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Reduced Phase Switch Capacity and Functional Adhesin Expression of Type 1-Fimbriated *Escherichia coli* from Immunoglobulin A-Deficient Individuals[▽]

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The mannose-specific adhesin of type 1 fimbriae is the most common adhesin in *Escherichia coli*. One receptor for this adhesin is the carbohydrate chains of secretory immunoglobulin A (S-IgA), and intestinal *E. coli* from IgA-deficient individuals has a reduced capacity to adhere to mannose-containing receptors. Here, we investigated the expression of the mannose-specific adhesin and its capacity to switch to the fimbriated phenotype in colonic resident and transient *E. coli* strains isolated from control ($n = 16$) and IgA-deficient ($n = 17$) persons. Resident *E. coli* strains from IgA-deficient individuals displayed weaker mannose-specific adherence to colonic cells than resident strains from control individuals (21 versus 44 bacteria/cell, $P = 0.0009$) due to three mechanisms: a lower carriage rate of the *fimH* gene (90% versus 97%, not significant), more frequent failure to switch on the *fim* genes (30% versus 6%, $P = 0.02$), and the reduced adhesive potential of *fimH*$^+$ isolates capable of phase switch (26 versus 46 bacteria/cell, $P = 0.02$). On the other hand, resident strains from IgA-deficient individuals displayed stronger mannose-resistant adherence than resident strains from control individuals ($P = 0.04$) and transient strains from IgA-deficient individuals ($P = 0.01$). The presence of S-IgA appears to favor the establishment of *E. coli* clones which readily express mannose-specific adhesins in the bowel microbiota.

Type 1 fimbriae are the most common adherence structures in *Escherichia coli* and are also found in other enterobacterial species (9, 11). Type 1 fimbriae carry adhesins that recognize terminal mannose residues in the Manα1-3(Man1-6)Manβ conformation (10). This trisaccharide is exposed on many glycoproteins, and type 1 fimbriae mediate adherence to, e.g., human small and large intestinal (3) and urinary tract (30) epithelial cells. The adherence is abolished in the presence of mannose and hence is termed mannose sensitive (MS). The role of the *E. coli* MS adhesin in virulence has been debated, but it may play a role in urinary tract infection (7, 18, 28). Other *E. coli* adhesins, including those associated with P and S fimbriae, confer mannose-resistant (MR) adherence to uroepithelial and colonic epithelial cells (3, 30, 45). MR adhesins are well-known virulence factors in urinary tract infection, septicemia, and meningitis (23, 27, 37). Furthermore, P fimbriae seem to facilitate colonization of the human bowel. Thus, strains that persist in the human intestinal microbiota (so-called resident strains) are more often P fimbriated and display MR adherence to colonic epithelial cells than strains that appear only transiently in the microbiota (4, 32, 33, 35, 43).

Bacteria can switch between a fimbriated and a nonfimbriated state, a process termed phase variation (13). Phase variation of type 1 fimbriae is mediated by a 314-bp invertible DNA element (*fim* switch) which contains the promoter for *fimA* (2) and whose position is regulated by two site-specific recombinases, FimB and FimE (26). Several environmental factors influence phase switch of type 1 fimbriae, including temperature and osmolarity (16, 36, 39). To maximize type 1 fimbriation, *E. coli* strains are usually cultured in static broth (7), in which case the hydrophobic fimbriae allow the bacteria to form a pellicle on the liquid-air interface and get full access to atmospheric oxygen. With successive passages in static broth, the proportion of fimbriated bacteria therefore increases (9, 36).

The normal niche for *E. coli* is the bowel microbiota of humans and animals (8). The gut contents are a rich source of secretory immunoglobulin A (S-IgA), which is produced at a rate of 2 to 5 g per day in an adult human being (1). S-IgA is heavily glycosylated, and many of its carbohydrate chains terminate with mannose and act as receptors for the MS adhesin of type 1-fimbriated *E. coli* (44). Thus, independent of the specificity of the S-IgA, type 1-fimbriated *E. coli* will interact with S-IgA antibodies through a lectin-carbohydrate interaction (44). Our previous findings indicate that the lectin-carbohydrate interaction is the main mechanism for the agglutinating activity of S-IgA against type 1-fimbriated *E. coli* in vitro (44).

About 1 individual in 600 lacks IgA in both serum and secretions but has normal levels of the other immunoglobulin isotypes (19). Approximately one-third of IgA-deficient individuals suffer from recurrent respiratory tract infections (5), but most are healthy and their IgA deficiencies are discovered accidentally, e.g., at blood donor screening. We have previously shown that *E. coli* isolated from IgA-deficient individuals displays reduced mannose-specific adherence to colonic epithelial cells in comparison with *E. coli* from age-matched controls (14). Two factors contributed to this effect. First, *E. coli*

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TABLE 1. Primers used for detection of *E. coli* virulence factor genes and the phase switch position

Virulence factor	Gene	Primer sequence (5′–3′)	Primer designation[a]	Size of PCR product (bp)	Reference
fimH adhesin	*fimH*	TGCAGAACGGATAAGCCGTGG	FimHf	506	25
		GCAGTCACCTGCCCTCCGGTA	FimHr		
P fimbriae	*papC*	GACGGCTGTACTGCAGGGTGTGGCG	pap 1	328	29
		ATATCCTTTCTGCAGGGATGCAATA	pap 2		
papG adhesin					
Class I	*papG*	TCGTGCTCAGGTCCGGAATTT	j96-193f	461	24
		TGGCATCCCCCAACATTATCG	j96-653r		
Class II	*papG*	GGGATGAGCGGGCCTTTGAT	ia2-383f	196	24
		CGGGCCCCCAAGTAACTCG	ia2-572r		
Class III	*papG*	GGCCTGCAATGGATTTACCTGG	prs-198f	258	24
		CCACCAAATGACCATGCCAGAC	prs-455r		
S fimbriae	*sfaD*	CTCCGGAGAACTGGGTGCATCTTAC	sfa 1	410	29
	sfaE	CGGAGGAGTAATTACAAACCTGGCA	sfa 2		
Dr hemagglutinin	*draA*	GCCAACTGACGGACGCAGCAC	Dr-321f	229	32
		CCCCAGCTCCCGACATCGTTTTT	Dr-550r		
fim switch region	Invertible DNA element	GAGAAGAGGTTTGATTTAACTTATTG	A	559	40
		AGAGCCGCTGTAGAACTGAGG	B		

