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Abstract
In order to determine whether the rat small intestine exhibits quantitative changes in the synthesis of the secretory component (SC) during growth, epithelial villus and crypt cells were isolated from jejunal segments at intervals after birth up to adulthood. SC concentration was measured in each cell fraction by immunoradiometric assay and compared to sucrase activity, an enzyme marker of the differentiated villus enterocyte. The following results were observed. (i) Adult rats showed a characteristic decreasing concentration gradient of SC from the crypts (mean concentration in crypt cells: 636 +/- 173 ng/mg protein) to the villus tip (mean concentration in villus cells: 152 +/- 17 ng/mg protein). This gradient was the reverse of that found for sucrase activity. (ii) In young sucklings (10 days old), SC was virtually absent in both villus and crypt cells, but its concentration progressively increased in weanling rats and reached adult levels by day 40 postpartum. (iii) The crypt to ...

Document type : Article de périodique (Journal article)

Référence bibliographique
Buts, Jean-Paul ; Delacroix, Dominique L.. Ontogenic changes in secretory component expression by villous and crypt cells of rat small intestine.. In: Immunology, Vol. 54, no. 1, p. 181-7 (1985)
Ontogenic changes in secretory component expression by villous and crypt cells of rat small intestine

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Accepted for publication 28 August 1984

Summary. In order to determine whether the rat small intestine exhibits quantitative changes in the synthesis of the secretory component (SC) during growth, epithelial villus and crypt cells were isolated from jejunal segments at intervals after birth up to adulthood. SC concentration was measured in each cell fraction by immunoradiometric assay and compared to sucrase activity, an enzyme marker of the differentiated villus enterocyte. The following results were observed.

(i) Adult rats showed a characteristic decreasing concentration gradient of SC from the crypts (mean concentration in crypt cells: 636 ± 173 ng/mg protein) to the villus tip (mean concentration in villus cells: 152 ± 17 ng/mg protein). This gradient was the reverse of that found for sucrase activity.

(ii) In young sucklings (10 days old), SC was virtually absent in both villus and crypt cells, but its concentration progressively increased in weanling rats and reached adult levels by day 40 postpartum.

(iii) The crypt to villus cell gradient of SC, absent in sucklings up to day 20, developed during the fourth postnatal week.

(iv) Treatment of 10-day-old suckling pups with pharmacological doses of either corticosterone or L-thyroxine for 3 consecutive days failed to induce the precocious synthesis of SC by jejunal enterocytes, but produced significant ($P < 0.01$) decreases in concentration. Under the same conditions, sucrase activity was markedly enhanced.

In conclusion, major changes in the ability of the immature crypt cell to produce the specific receptor for transepithelial transport of polymeric immunoglobulins occur during the fourth week of rat life. The initiation of this ontogenic process is not triggered by the dietary and hormonal changes known to control the maturation of other functions linked to the differentiated villus cell, such as sucrase activity.

INTRODUCTION

During the third postnatal week, the rat small intestine undergoes profound adaptive changes in morphology (Herbst & Sunshine, 1969; Buts & De Meyer, 1981), epithelial cell kinetics, mucosal enzyme activity (Herbst & Sunshine, 1969; Buts & De Meyer, 1984) and transport functions (Hennings, 1981). The physiological mechanisms which initiate and control these maturative events are still poorly understood. Initiation of the postnatal ontogenic events in the rat gastrointestinal tract is probably determined by an intrinsic genetic programme (Montgomery, Sybieki & Grand, 1981) which, itself, is influenced by environmental factors such as nutrients (Hennings, 1981) and hormones (Hennings, 1981, Buts, De Meyer & Kolanowski, 1983). So far, the potential effects of these maturative mechanisms on the expression of secretory component (SC) by intestinal epithelial cells has received very little attention. This highly glycosylated protein, specifically produced by mucosal and
glandular epithelial cells, plays a key role in the
secretory immune system (Brandtzæg, 1981). At the
basolateral surface of intestinal and other mucosal and
glandular cells, SC corresponds to the extramembranous domain (My 70,000) of the receptor (My
110,000) of polymeric IgA (P-IgA) and IgM (Mostov,
Kraehenbuhl & Blobel, 1980; Mostov & Blobel, 1982;
Kuhn & Kraehenbuhl, 1982; Crago et al., 1978;
Brown et al., 1977). It initiates the vesicular translocation
of these immunoglobulins (Kuhn & Kraehen-
buhl, 1982) from the lamina propria to the intestinal
lumen. This transport phenomenon only starts a few
weeks after birth when IgA plasma cells appear in the
lamina propria of the gut as a result of mucosal
stimulation by indigenous bacterial flora and food
antigens (Crabbé et al., 1968, 1970).

