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Mucosal barrier mechanisms. Interplay between secretory IgA (SIgA), IgG and mucins on the surface properties and association of salmonellae with intestine and granulocytes

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Summary. Rough *Salmonella typhimurium* 395 MR10 bacteria sensitized with SIgA were used to assess the effect of secretory IgA (SIgA) on bacterial association with the intestine of rat and with a column of hog gastric mucin, and on IgG-mediated surface properties and interaction with polymorphonuclear leucocytes.

It was found that SIgA increased the affinity for mucus belt of the intestine and for the mucin column, but reduced IgG-enhanced phagocytosis and surface hydrophobicity and charge of the bacteria.

It is suggested that the ability of SIgA to render bacteria mucophilic and to modify IgG-mediated reactions serve the purpose of secluding bacteria from contact with mucosal membranes and depress inflammatory reactions at the site of infection.

INTRODUCTION

The mucin layer has for a long time been known to act as a mechanical barrier against the penetration of particulate and molecular matter from the intestine (Florey, 1962; Heatley, 1959). The release of mucus is stimulated by a variety of substances, including bacterial toxins (Moon, Whipp & Baetz, 1971; Steinberg, Banwell, Yardley, Kensch & Hendrix, 1975) and immune complexes (Walker, Wu & Bloch, 1977). These studies show that mucus has a broad regulatory function in the maintenance of mucosal integrity. In the exclusion of different antigens from mucosal surfaces, secretory IgA (SIgA) has been found to play an important role (Bazin, 1976; Hansson, Ahlstedt, Andersson, Carlsson, Dahlgren, Lidin-Jansson, Mattsby-Baltzer & Svanborg-Edén, 1980; Russel-Jones, 1980; Stokes, Soothill & Turner, 1975). It has recently been observed that colostral SIgA reduced the negative surface charge and liability to hydrophobic interaction, and decreased the association of phagocytosis-sensitive salmonella bacteria with polymorphonuclear leucocytes PMNL, (Magnusson, Stendahl, Stjernström & Edebo, 1979). By contrast, hyperimmune IgG raised against the virulent, parent strain *Salmonella typhimurium* 395 MS, had a hydrophobic effect (Stjernström, Magnusson, Stendahl & Tagesson, 1977), and promoted phagocytosis *in vivo* as well as *in vitro* (Stendahl, Tagesson, Magnusson & Edebo, 1977).

IgA has been shown to modulate the bactericidal effects of IgG and IgM on *Neisseria meningitides* (Griffiss & Bertram, 1977) and on *S. typhimurium* (Eddie, Schulkind & Robbins, 1971).

Serum IgA, as well as colostral IgA, most likely

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SIgA, has also been found to suppress IgG-mediated, but not complement-dependent, phagocytosis of *Candida albicans* blastospores (Wilton, 1978). However, this suppression was probably due to a direct effect on the PMNL, since pretreatment of the blastospores with the IgA-preparation had no significant effect on the phagocytic uptake.

The present study was done to elucidate: (i) the effect of SIgA on bacterial attachment to mucosal surfaces by quantifying the association of SIgA-treatment of *S. typhimurium* 395 MR10 bacteria with rat intestine under normal circumstances and after pretreatment of the intestine with mucolytic agents, and (ii) the influence of SIgA on IgG-mediated surface properties and phagocytosis of the same bacteria.

MATERIALS AND METHODS

Bacteria

The rough (Rd-mutant) *S. typhimurium* 395 MR10 (Stendahl, Tagesson & Edebo, 1973) was maintained at 4° on agar slants before use. The bacteria were grown overnight (16 hr) at 37° in 10 ml of the following enriched glucose medium: glucose, 1 g; $Na_2HPO_4 \cdot 2H_2O$, 0·6 g; NaCl, 1·8 g; peptone (Difco), 10 g; beef extract(Difco), 5 g; and deionized water to 1000 ml, pH 7·4.

To study bacterial attachment to rat intestine two different isotopes, ^{51}Cr and ^{125}I were used to label the bacteria (Stendahl *et al.*, 1973). This was done in order to study the adhesion of untreated and SIgA-coated MR10 bacteria to the same part of the intestine of the rat. For the studies of the affinity for the mucin column, and for the phagocytosis experiment, bacteria were metabolically labelled by introducing 25 μl of [3, 4, 5-^{3}H]-leucine (NET 460, NEN Chemical GmbH, Dreieichenheim, F.R.G.) into the culture medium. Bacteria were washed four times (1000 ***g*** for 15 min) in PBS before use. They were quantified by the absorbance at 640 nm, as calibrated with viable count. Radioactivity was measured either in an auto-γ-scintillation counter (CG30, Intertechnique SA, Plaisir, France) or in an auto-β-scintillation counter (Isocap 300, Searle-Nuclear, Chicago, Ill).