[a] f, forward; r, reverse.

from IgA-deficient individuals carried the *fim* operon less often than did *E. coli* from control individuals. Second, *fimH*$^+$ *E. coli* from IgA-deficient individuals displayed reduced mannose-specific adherence in comparison with *fimH*$^+$ *E. coli* from control individuals (14).

In the present study we decided to further explore the differences in MS adhesin expression between IgA-deficient and control individuals. One aim was to investigate whether differences between *E. coli* strains obtained from IgA-deficient and control individuals were foremost evident among resident or transient strains. The second aim was to explore whether differences in capacity to switch to a fimbriated phenotype could underlie the difference in MS adherence capacity between *E. coli* strains from IgA-deficient and control individuals. For this purpose, the rectal microbiota of IgA-deficient and control individuals was sampled monthly over a period of 6 months. *E. coli* strains were isolated and characterized as resident (i.e., present in consecutive samples) or transient (i.e., present only on a single sampling occasion) in the microbiota. Resident and transient strains from IgA-deficient and control individuals were then compared with respect to possession of *fimH* and other adhesin genes, capacity to adhere via MS or MR mechanisms to the colonic cell line HT-29, and capacity to switch the type 1-fimbrial gene promoter to the "on" position.

MATERIALS AND METHODS

Subjects. Seventeen individuals (nine males, eight females) with selective IgA deficiency were included in the study (median age, 43 years; range, 18 to 68 years). IgA deficiency was defined as a serum IgA level of <0.05 g/liter in the presence of IgM at a level higher than 0.5 g/liter and IgG at a level higher than 7 g/liter, the lower limits of the normal ranges. They also had the following normal levels of the IgG subclasses (in grams/liter): IgG1, >4.22; IgG2, >1.17; IgG3, >0.41; and IgG4, >0.01 (19, 38).

Sixteen individuals (seven males, nine females) with normal levels of serum immunoglobulins served as a control group (median age, 46 years; range, 28 to 73 years). None of the individuals included had consumed antibiotics during the 3 months preceding the study. The study was approved by the Medical Ethics Committee of Göteborg University, Göteborg, Sweden.

Sampling of rectal microbiota and species identification. Rectal swabs were obtained monthly over a period of 6 months. The swabs were transported in Stuart's transportation medium to the laboratory, where they were streaked in a three-step manner on Drigalski agar, a medium selective for *Enterobacteriaceae* (41). After aerobic culture overnight at 37°C, the last three free-lying colonies were picked, which gives a 97% probability of including the dominant *E. coli* strain (31). After subculture on Drigalski agar for purity, the isolates were identified to the species level using API 20E (API Systems SA, La Balme Les Grottes, Montalieu-Vercieu, France), and those identified as *E. coli* were selected for study.

Strain typing by RAPD. *E. coli* isolates were typed to the strain level by random amplified polymorphic DNA (RAPD) (33, 34). In brief, a small amount of bacteria from an overnight culture was mixed with 6.0 μM of the primer GTGATCGCAG and 25 μl HotStarTaq master mix (QIAGEN, Spånga, Sweden). The PCR started with a 15-min incubation step at 95°C to activate the polymerase and continued with the following temperature profile: 94°C for 45 s, 30°C for 120 s, and 72°C for 60 s for four cycles; followed by 94°C for 5 s, 36°C for 30 s, and 72°C for 30 s for 26 cycles; with the extension step being increased by 1 s for every new cycle. The reaction was terminated at 72°C for 10 min and cooled to 4°C. The PCR products were separated on 8% ready-made Tris-glycine gels and visualized by silver staining (34).

All *E. coli* isolates from one individual were assayed together, and their PCR products were, when possible, separated on the same gel. Two isolates with identical profiles from the same individual were considered to belong to the same strain. Isolates were not compared between individuals.

Multiplex PCR for identification of adhesin genes in *E. coli* strains. The carriage of the *fimH* gene (the MS adhesin of type 1 fimbriae) was analyzed by PCR using previously published primers (25) (Table 1). In addition, each strain was characterized by multiplex PCR with respect to carriage of the following virulence genes: *papC* (P fimbriae); the class I, II, and III varieties of the P-fimbrial adhesin gene *papG* (recognizing subtle differences in receptor conformation); *sfaD* and *sfaE* (S fimbriae, F1C fimbriae); and *draA* (Dr hemagglutinin) (32). The primers used are listed in Table 1. Bacteria from colonies grown on tryptic soy agar (TSA) were added to a mixture containing HotStarTaq master mix (QIAGEN) and 0.45 μM of each primer pair in a final volume of 50 μl. The PCR program was started with an initial heat activation step for the *Taq* polymerase (95°C for 15 min). Thereafter, the PCR was run as described previously (32, 33). PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Determination of the *E. coli fim* switch position (on/off). The orientation of the invertible *fim* switch element was determined by PCR amplification followed by restriction cleavage. When the element is in the "on" position, the promoter is active and the fimbrial genes are transcribed, leading to formation of functional type 1 fimbriae with mannose-specific adhesins. When the element is in the "off" position, the fimbrial genes are not transcribed and the bacteria are nonfimbriated. A culture of a bacterial strain generally contains a mixture of fimbriated and nonfimbriated cells.