Previous experiments with mammals investigating
the intestinal production of SC during early stages of
development have been conducted with immunohistocytochemical methods (Nagura, Nakane & Brown,
1978). In rats, the presence of cytoplasmic SC could
only be detected in the jejunum after day 15 postpar-
tum (Nagura et al., 1978). Therefore, the present study
was designed (i) to measure, by a sensitive immunoradiometric assay, the cellular concentration of SC along
the villus-crypt unit in the adult rat jejunum; (ii) to
determine whether the rat small intestine exhibits
ontogenic changes in SC concentration during postnatal
growth; (iii) to assess the effects of pharmacological
doses of corticosterone and of L-thyroxin on intestinal
synthesis of SC in young suckling pups. Throughout
the study, sucrase-isomaltase was used as a marker of
differentiated epithelial villus cells and its activity was
measured simultaneously with SC concentration. As
SC, sucrase is a glycoprotein synthesized in the
dividing crypt cell but in an inactive form. Its biological
activity increases when the cell differentiates and
migrates from the crypt to the villus tip (Hauri,
Quaroni & Isselbacher, 1980). Ontogenic changes in
sucrase expression by villus cells are well character-
ized. In the rat, the activity of this enzyme is virtually
absent until day 13 after birth and is specifically
induced at day 14 corresponding to weaning (Hen-
nig, 1978).

**MATERIALS AND METHODS**

All experiments were performed on male rats of the
Wistar strain, bred in our own animal colony and
housed in opaque polystyrene cages with a 12-hr
light-dark cycle. The day of birth was defined as day 0.
Suckling pups remained from birth with their mother
and had free access to the nipples. Weanling rats were
separated from their mother by day 21 postpartum
and were fed *ad libitum* a solid stock diet (N 103, UAR,
Villemoisson-sur-Orge, France). During the entire
series of experiments, all rats had unrestricted access
to water. For Study I, six litters of five rats each, and
four adult rats were killed by decapitation between
09.00 and 10.00 hr at the following ages: 10, 14, 20, 28,
40, 60 and 150 days postpartum.

**Corticosterone treatment schedule (Study II)**

In order to determine whether variations in circulating
corticosterone levels could affect the intestinal syn-
thesis of the secretory component (SC), two litters (six
animals each) of 10-day-old sucklings, weighing 18–20
g were treated with 2.0 mg/100 g body wt/day of
corticosterone acetate (Sigma Chemical Co., St Louis,
MO). The injections were performed subcutaneously
at 08.00 and 20.00 hr on 4 consecutive days from day
10 until day 13. The last injection was given 1 hr before
the animals were killed. Littermates served as controls
and received an equivalent volume (0.1 ml) of the
hormone vehicle (0.9% NaCl) following the same schedule.

**Thyroxine treatment schedule (Study III)**

L-thyroxine sodium salt (Sigma Chemical Co.) was
dissolved in 0.005 M NaOH and injected subcutaneously
in volumes of 0.5 ml at a dose of 0.2 mg/100
 g body wt/day. The treatment schedule was identical
to that used for Study II, with the exception that only
one injection was performed daily at 09.00 hr. Control
animals (C) received 0.5 ml 0.005 M NaOH subcu-
taneously at the same time.

