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Increase in the Population of Duodenal Immunoglobulin A Plasmocytes in Axenic Mice Associated with Different Living or Dead Bacterial Strains of Intestinal Origin

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Various bacterial strains were tested for their ability to stimulate immunoglobulin A (IgA) plasmocytes to populate the duodenal lamina propria in axenic mice. The mice were associated with the strains for at least 4 weeks. The strains inhabiting the conventional mouse intestine and belonging to the genera *Lactobacillus, Streptococcus, Eubacterium, Actinobacillus, Micrococcus, Corynebacterium,* and *Clostridium* (including the extremely oxygen-sensitive ones) are only slightly or nonimmunogenic, whereas the strains belonging to the genera *Bacteroides* and *Escherichia* have an immunogenic effect. The same result was obtained with *Bacteroides* and *Escherichia* strains isolated from the digestive tract of other animal species. The kinetics of appearance of intestinal IgA plasmocytes are similar in axenic mice monoassociated with a stimulatory strain and in conventional mice. The association of two or more strains with axenic mice leads either to the same or a greater number of duodenal IgA plasmocytes as that obtained with the most stimulatory strain monoassociated with axenic mice. The maximum stimulation recorded in all of these trials represents about two-thirds of that observed in conventional mice and was obtained in the duodenum of gnotoxenic mice harboring four bacterial strains isolated from the conventional mouse microflora. The orally administered killed cells of two immunogenic strains, *E. coli* and *Bacteroides* sp., are as immunogenic as the living cells, provided that their concentration in the digestive tract is sufficient.

The lamina propria of conventional mouse intestine is richly endowed with plasmocytes, mainly immunoglobulin A (IgA) plasmocytes (3, 16, 27), proceeding from precursor cells developed in Peyer's patches after antigenic stimulation (9). These precursor cells may be transformed in situ into plasmocytes visible at the edge of Peyer's patches where they are more abundant than elsewhere (8), or they may take the lymphatic, mesenteric, and thoracic routes and reappear in the form of IgA plasmocytes accumulating all along the intestine. This "gut IgA cell cycle" has been demonstrated in mice (15) and rats (20). In axenic mice, the number of IgA plasmocytes is very low (7, 8). Three to 4 weeks after conventionalization of these mice, the intestinal IgA population reaches the size recorded in conventional mice (8). Accordingly, the intestinal microflora represents an important antigenic stimulus leading to accumulation of IgA plasmocytes in the lamina propria of the small intestine. Various authors have investigated the appearance of serum immunoglobulins in axenic animals after inoculation of their digestive tracts with different bacterial strains (2, 4, 5, 19). Other authors have studied the immune serum response obtained in conventional animals after parenteral vaccination with strains isolated from the intestinal microflora (6, 13). However, only few data are available on the in situ relationships between the different bacterial strains constituting the digestive microflora of the mouse and the development of the intestinal IgA immune system (IgA IIS) (21).

The purpose of the present work was to determine whether all strains of the intestinal microflora of conventional mice have the same immunogenic capacity towards the precursor cells present in Peyer's patches. With the aim of verifying whether the hypothesis of Foo and Lee (13) could also be applied to IgA IIS, bacterial strains from the digestive tract of other animal species were associated with axenic mice. These authors assumed that mice are "tolerant" towards bacterial strains belonging to its autochthonous intestinal flora, defined by Dubos et al. (10), whereas this is not true in the case of strains isolated from other animal species. Fur-

thermore, some authors believe that orally administered dead bacteria induce a poor or no intestinal immune response (12, 28). We therefore compared the development of IgA IIS in axenic mice receiving the same bacterial strains, either living or dead.

MATERIALS AND METHODS

Animals. The male and female axenic mice of the C3H and BALB/c strains used in these trials were supplied by Centre de Selection et d'Elevage des Animaux de Laboratoire (Orléans-La source). They were kept in small-sized Trexler-type plastic isolators, called "mini-isolators" (18). The mice were 2 to 5 months old, and a minimum of two animals was used for each trial. They were fed ad libitum a commercial diet (Usine d'Alimentation Rationnelle), sterilized by gamma irradiation (4 Mrads). Each mouse was inoculated per os with 1 ml of an 18-h culture of the bacterial strain studied (approximately 10^9 bacteria) after having been deprived of water for 18 h. Apart from the *Clostridium* strains which were inoculated simultaneously into the gut of the axenic mice, the other strains were inoculated successively every 48 h. Implantation was verified in the feces after each inoculation by counting in adequate culture media or by microscopic examination in the case of *Clostridium* strains. The gnotoxenic mice were killed 4 weeks after implantation of the last strain. Just before killing, feces were taken directly from the anus of each animal housed in the same mini-isolators, pooled, and used for the enumeration of the various inoculated strains. The dead bacteria were given in various concentrations in the drinking water of the axenic mice for a period of 4 weeks.

Bacteriological techniques. The bacterial strains used were isolated by the technique described by Raibaud et al. (23), from the digestive tract of unweaned young mice (s), the cecum of adult mice (S), and feces of pigs (P), hares (L), humans (H), and rats (R).