To determine the position of the promoter in *fimH*$^+$ strains, a 559-bp fragment containing the *fimA* promoter was generated by PCR and cleaved by endonuclease enzyme HinfI (40). Since the position of the cutting site depends on the

position of the invertible *fim* switch element, bacteria whose *fim* promoter is in the "on" position give rise to different cleavage fragments than bacteria whose promoter is in the "off" position.

E. coli strains were grown aerobically on TSA at 37°C overnight and thereafter passaged three times in Luria broth. Bacteria were harvested by centrifugation at 1,500 rpm for 20 min at 4°C, washed in phosphate-buffered saline (PBS) at room temperature, and thereafter centrifuged at 1,500 rpm for 4 min at 4°C. The bacterial pellet was suspended in 500 μl PBS and incubated for 12 min at 95°C to release bacterial DNA. The suspension was centrifuged at 13,400 rpm for 5 min, and the supernatant was frozen at −20°C until used as a template for PCR.

A previously described PCR assay (40) was used with some modifications. The primers used are shown in Table 1. The PCR mixture was prepared in a total volume of 50 μl consisting of 25 μl HotStarTaq master mix (QIAGEN), a 0.2 μM concentration of each primer, and distilled water. The PCR program started with 15 min at 95°C for activation of *Taq* polymerase, followed by 30 cycles of 94°C for 1 min (denaturation), 61°C for 70 s (annealing), and 72°C for 70 s (extension). The program terminated with a 3-min final extension at 72°C and cooling to 4°C.

The PCR products were digested by HinfI (New England Biolabs, Hitchin, Hertfordshire, United Kingdom) according to the manufacturer's instructions, and the digested PCR products were separated electrophoretically on an agarose gel (32).

Adherence to the colonic cell line HT-29. Adherence to the colonic cell line HT-29 was tested after culture of bacteria in static Luria broth to promote type 1-fimbrial expression (9, 36). Preliminary experiments were performed to determine the number of passages in static Luria broth required for expression of type 1-fimbrial MS adhesins in $fimH^+$ isolates. Six different $fimH^+$ strains were cultured in duplicate on TSA plates overnight and thereafter passaged up to 10 times in static Luria broth. Adherence was assessed after growth on TSA and after 1, 3, 5, and 10 passages in static Luria broth. Three passages were selected as the standard procedure. Thus, all isolates of *E. coli* were passaged three times in static Luria broth to select for bacteria expressing the MS adhesin and thereafter assessed for adherence to the human colonic cell line HT-29 in the absence and presence of mannose (14, 45). Briefly, a mixture of 5×10^8 bacteria, 5×10^5 HT-29 cells, and Hanks' balanced salt solution with or without 1% (final concentration) of *methyl*-α-D-mannoside was incubated for 30 min at 4°C with end-over-end rotation. The cells were washed and fixed with formalin, and at least 40 epithelial cells were examined by interference contrast microscopy (×500 magnification) (Nikon Optiphot; Bergström Instruments AB, Göteborg, Sweden). The number of bacteria adhering to each cell was counted, and the average number of adherent bacteria per cell was calculated. In each experiment, all isolates from one IgA-deficient and one control individual were assayed, and the person examining adherence was blinded as to their identity. The MS adherence was calculated by subtracting the mean number of bacteria per cell adhering in the presence of *methyl*-α-D-mannoside (MR adherence) from the mean number of bacteria per cell adhering in the absence of *methyl*-α-D-mannoside (total adherence). The transformant *E. coli* strains 506 MS (type 1 fimbriated) and 506 P (adhesin negative) (17, 22) were included as controls in each adhesion experiment. The control strains were cultivated on TSA plates supplemented with 20 μg/ml chloramphenicol.

Statistical methods. Proportions were compared using Fisher's exact test. Adherence data were compared using the Mann-Whitney U test.

RESULTS

Identification of resident and transient *E. coli* strains. Figure 1 shows the RAPD profiles of the *E. coli* isolates obtained from an individual who carried four different *E. coli* strains over the 6-month study period. Strain A appeared in the first sample only, whereas strains B, C, and D were each present on two consecutive sampling occasions. Strains found in an individual on at least two sampling occasions were defined as resident. Strains isolated on a single sampling occasion were defined as transient, provided that this was not the first or last sampling occasion. Thus, strains B, C, and D were all defined as resident, while strain A could not be classified as either resident or transient. RAPD patterns were not compared between strains from different individuals.

Persons with normal IgA levels carried an average of 4.3 different *E. coli* strains during the 6-month period, while the corresponding figure for IgA-deficient individuals was 3.8 strains ($P = 0.27$). Of the 68 strains isolated from the 16 control individuals, 36 were defined as resident and 18 as transient, while 14 could not be classified. Of the 64 strains isolated from the 17 IgA-deficient individuals, 31 were resident and 17 were transient, while 16 were unclassified.

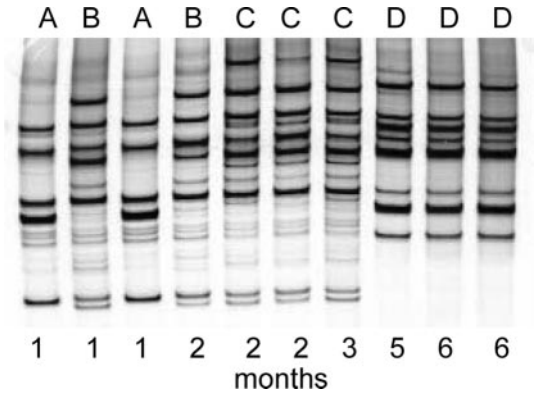

FIG. 1. RAPD patterns of 10 intestinal *E. coli* isolates obtained from a single individual over a period of 6 months. The subject carried four different strains during this period. Strain A appeared in the first sample only and was classified as neither resident nor transient, while strains B, C, and D were present on two sampling occasions and thus defined as resident strains. No *E. coli* was found in the sample obtained at 4 months.