Source of secretory IgA

SIgA was purified from colostrum. Colostrum was collected within 1–2 days post partum, to get a high content of SIgA. Preparation and quantification of SIgA was done as described previously (Magnusson *et al.*, 1979).

Sensitization of bacteria

For the study of adhesion to the mucin column and to rat intestine, $2{\cdot}5 \times 10^9$ bacteria per ml were sensitized for 30 min at 37°, with varying concentrations of SIgA and 500 μg per ml SIgA, respectively. The bacteria were washed by centrifugation (1100 ***g*** for 15 min) and resuspended in Krebs-Ringer's phosphate buffer with 10 mM glucose, pH 7·2 (KRG). SIgA was alternately reacted with ^{51}Cr- or ^{125}I-labelled bacteria to rule out isotope effects on the measurements of attachment to the intestine. To study the combined effect of SIgA and IgG on the envelope, the bacteria (1×10^9/ml) were incubated for 30 min at 37° in phosphate-buffered saline (PBS) with 50 or 100 μg per ml SIgA (IgG), washed at 1100 ***g*** for 10 min, incubated with 50 or 100 μg per ml IgG (SIgA) for 30 min at 37° and finally washed as before.

Animals

Female Spraque-Dawley rats (180-200 g body weight) were anaesthetized by an intraperitoneal injection of chlorbutol (25 mg per 100 g body weight). The abdomen was opened by a midline incision, and the contents gently pushed aside from a 10 cm segment of the distal ileum, which was ligated with silk immediately proximal to the caecum. Before tying the proximal ligature, a flexible cannula with an injection valve (Venflon, Ø 1·00/19G, Viggo AB, Helsingborg, Sweden; diameter 1·00 mm) was put into the segment through an insertion proximal to the ligature. At the other end of the segment was fixed a wider cannula (Venflon, Ø 2·00/14G, diameter 2·00 mm). The first cannula was connected to a syringe and to study bacterial attachment under normal circumstances, 0·5 ml ^{51}-Cr-labelled and 0·5 ml ^{125}I-labelled MR10 bacteria (5×10^8 per ml KRG) injected after coating either of them with SIgA. After 1 hr the segment of the intestine was cut off and opened. The following fractions were collected and analysed for radioactivity: the residual fluid (*ca* 1 ml), a first and second wash-out with 5 ml KRG, the mucus layer, gently scraped off with a curette, and suspended in 5 ml KRG, and the rest of the intestinal segment. To study the effect of mucolytic agents, the intestine was either pretreated for 3 days with bromohexine (BH, Bisolvon, Boehringer & Ingenheim: 100 mg/ml) dissolved in the drinking water (long term treatment) or for 15 min by 1 ml of dithiothreitol (DTT: 0·1 M, 15·4 mg/ml, short term treatment). Before application of the bacterial suspensions the intestinal segment was gently flushed with 2 ml of KRG. For light microscope

inspection of intestine was frozen isopentane chilled with CO_2 (-74°) and then transferred to formaldehyde (4%) -ethanol (95%) for 24 hr at 4°. Sections were stained with PAS and Harris haematoxylin at pH 2·6.

Preparation of the mucin column

Commercial hog gastric mucin (Lot 125327, Wilson & Co., Inc., Chicago, I11.) was coupled to Epoxy-activated Sepharose 6B (Lot number CB 8606, Pharmacia Fine Chemicals, Uppsala, Sweden) similarly to the general procedure proposed by the manufacturer. Three grams of the gel was suspended in distilled water (10 ml) and washed with the same fluid (300 ml) on a glass filter. The gel was then washed with PBS (50 ml) and distilled water (50 ml) and swollen in distilled water (10 ml). The moist gel was transferred to a solution containing hog gastric mucin (100 mg in 5 ml distilled water). Coupling was allowed to proceed on a shaker for 16 hr at 25°. The product was washed with distilled water (100 mg), borate buffer (100 ml, 0·1 M, pH 8·0, containing 0·5 M NaCl) and with acetate buffer (100 ml, 0·1 M, pH 4·0, containing 0·5 M NaCl). Excess of active groups in the gel was blocked by ethanolamine (1 M) for 16 hr. Finally the gel was washed with PBS (100 ml) and stored at 4° until used.