**Isolation of intestinal cells and assays**

Sequential cell release from villus tip to the crypt base
was performed following the Weiser's procedure
(Weiser, 1973) modified by Raul et al. (1977). Briefly,
rat jejunal segments (about 10 cm distant from the
pylorus) were removed, everted and submitted to
successive incubations of 10 min at 37°C in phosphate-
buffered saline (no Ca²⁺ or Mg²⁺) containing 1·5 M
EDTA and 0·5 M dithiothreitol, under agitation at
170 r.p.m. in a waterbath shaker. By successive
incubations, sequential fractions of isolated epithelial
cells were obtained. The released cells were collected as
described (Raul et al., 1977) washed twice and homo-
genized in 50 mM mannitol, 2 mM Tris, pH 7.4.
Homogenates were centrifuged at 1400 g for 10 min to remove cellular debris. The percentage of cells isolated in each successive fraction was determined by the proportion of cell protein isolated in a given fraction, assuming that the total sum of protein corresponds to 100% of cells. Protein was determined by the method of Lowry et al. (1951), and sucrase activity assayed according to the procedure of Messer & Dahlqvist (1966). Sucrase activity was expressed as mU/mg protein (Unit = μmole of substrate hydrolysed per minute at 37°C). SC concentration was measured in each cell fraction by a double antibody immunoradiometric assay (IRMA). The techniques for preparation and for labelling the IgG fraction of anti-SC goat antiserum have been reported in detail elsewhere (Delacroix & Vaerman, 1981; Delacroix & Vaerman, 1982). The goat anti-SC rat antiserum was kindly provided by Dr J. P. Vaerman (ICP, Brussels). The specificity of the goat anti-rat SC antiserum was demonstrated by immunohistochemistry. It did not stain IgA plasma cells from the lamina propria and only selectively reacted with intestinal columnar epithelial cells. Before the assay, the cell fractions were diluted five-fold, or more, in horse serum (20% in phosphate-buffered saline, pH 7.4). The successive steps of the IRMA have been described previously in detail (Delacroix & Vaerman, 1982). The standard curve was made with 11S sIgA purified from rat milk at the following concentrations in 20% horse serum: 0, 10, 50, 100, 150, 200, 250, and 400 ng/ml, (measured by OD at 280 nm, using \( E_{280}^{1%} 1 \text{ cm} = 13.8 \)). The IRMA measured all forms of SC (free and bound to immunoglobulins) and results were calculated as equivalents of 11 S sIgA concentration. The limit of sensitivity of the assay (+4 SD above the radioactivity of the zero standard) was 4 ng/ml. The final SC concentration in villus and crypt cells was expressed in ng/mg cell portion. All determinations of SC concentration were performed in duplicate. It must be outlined that the SC IRMA measured the SC antigenic content of the cells, whereas the sucrase assay only measured the biological activity of this enzyme.

**Calculations and statistical analysis**

The concentration of SC in villus cells calculated for each individual animal refers to the mean value of all SC concentrations measured in the successive 0–80% cell fractions. The concentration of SC in crypt cells refers to the mean of SC values measured in the successive 80–100% cells fractions.

All data are given as mean ± SE. Differences between means were tested for statistical significance (\( P < 0.05 \)) using the Student's t-test.

**RESULTS**

**Distribution of SC concentration along the villus-crypt unit (Study I)**

Histological sections of everted adult rat jejunal segments showed that when the cell isolation procedure was completed, only some crypt bases and the lamina propria persisted. Most of the cells released from the villus during the first 30 min of incubation were fully differentiated enterocytes with a well-developed brush border. Under our experimental conditions, a typical cell gradient from villus tip to the crypt base, as defined by Weiser (1973), was obtained. The gradient of sucrase activity, illustrated in Fig. 1, was similar to that reported by Raul et al. (1977) and validates our isolation procedure. Figure 1 clearly demonstrates that the villus-crypt gradient of SC concentration was the inverse of the gradient obtained for sucrase. SC concentration was low in upper villous cells and increased in the crypt zone at a level (80%) where the corresponding activity of sucrase decreased. Peak concentrations of SC were found in 95–98% of cell fractions, which corresponds to cells positioned at the lower part of the crypts. The mean concentration of SC was about four times higher in crypt cells (80–100% of cells isolated; 636 ± 173 ng/mg protein) than in upper villous cells (0–80%, 152 ± 17 ng/mg protein; \( P < 0.01 \), means ± SE of cell fractions derived from four animals).

**Changes in SC concentration during postnatal development (Study I)**

These changes are illustrated in Fig. 2. In the immature jejunum of 10-day-old rats, the presence of SC could not be detected. Small amounts of SC were measured in jejunal epithelial cells of the 14-day-old rat, the concentration being slightly higher in the crypt zone than in the villus zone (mean ± SE: 18.8 ± 5.9 ng/mg protein in crypt cells vs 11.8 ± 0.5 ng/mg protein in villus cells). During the third postnatal week, mean SC concentration progressively increased in both villus and crypt cells with the result that, by day 20 postpartum, there was no demonstrable typical adult gradient. The adult pattern of SC distribution along the villus-crypt unit appeared between days 20 and 30 (eight-fold increase of mean SC concentration in crypt
Figure 1. Distributive pattern of sucrase activity (●) and of SC concentration (○) along the villus-crypt unit in adult rat jejunum (four experiments). The 100% of cells isolated corresponds to the sum of the fractions expressed as protein. The percentage of cells isolated in each successive fraction was determined by the proportion of cell protein isolated in a given fraction.