Adhesin gene carriage. The frequencies of adhesin genes were compared between resident and transient *E. coli* strains from control and IgA-deficient individuals (Table 2). The MS adhesin gene was somewhat more common among resident than transient strains in both controls and IgA-deficient individuals, but the differences were not significant. In IgA-deficient individuals, genes encoding P and S fimbriae tended to be more common in resident than in transient strains (Table 2).

Mannose-sensitive adherence to HT-29 cells. Type 1-fimbrial expression is regulated by phase variation, and culture in static Luria broth enhances expression of MS adhesins (9). To determine the number of passages that were needed to drive $fimH^+$ strains into expression of MS adhesins, six $fimH^+$ strains were cultured on TSA, passaged 1, 3, 5, and 10 times in static Luria broth, and thereafter tested for adherence to HT-29 cells. As seen in Fig. 2, only one out of six strains showed any MS adherence after culture on TSA. MS adherence increased during the first three passages in four of the strains. Three of these strains continued to increase their expression of MS adhesins for up to 5 passages, and one strain for even up to 10 passages. Two strains failed to express any MS adhesins, even after 10 passages. Three passages were selected as a reasonable compromise between promoting adhesin expression and being practically feasible. Patterns of attachment of *E. coli* strains to the HT-29 cells are shown in Fig. 3.

All *E. coli* isolates from IgA-deficient and control individuals were passaged three times in static Luria broth and thereafter tested for adherence to HT-29 colonic epithelial cells in the presence and absence of *methyl*-α-D-mannoside, which permitted subdivision of the isolates into those with MS or MR adherence. For each resident strain, the average MS and MR adherence of all isolates of that strain was calculated. Transient strains contributed a single isolate, or in some cases more

TABLE 2. Prevalence of adhesin genes in resident and transient intestinal *E. coli* strains from control and IgA-deficient individuals[a]

Gene (virulence factor)	% of strains carrying gene in control individuals		*P* value	% of strains carrying gene in IgA-deficient individuals		*P* value
	Resident (n = 36)	Transient (n = 18)		Resident (n = 31)	Transient (n = 17)	
fimH (MS adhesin)	97	89	0.25	90	82	0.65
papC (P fimbriae)	33	28	0.80	29	6	0.07
sfaD or *sfaE* (S, F1C fimbriae)	14	22	0.50	26	6	0.13

[a] The carriage rate of genes encoding different adhesins was assessed by multiplex PCR in resident and transient *E. coli* strains obtained from 16 control and 17 IgA-deficient individuals. None of the examined strains carried the Dr hemagglutinin gene *draA*. Proportions were compared between resident and transient strains using Fisher's exact test.

than one isolate from a single sampling occasion. In the latter case, their MS and MR adherence was averaged. The average MS adherence levels of resident and transient strains from control and IgA-deficient individuals are shown in Fig. 4.

As shown in the figure, the average MS adherence of resident strains from control individuals was twice as high as the MS adherence of resident strains from IgA-deficient individuals (P = 0.0009). The MS adherence of transient strains was also higher in strains from controls than in those from IgA-deficient individuals, but the difference was not significant (P = 0.24). In both control and IgA-deficient individuals, resident strains displayed higher MS adherence than transient strains, but the differences were not significant (P = 0.19 and P = 0.10, respectively). When strains from IgA-deficient and control individuals were analyzed together, resident strains displayed significantly higher MS adherence than transient strains (P = 0.03).

Resident strains may display increased MS adherence for either of two reasons. First, strains with an inherent tendency to express MS adhesins may be superior colonizers. Second, the capacity to adhere might increase progressively during persistence in the microbiota due to upregulation of fimbrial expression. In the latter case, resident strains would express more MS adhesins because they have, on average, spent longer time in the microbiota than transient strains. To examine these two possibilities, we calculated the MS adherence of the first and last isolates of resident strains carrying the *fimH* gene.

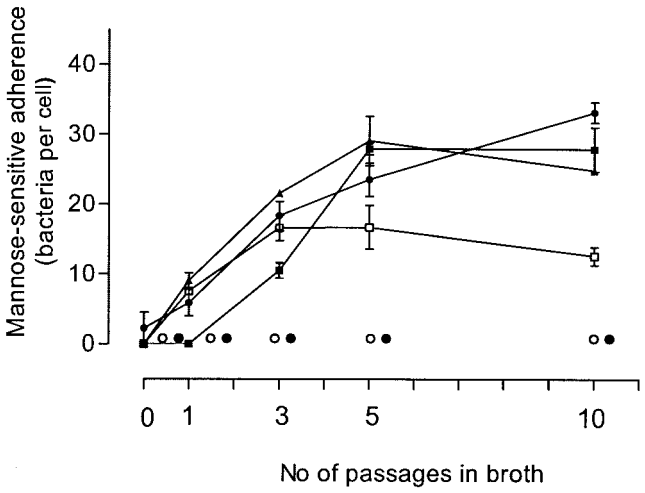

FIG. 2. Mannose-sensitive adherence to HT-29 cells of six *fimH*-positive *E. coli* strains after 0 (agar-grown bacteria), 1, 3, 5, and 10 serial passages in static Luria broth. For each strain, the mean value and the standard deviation (shown by I bars) for two parallel experiments are presented. Each symbol represents a certain *E. coli* strain.

