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Secretion of immunoglobulins and plasma proteins from the colonic mucosa: an *in vivo* study in man

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SUMMARY

There are no available data on immunoglobulins and albumin outputs into the normal human colon. We thus measured the intracolonic secretion rates of IgA, IgG, IgM, secretory component (SC) and plasma proteins (albumin (Alb), orosomucoid (Oro), transferrin (Transf) and α_2-macroglobulin (α_2-M)). Using a pancolonic perfusion technique in 10 healthy volunteers (six females, four males, mean age 24 years), concentrations and outputs of Alb, immunoglobulins, SC, Oro, Transf and α_2-M were measured in the rectal effluents by immunoradiometric assay. Monomeric (m) and polymeric (p) IgA distribution was analysed by sucrose density ultracentrifugation. The secretion of polymeric IgA (p-IgA) was 153 μg/min, i.e. 220 mg/day, exceeding that of other immunoglobulins (m-IgA 8·5 μg/min; IgG 33·5 μg/min; IgM 17 μg/min) and of non-immunoglobulin proteins (Alb 104 μg/min; Oro 9 μg/min; Transf 7 μg/min; α_2-M 4·5 μg/min). p-IgA was entirely linked to SC (secretory IgA) and 12% of SC was in free form. About 62% of total IgA was IgA2. For each protein, a relative coefficient of excretion (RCE) was calculated (colon to serum concentration ratio expressed relative to that of Alb). The p-IgA, IgM and m-IgA RCE were 277, 6 and 2·2 times higher than the values predicted from their molecular weight. RCE of non-immunoglobulin proteins also exceeded the values expected from a passive seepage from the vascular compartment. The intracolonic clearance of Alb extrapolated to 24 h was only 3·7 ml/day. These results show the high local production and/or the facilitated transport to the colonic lumen of p-IgA, and are in very good agreement with the distribution of plasma cells in the colonic mucosa.

Keywords colonic perfusion immunoglobulins immunoglobulin A mucosal immunology secretory component

INTRODUCTION

Immunologic components of the large bowel, and notably local immunoglobulins, play a large role in normal colonic mucosa defence and in the pathophysiology of colonic diseases, such as ulcerative colitis and Crohn's disease [1]. However, only plasma cell counts [2–4] and *in vitro* immunoglobulin secretion studies [5–8] have been performed with normal and pathologic colonic mucosa. There is no human *in vivo* intracolonic immunoglobulin secretion study in health and disease, and it is hazardous to extrapolate the morphological and *in vitro* data to *in vivo* immunoglobulin secretion rates. Indeed, several cytokines govern the secretion rates of immunoglobulins by plasma cells [9]. Previous studies in health and disease [10–12] showed that intestinal perfusion under an occluding balloon was a safe and accurate method for quantification of the secretion of immunoglobulin and other serum proteins in a segment of the small intestine. Thus, we applied this technique to the large intestine in healthy volunteers in order to establish normal colonic secretion rates for immunoglobulins and other serum proteins, and make possible further comparisons with patients suffering from inflammatory bowel disease.

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SUBJECTS AND METHODS

Subjects

Ten caucasian healthy volunteers (four males and six females) with a mean age of 24 years (range 20–30 years) were included in the study. They had no evidence of gastrointestinal, allergic or immunologic disease, and none was taking drugs of any

kind. The protocol of the study was approved by the Ethics Committee of the Saint-Lazare Hospital, and written informed consent was obtained for all subjects.

Colonic perfusion

The colonic perfusion was performed using a tube as described by Devroede & Phillips [13], and modified by Rambaud *et al.* [14]. The four-lumen perfusion tube was tracted by an inflatable balloon with a mercury bag. The colonic perfusion point was at 10 cm above the tracting bag, and immediately under an inflatable balloon able to occlude the terminal ileum. An aspiration point was located just above the balloon to collect ileal fluid and to infuse every 15 min a bromosulphonphthalein solution to test the efficacy of lumen occlusion. Thereafter, the subjects stayed in a semi-recumbant position.

The tube progression was monitored by radioscopy until the mercury bag reached the caecum (orocaecal distance 220 ± 26 cm, mean ± s.d.). During the 24 h required for the transit, subjects were fed a standard diet. On the day of the study, after an overnight fast, the colon was rinsed rapidly with 2–3 *l* of 154 mM sodium chloride until anal effluent was clear. After the inflation of the ileal occluding balloon, the colon was perfused at the rate of 15 ml/min, with a solution containing 130 mmol/*l* sodium, 20 mmol/*l* potassium, 100 mmol/*l* Cl, 48 mmol/*l* bicarbonate and 10 g/*l* polyethyleneglycol 4000 (PEG) as a water movement marker. After a 90-min equilibration period, six 10-min samples were collected consecutively by a rectal tube and kept on ice. Any sample contaminated by ileal content was discarded and replaced by a prolongation of the perfusion. Diisopropylfluorophosphate, a potent protease inhibitor, was added to each sample. Absence of blood contamination was confirmed in all samples (Hemotest, Ames, France). A serum sample was obtained from each patient after the third 10-min period. Serum and perfusate samples were stored at −20°C.