Figure 2. Ontogenic changes of mean sucrase activity and of mean SC concentration in rat jejunum enterocytes of the villus zone (0–80% cell isolated) and of the crypt zone (80–100% cells isolated). Each value refers to mean ± SE derived from four to five individual observations. Lack of error bar indicates that SE is too small to depict on the figure. For each individual observation, the SC concentration represents the mean value of all SC concentrations measured in duplicate in the successive 0–80% cell fractions for villus cells and in the 80–100% cell fractions for crypt cells.
cells and four-fold increase in villus cells) and the gradient was completed by day 40 postpartum. This developmental pattern differs in its timing to that observed for sucrase. Jejunal sucrase activity, virtually absent in 10-day-old sucklings, increased markedly in villus cells between days 14 and 20, so that by day 20, there was an adult differentiated villus-to-crypt gradient of enzyme activity.

Effects of corticosterone and L-thyroxine treatments (Studies II and III)

Compared to the respective control groups, final body weight was significantly lower in 13-day-old animals treated with L-T\textsubscript{4} (controls: 22 ± 0.65 vs treated group: 19 ± 0.91 g, \(P < 0.05\)) and was slightly decreased in the group treated with corticosterone (controls: 25.8 ± 1.07 vs 24.1 ± 0.3 g, not significant). All animals appeared healthy and did not develop obvious signs of hormonal toxicity (i.e. tremour, diarrhoea, etc.).

Both hormonal treatments were found to enhance precociously the specific activity (expressed per mg protein) of the enzyme in villus (corticosterone: six-fold increase and L-T\textsubscript{4} 12-fold increase over control values) and in crypt cells (corticosterone: six-fold; L-T\textsubscript{4} seven-fold). Changes in sucrase activity appeared to be the result of an hormonal effect on the enzyme itself, since total mucosal protein content and the total amount of cell protein released during each isolation procedure (corresponding to 100% of cells isolated) was similar in experimental and control groups (L-T\textsubscript{4}: 7.87 ± 0.39 vs controls: 7.84 ± 0.57 mg protein; corticosterone: 8.73 ± 0.44 vs controls 9.10 ± 0.61 mg protein, not significant). These data are in agreement with the findings reported by others (Henning & Sims, 1979; Henning, 1978) and support evidence that, at the doses given and under our experimental conditions, corticosterone and L-T\textsubscript{4} were effective on the immature jejunal cell. However, these hormonal treatments affected cellular SC concentration in a different way to that observed for sucrase (Fig. 3). In corticosteronetreated rats, the mean SC concentration measured by day 13 was reduced to a level of 7.5% (\(P < 0.01\)) of the controls in the villus zone, and to 33% (\(P < 0.01\)) of the controls in the crypt zone. Likewise, for L-T\textsubscript{4} treated animals, the mean SC concentration fell to a value corresponding to 10.2% (\(P < 0.01\)) of the controls in the villus zone and to 36% (\(P < 0.05\)) of the controls in the crypt zone.

**DISCUSSION**

Our quantitative data provide further (Brandtzaeg, 1974) evidence that in the adult rat jejunum, SC is synthesized in the epithelial undifferentiated crypt cell and decreases following a concentration gradient in the epithelium covering the villi with a mean concentration four times higher in the crypt zone than in the villus zone. The SC gradient was the reverse of the sucrase gradient, an enzyme marker of the differentiated villus enterocyte.

At birth, there is no appreciable amount of IgA-producing cells in the rat intestinal mucosa so that, during the suckling period, the intestinal immune barrier is highly dependent on IgA antibodies provided by breast milk. Plasma cell secreting IgA antibodies in lamina propria only appear at the beginning of the third postnatal week (Nagura et al., 1978) as a result of the antigenic stimulus of the mucosa by the normal gut microflora (Crabbé et al., 1968, 1970). The present study demonstrates that the maturation of the secretory immune system of the infant rat also requires major adaptive changes of intestinal epithelial cells with respect to their ability to produce SC, the receptor
for transepithelial transport of p-IgA. SC was virtually absent in villus and crypt cells of suckling pups, and only became detectable by day 14 postpartum. Although the developmental pattern of SC, illustrated in Fig. 2, resembled that observed for sucrase, the abrupt rise in SC concentration and the development its typical crypt-to-villus gradient occurred about 1 week later (day 20–30) than for sucrase activity (day 14–20). Interestingly, maximal SC concentrations in crypt cells were reached by day 40–50 postpartum, that is the period corresponding to an adult homing pattern of the lamina propria by IgA plasmacytes (Crabbé et al., 1970). The ontogenic model for SC expression, depicted in Fig. 2, is in good concordance with the study of Nagura et al. (1978) who could not detect SC in jejunal epithelial cells of the suckling rat by immunocytochemistry until the third week of life. These authors also found that SC-IgA molecules from rat milk bind to specific receptors on the surface of the intestinal epithelium covering the villi of the suckling rat. We did not detect this external source of SC in homogenates of isolated villus cells of sucklings, suggesting that very little of this SC-IgA is internalized into the cell, and that dithiothreitol was efficient to remove exogeneous SC during our cell isolation procedure.