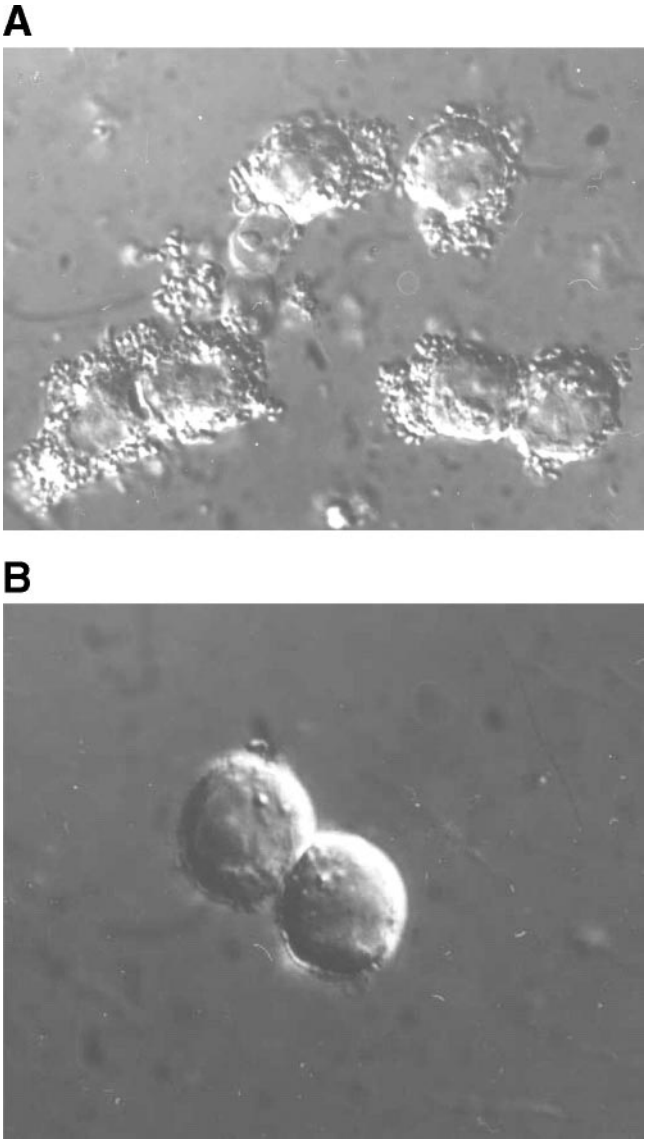

FIG. 3. Attachment of *E. coli* strains to cells of the HT-29 cell line. (A) A type 1-fimbriated *E. coli* strain. (B) An adhesin-negative control strain.

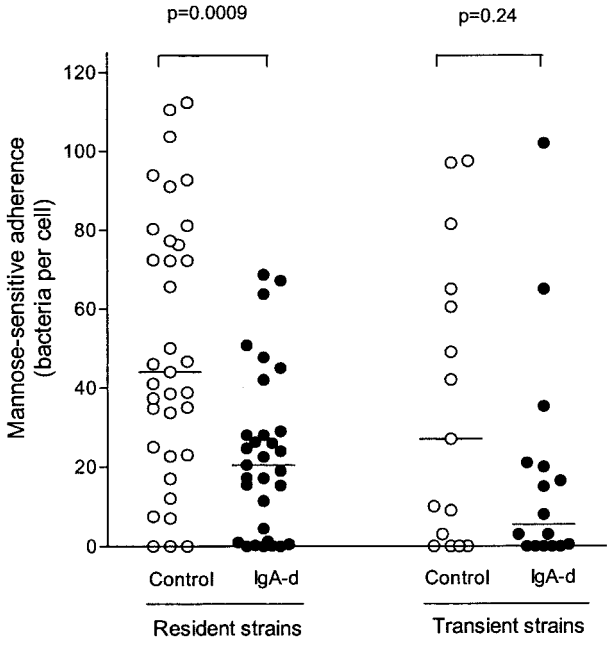

FIG. 4. Mannose-sensitive adherence to HT-29 cells of 50 *E. coli* strains (35 resident and 15 transient) obtained from 16 healthy controls and 47 strains (31 resident and 16 transient) obtained from 17 IgA-deficient (IgA-d) individuals. Each circle represents the mean adherence value for all isolates belonging to one strain. The median values are represented by horizontal bars, and the groups were compared using the Mann-Whitney U test. Data are missing for one resident and three transient strains from control individuals and for one transient strain from an IgA-deficient individual.