Chromatography on the mucin column

Ordinary Pasteur pipettes were used as column support. The tip of the pipette was filled with siliconized glass-wool (Varian, Number 82-006000-00) on which was laid a nylon net (70 μm pores) and about 1 ml of the gel. Thus, about 1 ml of space was left for the eluant. After application of the bacteria ($2{\cdot}5 \times 10^9$ per ml) in 0·2 ml of the indicated buffer, elution was made with 0·4 portions of the same buffer. The concentrations of SIgA are related to $2{\cdot}5 \times 10^9$ bacteria per ml. At pH 7·3 PBS was used as eluant and at pH 4·0 acetate buffer (0·05 M).

In vitro *assay of phagocytosis*

Polymorphonuclear leucocytes (PMNL) were obtained from the intraperitoneal cavity of guinea-pigs after injection of 20 ml 0·1% (w/w) glycogen solution interperitoneally (Stendahl *et al.*, 1973), and rinsing 16 hr later with saline containing heparin (15 i.u. per ml; Vitrum, Stockholm, Sweden). After lysing contaminating red cells, about 2×10^7 PMNL in complete Krebs-Ringer's phosphate buffer with Mg^{2+} and Ca^{2+} and 10 mM glucose, pH 7·2, were added to tissue culture petri dishes (50 × 13 mm; Flow Laboratory) with six Millipore filters (HAWPO 1300, Millipore) and allowed to settle for 30 min. After preincubation for 20 min at 37° with 4 ml KRG, 0·5 ml of the suspension of [^{3}H]leucine-labelled bacteria (2×10^9 per ml in PBS) was added. After mixing, a sample was withdrawn and the 0 min filter taken. Filters were subsequently removed at 30, 60 (two) and 120 (two) min, washed gently in three beakers with saline, dried and finally put into a scintillation vial with 2 ml water and 5 ml Aquasol (NEN) (Magnusson *et al.*, 1979). Two parallel experiments were done. The percentage of PMNL was never below 70% when used in the experiments.

Two-phase partitioning

The procedure to obtain the aqueous two-phase systems used has been described elsewhere (Magnusson *et al.*, 1979; Kihlström & Magnusson, 1980). the basal system contained 4·4% (w/w) poly ethylene-glycol 6000 (PEG; Carbowax 6000, Union Carbide, New York, N.K.) and 6·2% (w/w) dextran T500 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) in 0·03 M tris (hydroxymethyl) aminomethane buffer (tris), pH 7·0. After equilibrating the two-phase system over night at 4° in a separation funnel, the bottom phase (rich in dextran) and the top phase (rich in PEG) were collected and stored separately at 4°. For the partitioning studies, 2 ml of the bottom and 2 ml of the top phase were pipetted into graduated (volume) test tubes. To assess hydrophobic interaction, 0·2 ml 2% (w/w) PEG-palmitate (0·13 mmol palmitic acid per gram PEG; Johansson, 1976) in PBS was added. In the systems with charged PEG, 12·5% of the PEG had been exchanged with positively charged bis-trimethylamino-[$(CH_3)_3N^+$-] PEG (TMA-PEG), Johansson, 1970, already in the preparation of the stock solutions. One tenth millilitre of the bacterial suspensions was then added to the test tubes with the different two-phase systems, and the tubes inverted (twenty times) for mixing. They were then kept at 4° for 30 min for separation of the phases. After determining the volumes of the phases, 0·5 ml aliquots were withdrawn from the two phases, and after mixing with a Vortex homogenizer from the rest, containing also material adhering to the interface, to determine the distribution of bacteria.

The variation between two experiments was less than 5%. The results of two-phase partitioning (TPP) was expressed by (i) the percentage material in the top (T) and bottom (B) phase [the remainder making 100%, is found at the interface (I)] and (ii) the change of the distribution, relatively the distribution in the basal system, by the addition of TMA-PEG or P-PEG,

calculated as accumulation of new material in T plus removal of material from B. Principally, the latter index consists of three components—the transfer of material: (a) from B to I, (b) from B to T, and (c) from I to T. If particles are unaffected by ligand PEG, the index equals zero; it becomes 200 if the entire particle population is moved from a position in B in the basal system containing ligand PEG. This index was chosen so as to monitor all three of the components above. Any index based solely on the transfer to T ignores component (a), while one based on the transfer of particles from B would ignore component (c).

