated by this maternal factor. Prostaglandin E_2 and alpha-fetoprotein in neonates have been shown to inhibit class II antigen expression by APC *in vitro* (Lu and Unanue, 1985). The immune system early in the ontogeny of an organ is speculated to play an important role in self-tolerance and notself-defense (Lu and Unanue, 1985). The delayed expression of class II antigen positive cells in fetal and neonate tissues may have evolved to allow self-tolerance as new antigens are still being expressed on differentiating tissue during development. Since small intestine develops in a cranio-caudal direction, duodenal and jejunal enterocytes differentiate at an earlier age than those in the ileum. Therefore, it is reasonable to assume that the proximal small intestine express classes II antigens at an earlier age in comparison with that of the ileum epithelium.

Ontogenic studies of these antigens on the enterocytes are important to understand autoimmune diseases. A study on the ontogeny of MHC class II antigen-bearing accessory cells in autoimmune MRL-1pr mice found that there was inappropriately early ontogenetic appearance of the class II bearing accessory cells in spleen (Lu and Changelian, 1983); such cells might contribute to the development of autoimmunity in these animals.

The class II antigen expression by enterocytes appears to be related to an increase in the number of intraepithelial lymphocytes of suppressor/cytotoxic phenotype in rats (Cerf-Bensussan et al., 1984). Ontogenic studies of these cells have revealed that the number of intraepithelial lymphocytes increases with age. Hence, the onset of the antigens' expression by enterocytes might be related to the number of these lymphocytes that are in close proximity of enterocytes at the time. The class II antigen positive cells increase with age in the cranio-caudal direction in the lamina propria of the small intestine and the presence of these cells might influence the enterocytes to express these antigens (Mayrhofer et al., 1983). Our results are indicative of regional variation in the development of class II antigens, suggesting differences in antigen handling by enterocytes of postnatal and adult mice which might help correlate the onset of immune competence in the small intestine with expression of the class II antigens.

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Accepted September 10, 1991