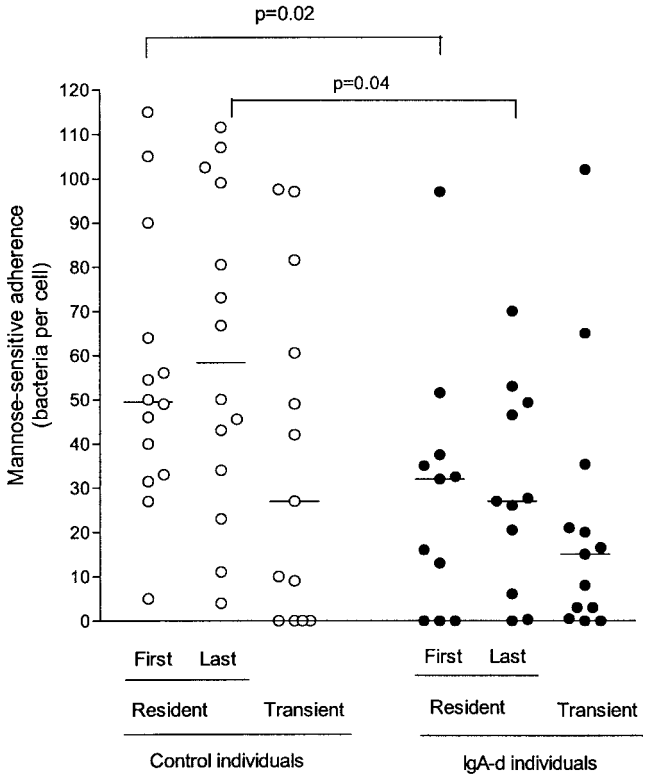

FIG. 5. Mannose-sensitive adherence to HT-29 cells of *fimH*-positive *E. coli* strains (28 resident, 13 transient) isolated from 16 healthy controls and 35 strains (22 resident, 13 transient) from 22 IgA-deficient (IgA-d) individuals. The adherence of the first and last isolate of resident strains and of each transient strain is represented by a circle. The median adherence for each group of strains is indicated by a horizontal bar, and the groups were compared using the Mann-Whitney U test.

fimH$^+$ strains which were already present on the first sampling occasion were excluded, as their time of persistence in the microbiota was unknown. The result of this analysis is shown in Fig. 5. As is evident from the figure, *fim*$^+$ strains resident in the microbiota of control individuals already had high MS adherence when they were first isolated, and their MS adherence increased only marginally over time ($P = 0.30$). Conversely, *fimH*$^+$ strains resident in IgA-deficient individuals expressed lower MS adherence than the corresponding strains from control individuals at the outset ($P = 0.02$), and the difference remained ($P = 0.04$ for the last isolate) (Fig. 5). For transient *fimH*$^+$ strains, the difference in MS adherence between those isolated from IgA-deficient and those isolated from control individuals was small (Fig. 5) and not significant ($P = 0.60$). We concluded that strains capable of long-term persistence in individuals with S-IgA in their secretions had an inherently strong capacity to express MS adhesins.

Orientation of the *fim* switch element in *E. coli* strains from IgA-deficient and control individuals. The weak capacity for MS adherence of *fimH*$^+$ strains from IgA-deficient individuals could be due to a decreased capacity of the strains to switch to the fimbriated phase, a reduced binding capacity of the adhesins, or both. To test this, we examined the capacity of strains from IgA-deficient and control individuals to switch to the fimbriated phase after three passages in static broth. Bacterial DNA was obtained from the culture, and the position of the invertible element containing the *fim* promoter was examined by PCR followed by restriction cleavage. Figure 6 shows an example of four strains analyzed by this method. Different restriction fragments are obtained from the *fimH* promoter region depending on the position of the *fim* switch element. Strains A and B show the presence of the phase switch element in the "off" position as evidenced by the 200- and 359-bp fragments. In contrast, bacterial cultures from strains C and D display in both the "on" and "off" positions.

After three passages in static broth, bacterial cultures of *fimH*$^+$ strains contained either cells that all had the promoter in the "off" position or a mixture of cells with the promoter in the "on" and "off" positions. If all cells were in the "off" position, we considered that the strain had a block in the capacity to switch to the fimbriated phase. Nine percent of the 53 *fimH*$^+$ strains from control individuals and 27% of the 49 *fimH*$^+$ strains from IgA-deficient individuals examined appeared to be locked in the "off" position ($P = 0.04$). Table 3 shows the proportions of resident and transient *fimH*$^+$ strains from control and IgA-deficient individuals that revealed a phase switch block. A fairly large proportion of both resident and transient strains from IgA-deficient strains appeared incapable of switching to the fimbriated phase. When resident strains from control and IgA-deficient individuals were compared, the strains from the

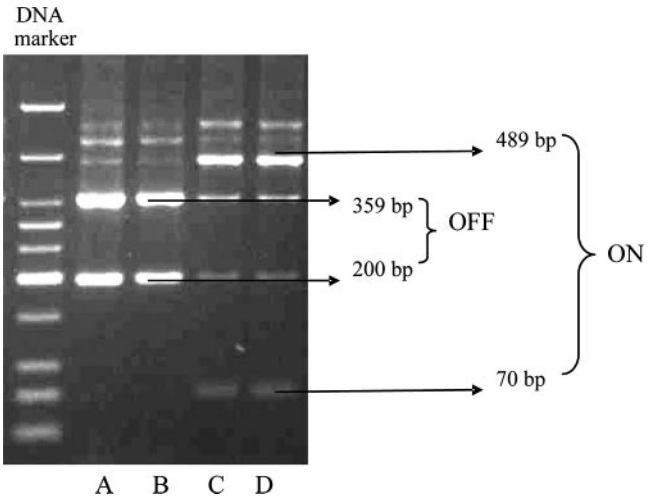

FIG. 6. Detection of the phase switch orientation of *fimH*$^+$ *E. coli* strains using PCR followed by restriction enzyme cleavage. The left lane contains DNA molecular size markers. The cleaved PCR product of strains A and B reveal fragments of 359 and 200 bp, generated from bacterial cultures only in the phase switch "off" position. Strains C and D show cleavage products representing bacterial cultures in both the "on" and "off" switch positions. The PCR products were digested by restriction enzyme HinfI, separated by agarose gel electrophoresis, and stained with ethidium bromide.

IgA-deficient individuals had a phase switch block significantly more often (Table 3).

We next examined whether *fimH*$^+$ strains that were able to switch on their fimbrial production differed in MS adherence depending on whether they were isolated from IgA-deficient or control individuals. Figure 6 shows the MS adherence of strains that displayed the *fim* promoter in both the "on" and "off" positions after three passages in static broth, indicating that they could produce fimbriae with MS adhesins. As shown in the figure, the difference in MS adherence between resident and transient strains from control individuals was now gone. Thus, strains that were able to switch their *fimH* promoter to the "on" position displayed equally strong adherence levels whether they had been resident or transient in the microbiota.