Hydrophobic interaction chromatography (HIC)

The tendency to hydrophobic interaction of the bacteria was also assessed by their affinity for an Octyl-Sepharose gel (Pharmacia, Sweden); Stjernström *et al.*, 1977). Ordinary Pasteur pipettes were used as columns; the tip was filled with siliconized glass wool, on which followed a nylon net (70 μm pores) and 1 ml of the gel. After equilibration of the column with 0·1% (v/v) Triton X-100 in 0·01 M phosphate buffer (pH 6·8), a 0·5 sample with bacteria (2×10^8) was added, and elution continued with 6 ml of the equilibration buffer. The fraction of bacteria eluted was operationally defined as 'hydrophilic' and the fraction remaining on gel as 'hydrophobic'.

RESULTS

Association of the MR10 bacterial with rat intestine

When the rat intestine was flushed with buffer it was observed under the light microscope that on sections stained for acidic mucins that mucus coverage of the intestinal cells was gradually removed (not shown). Therefore, to characterize the partition of the bacteria between the intestinal liquid and the mucosal membrane with the mucin cover, the bacteria in the remaining liquid and in the first 5 ml wash-out, which seemed to leave the mucin layer intact, were regarded as the non-adhering fraction I and the bacteria in the subsequent wash-outs, in the mucosa which had been scraped off and in the remainder of the rigid intestine defined as the adhering fraction II (Fig. 1). To assess the effect of SIgA on the partition between fractions I and II, the quotient (times one hundred) between the percentage of SIgA-sensitized and untreated MR10 bacteria in I and II were determined for each rat, so as to randomize variations between individual animals; a considerable variation was observed in the relative

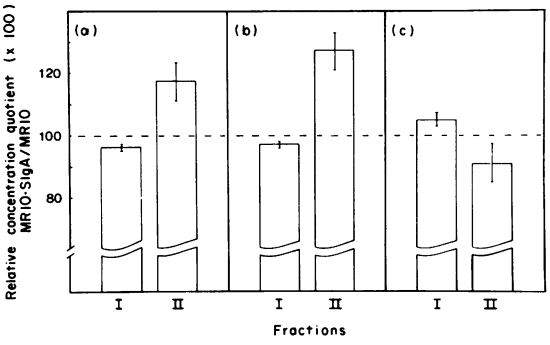

Figure 1. Effect of SIgA sensitization (500 μg/ml) of rough *Salmonella typhimurim* 395 MR10 (2×10^9/ml) on bacterial association with (a) untreated, (b) bromohexine-treated (100 mg/ml for three days in the drinking water) and (c) dithiothreithol-treated (0·10 M for 15 min) intestine as expressed by the quotient (one hundred times) between the percentage of SIgA-coated and untreated bacteria in fractions I and II. A value > 100 means increased affinity by SIgA binding, the number of experiments were eight, two and three, respectively.

amount of bacteria in I and II of different animals. No differences were found in the distribution between I and II, when the radioactive labelling was reversed (not shown). In stained sections (PAS + Harris's haematoxylin, pH 2·6) the normal intestine had an even coat of mucus and the majority of the goblet cells retained their content (Fig. 2a). In the BH-treated animals the mucus layer appeared similar to the control situation, but the goblet cells were more actively releasing acidic mucins (Fig. 2b). In the DTT-treatment intestine, the mucosal coat was impaired and the goblet cells were either empty or forcedly excreting their content (Fig. 2c). As judged by Fig. 1, SIgA increased the affinity for the mucosal fraction (II), possibly to the mucin layer, since (i) repeated washing removed the bacteria almost completely in the control situation, and since (ii) the control (Fig. 1a) and the BH-treated animals (Fig. 1b) with intact mucin coverage of the intestinal cells showed the same tendency. In the DTT-treated intestine with a disintegrated mucosal coat, the reverse pattern was found (Fig. 1c).

Affinity of SIgA-sensitized MR10 bacteria for a mucin column

The retention of bacteria on the column with hog gastric mucin (pH 7·3) increased with the concentration of SIgA at sensitization, indicating a non-specific

Table 1. Hydrophobic interaction chromatography (HIC)* on Octyl-Sepharose of *S. typhimurium* 395 MR10 sensitized with hyperimmune rabbit anti-MR10 IgG and/or human colostral SIgA expressed by the percentage of bacteria† eluted with 0·10% (v/v) Trition X-100 (in 0·01 M phosphate buffer, pH = 6·8) and the percentage adhering to the column material

Bacteria	Percentage of bacteria	
	Eluted‡	Adhering§
MR10	47	53
MR10·IgG (100 μg/ml)	19	81
MR10·SIgA (100 μg/ml)	80	20
MR10·IgG (100 μg/ml) SIgA (100 μg/ml)	27	73
MR10·SIgA (100 μg/ml) IgG (100 μg/ml)	56	44

* One representative experiment on the same batch of human colostral SIgA as was used for TPP (Table 2) and phagocytosis (Table 3).
† Determined by viable count.
‡ 'Hydrophilic' population.
§ 'Hydrophobic' population.