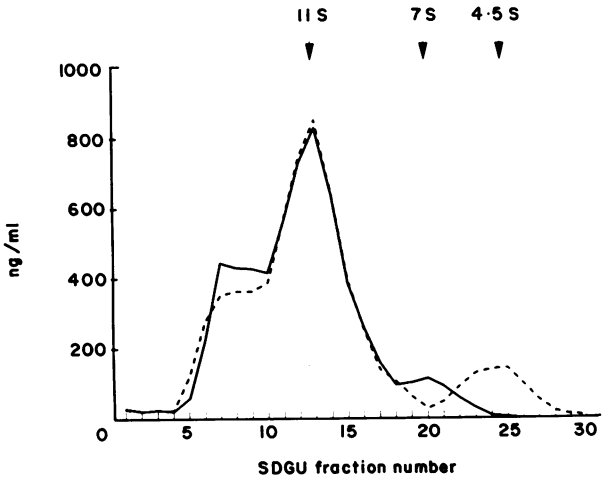

Fig. 1. IgA and secretory component (SC) levels on sucrose density gradient ultracentrifugation of colonic perfusate (pooled, n = 10). The arrows indicate the sedimentation positions of 4·5S free SC, 7S m-IgA and 11S s-IgA. SDGU, Sucrose density gradient ultracentrifugation. —, IgA; - - - -, SC.

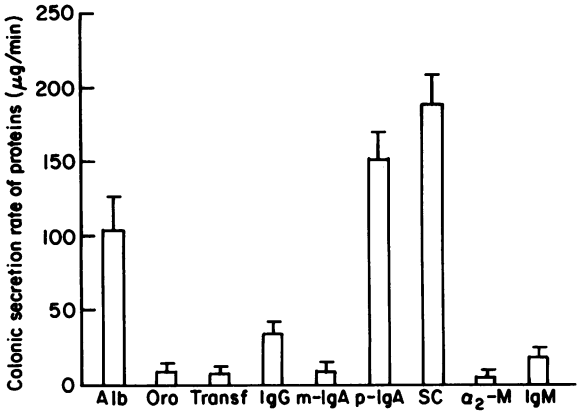

Fig. 2. Colonic secretion rate of immunoglobulins and other serum proteins measured by a pancolonic perfusion. Columns represent means ± s.e.m. (n = 10). Alb, Albumin; Oro, orosomucoid; Transf, transferrin; SC, secretory component; α_2-M, α_2-macroglobulin.

Assays

On the whole, the IgA, IgG, IgM, secretory component (SC), albumin (Alb), orosomucoid (Oro), transferrin (Transf) and α_2-macroglobulin (α_2-M) concentrations in serum and colonic samples were measured using the methods, reagents and standards described previously [11,12,15] for measurement of serum, salivary, biliary and jejunal concentrations of these proteins. Briefly, serum concentrations of Alb, Transf, Oro, α_2-M, IgA, IgM, IgG were measured by turbidimetry. All proteins in colonic samples, including SC, IgA1 and IgA2, were measured by a sensitive immunoradiometric assay (IRMA) because of their small concentrations in the perfusates. The proportions of monomeric and polymeric IgA were also determined by IRMA after sucrose density gradient ultracentrifugation (SDGU). The influence of the size of IgA in IRMA was taken into account by multiplying all results obtained by IRMA in colonic fluid by a factor of 2·3 [11,12,16]. The proportions of free and bound SC were determined according to the same methods as for m-IgA and p-IgA [11,15]. Polyethylene glycol was assayed by the turbidimetric method [17].

Statistical analysis

The fluid flow rate (FRs) at the sampling point was calculated as follows: FRs = FRp × (PEGp)/ (PEGs), where FRp is the fluid flow rate at the perfusion point, and (PEGp) and (PEGs) are the PEG concentrations at the perfusion and sampling points, respectively. Net anal output of water during colonic perfusion was 12·9 ± 0·4 ml/min.