TABLE 3. Proportion of *fimH*$^+$ *E. coli* strains displaying the switch element only in the OFF position after three passages in static broth[c]

Strain source	Relative frequency of switch-blocked strains (%)	
	Resident	Transient
Control individuals	6	15[a]
IgA-deficient individuals	30[b]	25

[a] $P = 0.60$, in comparison with resident strains from control individuals.
[b] $P = 0.03$, in comparison with resident strains from control individuals.
[c] The position of the *fim* switch element was examined by PCR and restriction cleavage (40) in 44 strains from control individuals (31 resident and 13 transient) and 35 strains from Ig-A deficient individuals (23 resident and 12 transient). Before the position of the switch element was assessed, all strains were passaged three times in static broth in order to induce switch to the ON position. The table shows the proportion of strains in which the bacterial mixture yielded only a PCR product representing the promoter in the OFF position, which was interpreted as a phase switch block. Proportions were compared using Fisher's exact test.

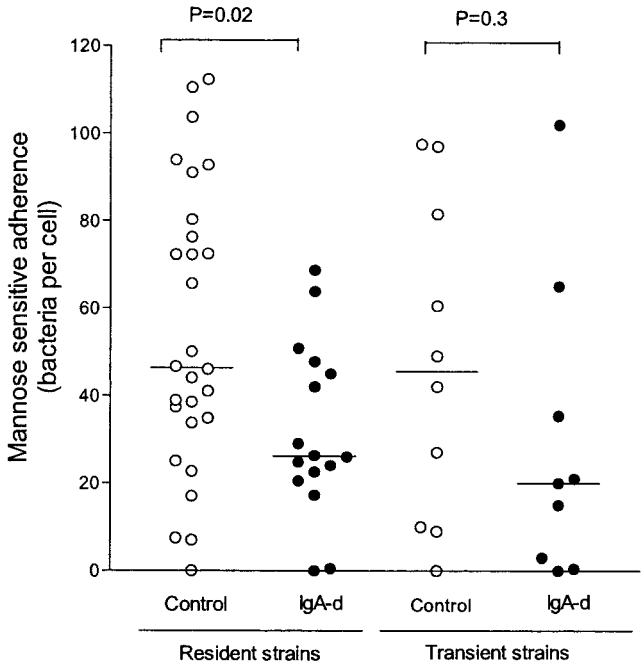

FIG. 7. Mannose-sensitive adherence to colonic HT-29 cells of *fimH*$^+$ strains capable of switching their promoter to the "on" phase switch position. Thirty-eight *E. coli* strains (28 resident, 10 transient) obtained from 13 control individuals and 25 strains (16 resident, 9 transient) from 14 IgA-deficient (IgA-d) individuals were included in the analysis. Each circle represents the mean value for all *E. coli* isolates belonging to one strain, and the horizontal bar represents the median. Adherence levels were compared between groups using the Mann-Whitney U test.

However, the difference between strains from IgA-deficient and control individuals remained (Fig. 7). Thus, *fimH*$^+$ resident strains from IgA-deficient individuals that could switch on their fimbrial expression displayed reduced MS adherence to HT-29 cells in comparison with corresponding strains from healthy controls ($P = 0.02$) (Fig. 7).

MR adherence to HT-29 cells. The MR adherence of resident and transient *E. coli* strains from control and IgA-deficient individuals is shown in Fig. 8. Resident strains from IgA-deficient individuals on average displayed higher MR adherence than resident strains from control individuals ($P = 0.04$). In IgA-deficient individuals, resident strains displayed higher MR adherence than did transient strains ($P = 0.01$). This was not seen in strains from control individuals. The MR adherence levels did not differ between the first and last isolates of resident strains retrieved from either control or IgA-deficient individuals (data not shown).

DISCUSSION

We have earlier shown that *E. coli* isolated from IgA-deficient individuals has a reduced capacity to adhere via mannose-specific mechanisms to colonic epithelial cells in comparison with *E. coli* from individuals with normal IgA levels in serum and secretions (14). Two factors in IgA-deficient individuals contributed to this finding: a lower prevalence of *E. coli* carrying the genes for type 1 fimbriae and the MS adhesin and