Table 2. Aqueous two-phase partitioning (TPP)* of *S. typhimurium* 395 MR10 sensitized with rabbit hyperimmune anti-MR10 IgG and/or human colostral SIgA in dextran-PEG systems containing positively charged TMA-PEG and hydrophobic P-PEG, as expressed by the percentage of bacteria in (i) top (T) and bottom (B) phase in the basal phase system (rest of material at interface I) and (ii) change of partition on addition of TMA-PEG or P-PEG.

Bacteria	Partition (%)		Change of partition	
	T	B	TMA-PEG	P-PEG
MR10	3	71	41	83
MR10·IgG (50 μg/ml)	4	73	31	73
MR10·SIgA (50 μg/ml)	2	57	22	46
MR10·IgG (50 μg/ml)· SIgA (50 μg/ml)	5	73	28	57
MR10·SIgA (50 μg/ml)· IgG (50 μg/ml)	4	74	22	51

* One representative experiment with same batch of colostral SIgA as was used for the HIC (Table 1) and phagocytosis experiments (Table 3).

affinity of SIgA for acidic mucin (Figs 3a and b). When the pH was reduced to 4·0, the retention of bacteria was more pronounced. Still the affinity was greater after binding SIgA to the bacterial surface (not shown).

Effects of hyperimmune anti-MR10 IgG and colostral SIgA on:

Physicochemical surface properties of MR10 bacteria. When assayed with hydrophobic interaction chromatography (HIC), rabbit anti-MR10 IgG rendered the bacteria more hydrophobic, i.e. a larger proportion of the bacteria adhered to the column with Octyl-Sepharose, whereas colostral SIgA had a hydrophilic effect. One representative experiment is shown in Table 1. Treating the bacteria first with IgG, and then with SIgA made them more hydrophobic than MR10 as such, but less hydrophobic than with IgG alone. Reversing the order of sensitization with immunoglobulin gave a hydrophilic effect, but not as strong as with SIgA alone.

Table 3. Phagocytosis* by guinea-pig PMNL of [^{3}H]-leucine-labeled *S. typhimurium* 395 MR10 sensitized with hyperimmune rabbit anti-MR10 IgG and/or human colostral SIgA after different incubation periods

Bacteria	Percentage† retained activity per filter for incubation periods (min)		
MR10	0·12 (1)	0·31 ± 0·01 (2)	0·51 ± 0·09 (2)
MR10·IgG (50 μg/ml)	0·21 (1)	0·60 ± 0·06 (2)	0·85 ± 0·14 (2)
MR10·SIgA (50 μg/ml)	0·11 (1)	0·24 ± 0·02 (2)	0·45 ± 0·02 (2)
MR10·IgG (50 μg/ml)·SIgA (50 μg/ml)	0·19 (1)	0·38 ± 0·06 (2)	0·54 ± 0·02 (2)
MR10-SIgA·(50 μg/ml)·IgG (50 μg/ml)	0·15 (1)	0·45 ± 0·02 (2)	0·61 ± 0·08 (2)

* One representative experiment; another experiment with a different batch of colostral SIgA showed the same qualitative characteristics.
† ± Range ($n = 2$); number of samples within parenthesis.