The stimuli and mechanisms that initiate and control the ontogenic changes in SC expression by the crypt cell are currently unknown, but the possible interaction of the weaning diet, of certain hormones and of immune factors have to be considered. The weaning diet is an important modulator for the intestinal development, and the amount of iron consumed during the weaning process is a critical determinant for the intestinal cell concentration of SC measured at the age of 28 days (Buts et al., 1984). However, it seems unlikely that the rise in SC concentration between days 20 and 30 could be initiated by the transition from rat milk (low in iron) to standard chow (high in iron). Indeed, SC was measured in villus and crypt cells of the 14-day-old rat, whereas the presence of solid food in the stomach is only detectable after day 16 postpartum (Henning, 1981). Moreover, during ‘active phase’ of weaning, when the consumption of solid food is known to increase markedly (day 16–21), the intestinal concentration of SC remained low and, by day 20, there was no evidence of a crypt-to-villus cell gradient. Alternatively, we attempted to determine whether the changes in jejunal SC concentration could be triggered by the normal rise in circulating levels of corticosterone and of thyroxine, which occurs shortly before weaning begins (Henning & Sims, 1979; Buts et al., 1983; Samel, 1968).

The applied hormonal treatments were effective on immature jejunal cells, as demonstrated by the rapid and precocious induction of sucrase activity. However, both hormones failed to enhance the intestinal production of SC, although they produced significant decreases in mean SC concentration in villus and crypt cells. Exogenous glucocorticoids exert differential effects on structure and function of both immature (Buts & De Meyer, 1984; Buts et al., 1983) and mature (Scott et al., 1981) rat jejunum. Recent data from in vivo and in vitro experiments on human (Scott & Peeters, 1983) and on rat adult jejunum (Scott et al., 1981) indicate that the glucocorticoid response is specific to particular cells and limited to a small number of gene products in each cell type. The specificity of the effects of glucocorticoids on immature jejunal cells is reaffirmed by our study, showing that certain surface membrane glycoproteins synthesized by the enterocyte (such as sucrase) respond to glucocorticoid stimulation, while other glycoproteins such as SC do not. The chronological difference between the postnatal development of sucrase and SC, and the specificity of glucocorticoid- and L-T4-mediated responses of the immature jejunal cell implies that other controlling factors, such as the intrinsic genetic programme, are of importance.

Alternatively, potential immune stimuli for SC expression by the cell could be the disappearance of exogenous supply in SC-IgA antibodies from rat milk and/or the appearance of an endogenous production of IgA in the lamina propria consecutive to the development of the microbial gut flora (Crabbé et al., 1968, 1970). Both possibilities need to be tested by further investigation, although the production of SC by the epithelial cell is known to be quantitatively independent of the number of p-IgA molecules presented to these cells (Nagura, Nakane & Brown, 1980).

Taken together, our data suggest that the maturation of the intestinal secretory immune system of the suckling rat is dependent not only on homing of the intestinal lamina propria by IgA plasma cells but also on major changes in expression of SC by the crypt cell, beginning in the third postnatal week. After its appearance, intestinal SC is committed to an adult pattern of distribution along the villus-crypt unit during the fourth week of life. The stimuli that trigger these ontogenic changes are currently unknown, but are clearly distinct from those (dietary changes, corticosterone and L-T4) initiating the maturation of other
surface membrane glycoproteins (such as sucrase) produced by the crypt cell.

**ACKNOWLEDGMENTS**

The authors would like to express their gratitude to Jean-Pierre Dehennin and to Nadine De Keyser for excellent technical assistance, and to Penelope Brock for helpful comments on the manuscript. They are indebted to Francis Raul for assistance in conducting the preliminary assays of the cell isolation procedure.

This research was supported by the Nestle Nutrition Research Grant Program and by the Fonds de Recherche Scientifique Médicale (FRSM), Belgium, Grant 3/4551/82. Dominique L. Delacroix is a Senior Research Assistant at the FNRS, Belgium.

**REFERENCES**