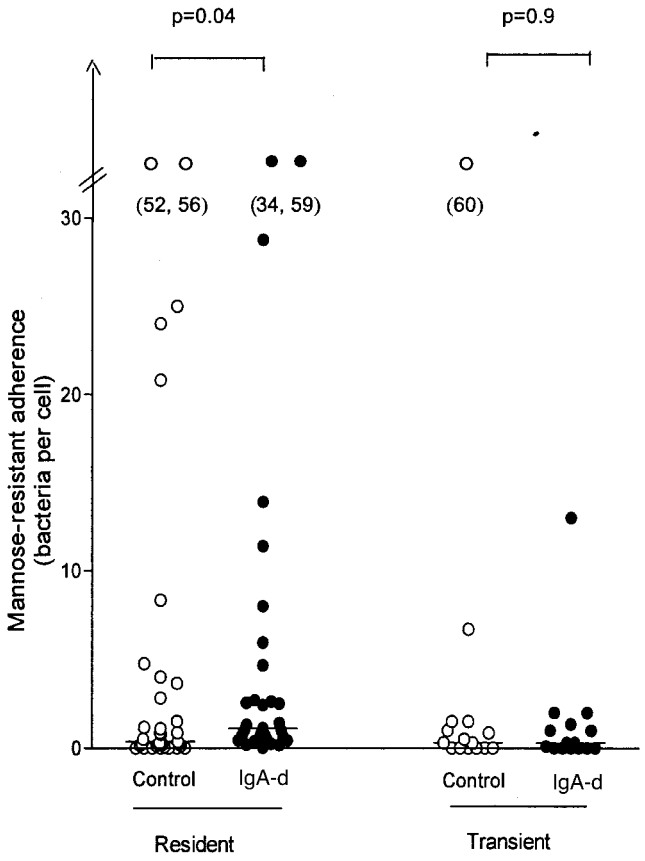

FIG. 8. Mannose-resistant adherence to HT-29 cells of 50 strains (35 resident, 15 transient) obtained from 16 healthy controls and 47 strains (31 resident, 16 transient) obtained from 17 IgA-deficient (IgA-d) individuals. Adherence was assessed after three passages in static broth culture. Each circle represents the mean value for all *E. coli* isolates belonging to one strain. The median values are given in parentheses for each group of strains. The mannose-resistant adherence levels were compared using the Mann-Whitney U test.

a reduced MS adherence to colonic cells by strains that had the genes for this adhesin (14).

Here, we confirm and extend these findings in a longitudinal study. We isolated *E. coli* from rectal swabs obtained monthly from IgA-deficient and control individuals. Strains found on more than one occasion in an individual were defined as resident, while strains found only in a single sample were defined as transient. We found that the differences in MS adherence between *E. coli* strains from IgA-deficient and control individuals were most marked and highly significant when resident strains were examined. Such strains are likely to be best adapted to the human colonic milieu. This reinforces the findings of our previous cross-sectional study (14) and suggests that a superior capacity to adhere to mannose-containing receptors is beneficial for long-term persistence in the colon, especially when S-IgA is present in the secretions. Since the capacity for strong adherence was evident in the first isolate of resident strains, strains with a superior capacity to produce mannose-binding adhesins may be positively selected for persistence. Strains that have a less prominent adherence capacity may not be able to establish residence in the microbiota and may disappear in a short time.

The substantially reduced adherence to mannose-containing receptors on human colonic cells of the HT-29 cell line of *E. coli* from IgA-deficient versus age-matched control individuals derived from a combination of three factors. First, there was a slight and nonsignificant reduction in the proportion of strains in IgA-deficient individuals that carried the *fimH*$^+$ gene cluster, which was also observed in our previous study, where this difference was significant (14). Second, in IgA-deficient individuals, the proportion of strains carrying the *fimH* gene cluster in their genome that appeared to be incapable of switching to the fimbriated phase was larger than that of *fimH*$^+$ strains from control individuals. The *fim* switch experiments were performed after three passages of the bacteria in Luria broth which, according to our adherence experiments, was sufficient to induce expression of type 1 fimbriae in strains capable of expressing these adhesins. The third factor was the reduced MS adherence of *fimH*$^+$ switch-capable strains from IgA-deficient individuals in comparison with corresponding strains from control individuals.

A limitation of this study was that we could not quantify the proportion of the bacteria that had switched to the fimbriated phase. Thus, we cannot exclude the possibility that there was a quantitative difference in the proportions of bacterial cells that had their promoter switched to the "on" position between switch-capable strains from IgA-deficient and control individuals. Using RT-PCR, it should be possible to quantify whether the proportion of bacterial cells that are in the "on" and "off" position differs between isolates retrieved from IgA-deficient and control individuals. Another possibility would be that the *fimH* adhesins of *E. coli* colonizing IgA-deficient individuals have reduced binding capacity to colonic receptors. Slight changes in adhesin conformation, conferring a broader receptor specificity for the MS adhesin, have been demonstrated among *E. coli* strains isolated from urinary tract infections, suggested to be due to "pathoadaptive mutations" (21). In a future study, we will attempt to examine whether *fimH* adhesin genes in *E. coli* from IgA-deficient and control individuals differ in sequence.

The reason *E. coli* with poor MS adherence can persist better in IgA-deficient than in control individuals can only be speculated upon. The MS adhesin allows the bacteria to adhere to colonic epithelial cells. S-IgA acts as a competing receptor blocking this adherence. Competition from S-IgA may force bacteria to produce more MS adhesins in order to adhere to mucosal receptors, while a moderate expression of the MS adhesin might be sufficient to attach to colonic cells in IgA-deficient individuals. Another possibility is that the interaction between MS adhesins and mannose residues on the carbohydrate chains of S-IgA is actually beneficial for the bacteria and that the bacteria which obtain a coat of S-IgA have an advantage over other strains. Coating of bacteria by IgA reduces their surface hydrophobicity (12), and other factors rendering the bacteria more hydrophilic, such as capsule and smooth O antigen, enhance the colonizing capacity of *E. coli* in rodent models (20, 42). It is also possible that the weak interaction between mucin molecules and S-IgA covering the bacteria would position the bacterium in a favorable niche in the mucus layer (6).

We noted that resident strains from IgA-deficient individuals displayed significantly higher mannose-resistant adherence

than both resident strains from control individuals and transient strains from IgA-deficient individuals. We have previously reported that *E. coli* from IgA-deficient individuals more often carries genes for both S fimbriae and hemolysin than *E. coli* from control individuals (15).

In summary, our results indicate that the presence of S-IgA in colonic secretions modulates the colonic microbiota and determines which subgroups of strains may establish residence in the colon. Indeed, preliminary evidence indicates that the microbiota of IgA-deficient individuals is phylogenetically different from *E. coli* colonizing control individuals (F. Nowrouzian et al., unpublished observation). Taken together, our findings indicate that S-IgA plays a significant role in regulating large intestinal microbial ecology.

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