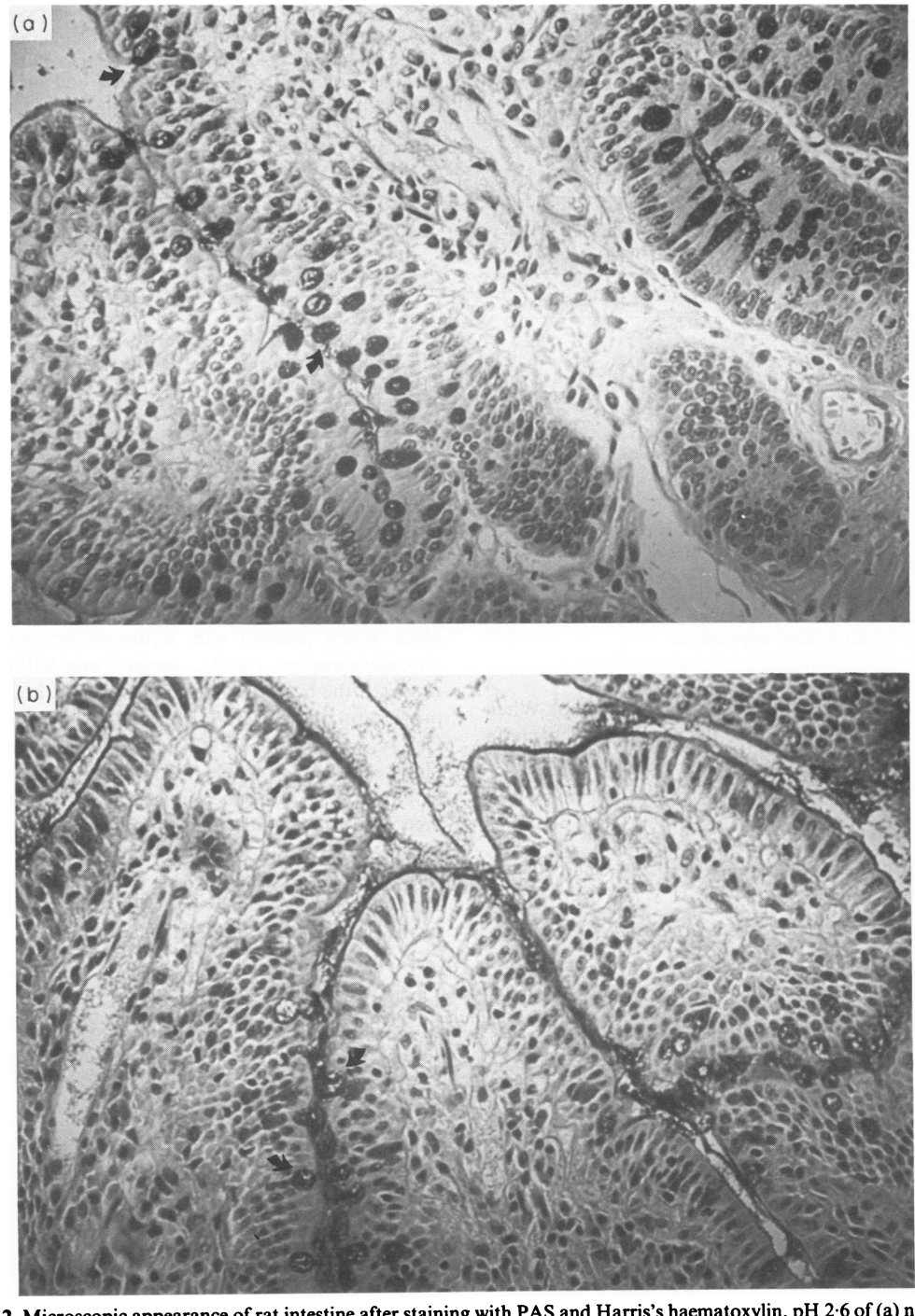

Figure 2. Microscopic appearance of rat intestine after staining with PAS and Harris's haematoxylin, pH 2·6 of (a) normal, (b) bromohexine-treated (100 mg/ml for three days) and (c) dithiothreitol-treated (0·1 M for 15 min) intestine. The goblet cells (arrow) and the mucus appear dark (stained red), as well as the cell nuclei (stained blue).