The secretion rate of each protein (PSR), expressed as μg/min, was calculated according to the formula: PSR = FRs × (protein), where (protein) is the concentration of the protein at the sampling point. The relative coefficient of excretion of each protein (RCE) was obtained from: RCE = ((protein in perfusate)/(protein in serum))/(Alb in perfusate)/(Alb in serum). As transport of Alb to the digestive tract lumen is entirely passive [11], this coefficient expresses the secretion rate independently of the serum protein concentration and of the net water transcolonic movement. If a protein is passively transported from the plasma to the colonic lumen, its

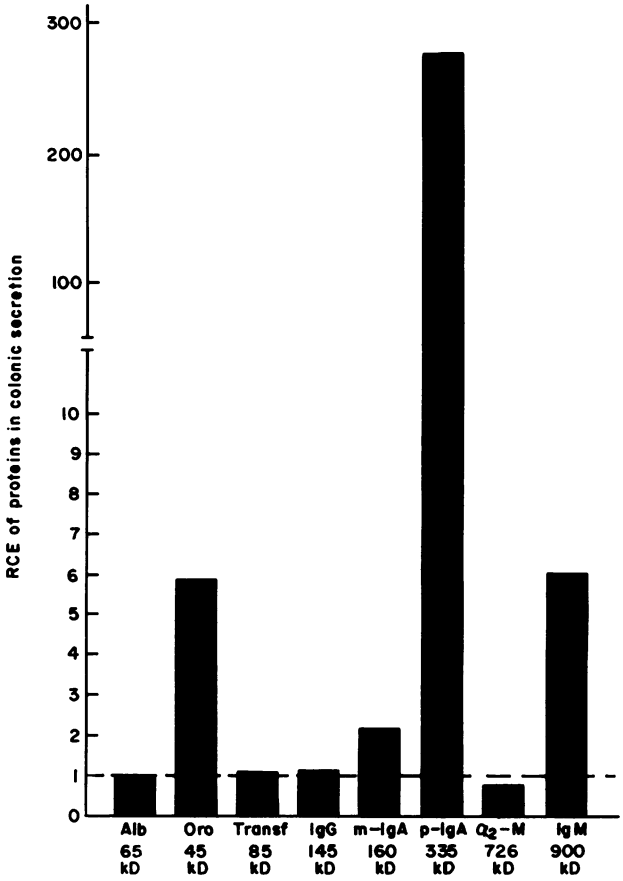

Fig. 3. Relative coefficient of excretion (RCE) of proteins in colonic secretions (n = 10). The horizontal dotted line represents the RCE of albumin, taken as a unit. Alb, Albumin; Oro, orosomucoide; Transf, transferrin; α_2-M, α_2-macroglobulin.

RCE value is near 1 and inversely related to its molecular weight. An RCE higher than this value indicates partial or total local gut synthesis and/or facilitated transport [15]. Colonic clearance of Alb was calculated as PSR Alb/(Alb in serum), and was expressed as ml/day. For each subject, results of the six 10-min collection samples were averaged and final results were expressed as arithmetic means of the averaged values observed in the 10 subjects ± s.e.m.

RESULTS

Molecular forms and subclasses of IgA and SC in colonic perfusion fluid

In the sucrose density gradient separation of the perfusate (Fig. 1), IgA sedimented as a major peak (89 ± 5%) at 11S and ⩾ 11S, and a minor peak at 7S (11 ± 5%). Both 11S and ⩾ 11S IgA sedimented with most SC, corresponding to secretory IgA (s-IgA). SC in perfusates sedimented with 11S and ⩾ 11S p-IgA (88·2 ± 2·3%) as well as at the 4·5S position, corresponding to free SC (11·8 ± 2·3%). IgA2 contributed 62 ± 2% to total IgA in the perfusate.

Absolute colonic secretion rates

In the perfusate, SC and p-IgA were present at a greater concentration than all other proteins. No evidence of proteolysis was observed by SDGU of IgA and SC. Polymeric IgA contributed 76% of total immunoglobulin in the perfusate (IgG/p-IgA = 0·22). Absolute secretion rates of proteins from the colonic mucosa are shown in Fig. 2. About 153 ± 15 μg of p-IgA, 190 ± 18 μg of total SC, 8·5 ± 5 μg of m-IgA, 33·5 ± 7 μg of IgG and 17 ± 4 μg of IgM were secreted per min into the large intestine. The secretion rates of non-immunoglobulin proteins were 104 ± 15 μg/min, 9 ± 2 μg/min, 7 ± 1 μg/min and 4·5 ± 1 μg/min for Alb, Oro, Transf and α_2-M, respectively. The mean intra-individual coefficient of variation of secretion rates ranged from 14 ± 3% for Oro to 35 ± 14% for IgG, and was 15 ± 10% for p-IgA.

Relative coefficients of excretion (RCE) of proteins

The RCE calculation for each protein can give an idea of its origin and transport into the colon. The p-IgA, IgM and m-IgA RCE were 277, 6·0 and 2·2 times higher than the values predicted from their respective molecular weights, respectively, whereas IgG RCE was close to the predicted value for passive diffusion (Fig. 3). Of the non-immunoglobulin proteins, Oro RCE was surprisingly high (5·9), whereas Transf RCE was slightly above 1. The mean clearance of Alb was 3·7 ml/day.