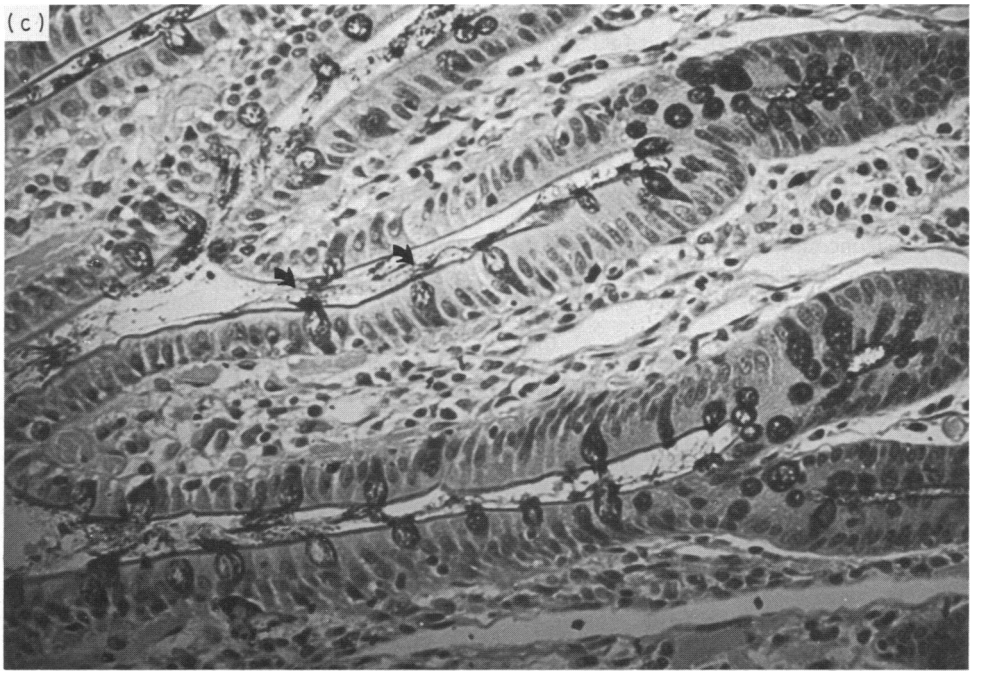

Figure 2c.

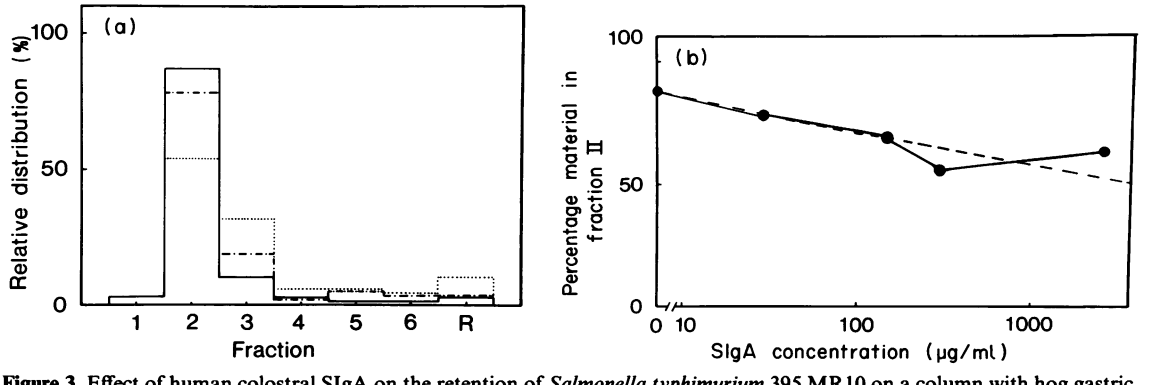

Figure 3. Effect of human colostral SIgA on the retention of *Salmonella typhimurium* 395 MR10 on a column with hog gastric mucin at pH 7·3, as described (a) by the elution profiles (0·4 ml fractions) after sensitization of the bacteria ($2·5 \times 10^9$/ml) with 0(——), 31 (- · -), and 320 (· · ·) μg/ml SIgA. (R) Remainder on the column) and (b) by the percentage of bacteria in fraction II after sensitizing with different concentrations of SIgA.

A parallel TPP experiment with the same preparations of IgG and SIgA indicates that IgG slightly reduced the surface hydrophobicity of bacteria, as reflected by the reduced ability of P-PEG to transfer the bacteria from the bottom phase towards the top phase (Table 2). When they were incubated with SIgA and then with IgG, or *vice versa* they became clearly more hydrophilic but less hydrophilic than with SIgA alone (Table 2). Concomitantly, the negative surface charge was diminished as described by the capacity of positively charged TMA-PEG to move the bacteria towards the top phase (Table 2).

Phagocytosis of MR10 bacteria. A phagocytosis experiment is shown in Table 3. Another experiment with a new source of PMNL and SIgA displayed essentially the same features. It is evident that IgG increased the association of MR10 bacteria with PMNL at all times of incubation ($P < 0{\cdot}025$ at 60 min, and $P < 0{\cdot}10$ at 120 min; Student's t test). By contrast, SIgA reduced the phagocytosis ($P < 0{\cdot}05$ at 60 min; NS at 120 min). SIgA also seems to be capable to moderate the effect of sensitizing with IgG, both if it was added before IgG ($P < 0{\cdot}025$ at 60 min and $P < 0{\cdot}25$ at 120 min) or after IgG ($P < 0{\cdot}10$ at 60 min and 120 min). Post-treatment with SIgA was apparently a little more effective, but the level of significance was low ($P < 0{\cdot}25$ at 60 and 120 min). SIgA alone was more anti-opsonic than the combined sensitization with IgG and then SIgA ($P < 0{\cdot}10$ at 60 min and $P < 0{\cdot}05$ at 120 min), or with SIgA and then IgG ($P < 0{\cdot}01$ at 30 min and $P < 0{\cdot}10$ at 120 min).

DISCUSSION

Mucin-secretory cells play a vital role in the control of infections, and mucins produced on gastrointestinal mucosa seem to provide a delicate protective layer on the living epithelium beneath (Florey, 1962). In this communication we present some evidence that SIgA promoted attachment of bacteria to intact rat intestine (Fig. 1a and b) but counteracted the association with the intestine with an impaired mucosal layer, i.e. when pretreated with dithiothreitol (Fig. 1c). Furthermore, SIgA made the bacteria mucophilic, as indicated by the effect on the partition between fractions I and II, (Fig. 1) and the retention on a column with gastric mucin (Fig. 3a and b). The absence of effect of bromohexine is regarded as an adaptation of the long term treatment with the mucolyticum (Bruce & Kumar, 1968; Williams & Chir, 1978). Heat-inactivated bacteria were used to allow to distinguish SIgA-mediated effects on adhesion from effects on bacterial motility (Russel-Jones, 1980), and radioactive labelling with ^{51}Cr and ^{125}I (γ-radiation) to avoid quenching due to the different physical character of the samples taken from the intestine. Fractions I and II were chosen to monitor the effect of SIgA on bacterial association with the intestine since microscopic examination of tissue sections indicated that this protocol removed non-adhering bacteria, left the mucus gel intact, and randomized differences between the experimental animals. The present results raise the question of the mechanism of the SIgA-enhanced association between two apparently hydrophilic surface structures on the bacteria and on the intestine. Lectin-like binding (Etzler, 1979) or exclusion of unlike carbohydrates, which might trap the incompatible structures (Dea, Morris, Ress, Welsh, Barnes & Price, 1977) are two possible candidates.

We have previously shown for the parent, virulent strain, 395 MS, that hyperimmune rabbit anti-MS IgG promoted phagocytosis *in vivo*, and increased surface charge and hydrophobicity of the otherwise virtually uncharged and hydrophilic bacteria (Magnusson & Johansson, 1977; Stjernström *et al.*, 1977). In contrast, the deep-rough mutant 395 MR10 (Rd chemotype) is phagocytosis-sensitive (Stendahl *et al.*, 1973), negatively charged and liable to hydrophobic interaction (Magnusson & Johansson, 1977). However, phagocytosis, as well as charge and hydrophobicity was decreased by sensitization with human colostral secretory IgA (Magnusson *et al.*, 1979), which is also confirmed in the present investigation (Tables 1, 2 and 3). This study was, however, done to elucidate effects on physicochemical surface properties and phagocytosis of reacting MR10 bacteria with IgG and then with SIgA or *vice versa*. It should again be stressed that IgG and SIgA were from different sources but that they both had affinity for the MR10 bacteria. The results show that anti-MR10 IgG enhanced phagocytosis (Table 3) and promoted hydrophobic interaction as measured with HIC on Octyl-Sepharose (Table 1). When assayed with aqueous TPP, IgG achieved a slight decrease in the exposure of negatively charged groups (smaller effect of positively charged TMA-PEG), as well as in the surface hydrophobicity (less effect of hydrophobic P-PEG; Table 2). The discrepancy between HIC and TPP with P-PEG was probably due to different capacity of the two probes to interact with hydrophobic but chemically distinct

surface structures on the bacteria. If the hydrophobic entities on the IgG-coated cells are very near each other, the bulky comparatively hydrophilic PEG portion could interfere with the binding of another P-PEG molecule to the most adjacent site, whereas in HIC the primary restriction to binding is the number of Octyl groups per sugar molecule. A significant observation has been made by Brandtzaeg & Tolo (1977) who demonstrated that anti-albumin IgG in serum blocks the mucosal penetration of albumin, but concomitantly enhances the passage of a second tracer, transferrin. These results argue in favour of a pathogenic potential of local IgG mediated inflammatory reactions (Auer, 1920; Brandtzaeg & Tolo, 1977).

To conclude, we have found two protective properties of SIgA at mucosal membranes. Firstly, it increased the contact with the mucus belt, which can be viewed as an adaptation to the surrounding conditions (Heremans, 1974; Williams & Chir, 1978), (Fig 1a and c). Thus mucus affinity helps the purpose of immune exclusion at mucosal membranes (Stokes, Soothill & Turner, 1975).

Secondly, SIgA modulated the IgG-mediated phagocytosis, as well as general surface properties of the MR10 bacteria (Table 1, 2 and 3), thereby possibly depressing local inflammatory reactions.

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