DISCUSSION

The colonic perfusion method used in our study was a modification of the Devroede's technique [13]. A distal ileum occluding balloon was added to avoid completely contamination of the colonic perfusate by immunoglobulins, plasma proteins and digestive enzymes coming from above [14]. In spite of this modification, the transcolonic movement of water was similar to values previously reported [18] with the same perfusing solution and the same rate of infusion. No evidence of proteolysis was observed by SDGU of IgA and SC, and the mean intra-individual coefficients of variation of protein secretion rates were reasonably low. Monomeric IgA, p-IgA and free and bound SC were considered separately, with appropriate correction for the influence of their size in IgA immunoassays [16]. This was not the case in the work of Bull *et al.* [19], whose results markedly differed and obviously underestimated IgA compared with IgG.

Total IgA was 161·5 μg/min and accounted for 77% of total immunoglobulin secretion rate into the large intestine; p-IgA was entirely bound to SC (s-IgA) and represented 89% of total IgA. SC was also present in free form, suggesting that it is not rate limiting for p-IgA transport in the normal colon. This pattern of IgA and SC secretion in the large intestine was similar to that found in a 40-cm segment of jejunum [11].

It is known that the proportion of IgA2 subclass is higher in external secretions than in serum [20]. Our study shows that the colonic secretion has the greatest percentage of IgA2 (62%), compared with jejunum (35%) and other organs [11, 21]. This predominance of IgA2 in colonic secretion is to be considered in light of its resistance to most bacterial IgA proteases that only cleave IgA1 [22].

The local origin and SC-facilitated transport in the colon of p-IgA has been previously deduced from *in vitro* experiments [5,23] and immunohistochemical studies of colonic lamina propria. Ninety per cent of plasma cells produce IgA [2,3], with a ratio IgA2/IgA1 + IgA2 of 60% [21,24,25]. Synthesis of

SC by colonocytes is also well documented [26–28]. Our RCE calculations further suggest a mainly local synthesis and SC-mediated transport of p-IgA, since p-IgA was secreted 277-fold more selectively in colon than Alb, and entirely cosedimented with SC. The RCE value of colonic p-IgA was close to the value found in the jejunum [11], where p-IgA is also entirely linked to SC in the lumen. In contrast, m-IgA is not transported by SC [29]. Its RCE, although only 0·8% that of p-IgA, was higher than that expected solely from its molecular weight, which suggests a double origin, local and plasmatic. The same finding has been reported for the jejunum, where isotopic studies estimated the local production to represent about two-thirds of the whole m-IgA present in the lumen [11]. In fact, a large part of m-IgA synthesized in the colonic wall is probably secreted into lymph and then blood, since the percentage of IgA plasma cells not synthesizing J chain in the gut lamina propria has been estimated to be approximately 10% of total IgA-secreting cells [30].

Intracolonic secretion of IgM and IgG were 8% and 16% of total immunoglobulin, respectively. The IgM RCE of 6 reflects a purely local production, as the high molecular weight of IgM precludes its significant transport from plasma. This was confirmed by the good agreement between the IgM secretion rate and the proportion (6%) of IgM cells in the colonic mucosa [2–4]. IgM transport is known to be SC-mediated [27], but this was not detected in our SDGU study because of the very low concentration of this immunoglobulin in the colonic perfusate. IgG RCE was 1·17, indicating a prominent seepage from plasma. Indeed, the percentage of IgG cells in colonic mucosa is only 3% [2–4].

RCE of non-immunoglobulin proteins exceeded the values expected for a simple molecular weight-affected seepage from plasma. Moreover, due to its high molecular weight, α_2-M should not significantly cross the endothelial barrier. Indeed, local production of α_2-M as well as of Transf is likely [31–34], but a local production or facilitated transport of Oro is a new finding. Curiously, Oro-RCE was four times higher than in the jejunum [11].

Finally, we found a colonic clearance of Alb of 3·7 ml/day. This figure is very small compared with that of stomach clearance, estimated to be between 45 and 70 ml/day [35] and small bowel clearance (estimated to be between 20 and 30 ml/day).

In conclusion, perfusion studies of the whole large intestine confirm that this organ is an important site of secretion of p-IgA, with a predominance of IgA2. Local production by plasma cells in the lamina propria is probably the main source of the secreted p-IgA. The colonic p-IgA is virtually entirely transported by SC, which, however, is not a rate limiting factor of secretion of p-IgA as secretory IgA. Intracolonic secretion of IgM is weak, and also of local origin. RCE of α_2-M, Transf and unexpectedly Oro, seem to imply local synthesis.

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