The *Micrococcus* s6 and *Corynebacterium* s5 strains were isolated from a 10^{-6} dilution of medium H_1 (23); the *Lactobacillus* s4 and P20 strains were isolated from 10^{-9} and 10^{-8} dilutions, respectively, of medium $GAPTG_{10}$ (24); *Streptococcus* s1 was isolated from a 10^{-7} dilution of medium B′ (25); *Streptococcus* s2 was isolated from a 10^{-8} dilution of medium AGAT (22); *Actinobacillus* s3 was isolated from a 10^{-8} dilution of medium E (23) containing 0.0075% bacitracin (Nutritional Biochemicals Corp.); *Escherichia coli* s7 and S11 were isolated from 10^{-8} and 10^{-6} dilutions, respectively, of medium desoxycholate agar (Difco); *Bacteroides* s8, S12, P21, H50, R30, and L40 were isolated from 10^{-8}, 10^{-9}, 10^{-9}, 10^{-9}, 10^{-8}, and 10^{-8} dilutions, respectively, of medium B′ containing 0.013% neomycin sulfate (Nutritional Biochemicals Corp.); and *Eubacterium* S10 was isolated from a 10^{-9} dilution of medium B′ containing 0.09% streptomycin (Specia). The *Shigella flexneri* strain used was a streptomycin-resistant mutant selected from a strain belonging to the collection of the Pasteur Institute, Paris. An *E. coli* K-12 strain was also supplied by the Pasteur Institute. They were of human origin and had been cultivated in liquid medium C (11).

The enumerations of the various strains were made in the media used for isolations. The technique used to enumerate them in the different intestinal segments and in the feces has been described elsewhere (23). The *Clostridium* strains used in these trials, C1 to C15, were all isolated from a 10^{-9} dilution of conventional mouse cecum using the technique and medium described by Aranki et al. (1). The total number of bacteria in conventional mice was also estimated by this method. All the *Clostridium* strains were extremely oxygen sensitive except the strains C1, C6, C9, C11, and C12, which could be manipulated outside a Freter anaerobic glove box. The extremely oxygen-sensitive *Clostridium* strains cannot be established alone in axenic mice. However, this establishment becomes possible in the digestive tract of axenic mice monoassociated for a few days with some "helper" bacterial strains such as *S. flexneri* or *Clostridium* C1. A microscopic examination of a 10^{-2} fecal dilution showed that various morphological types of *Clostridium* had become established, but they were not enumerated separately.

The dead *E. coli* s7 and *Bacteroides* s8 cells were obtained by the following procedure: the centrifugation pellet of a 48-h culture of 3 liters of liquid medium C (*E. coli*) and A (*Bacteroides*) was recovered by centrifugation, washed twice with sterile water, and suspended in 30 ml of water. The viable bacteria were counted at this step of the operation. The pellet was then either heated in sealed glass ampoules (10 min at 65°C and 15 min at 65°C, respectively, for *E. coli* and *Bacteroides*) or lyophilized and subjected to gamma irradiation (4 Mrads) in 15-ml bottles. The killed cells were thereafter diluted at the desired concentration in the drinking water of the animals. To obtain killed conventional mouse microflora, a 1:10 dilution of conventional mouse feces was ground, filtered through gauze, and then either heated in sealed ampoules for 30 min at 100°C or irradiated (4 Mrads) after lyophilization. A final 1:100 dilution of this killed flora was made with the drinking water of the animals.

Immunohistochemical techniques. After killing animals by cervical elongation, the digestive tract was unrolled. It was fixed in 95°C alcohol, 1-cm-long samples, excluding the Peyer's patches, were taken from the duodenum, and eventually other samples were taken from the middle and the terminal part of the small intestine and from the Peyer's patches. The intestinal fragments were prepared by the technique described by Sainte-Marie (26). The paraffin used for immersions and embeddings was "Histomed special" (Labo-Moderne, Paris, France). Tissue sections, 3 to 4 μm thick, were obtained with a Leitz microtome. Rhodamine-conjugated rabbit antiserum anti-mouse alpha chains was generously supplied by B. Lisowska-Grospierre (17). The tissues were examined by means of a Leitz Orthoplan cell microscope fitted with an Opak-fluor vertical illuminator (E. Leitz, Wetzlar, Federal Republic of Germany) (magnification, ×250). The results obtained represent the arithmetic mean of the fluorescent cells observed in 50 to 100 microscopic fields. The results do not take into account the number

of IgA plasmocytes found in the villi adjacent to Peyer's patches, unless otherwise noted.

RESULTS

Choice of the axenic mouse strain and of the experimental design. The number of IgA plasmocytes (Table 1) in the axenic C3H mice was lower than that of the BALB/c mice reared in the same conditions. The C3H strain was therefore chosen for these experiments. The ratio between the number of IgA plasmocytes in the duodenum of conventional and axenic mice was 10, and this is in keeping with the results of Crabbé et al. (7).

According to our study, the kinetics of appearance of IgA plasmocytes in the duodenum of axenic mice monoassociated with a stimulatory strain, *E. coli* s7 (Fig. 1), is the same as that described by Crabbé et al. (8) in conventional mice. The number of IgA plasmocytes stabilizes at a plateau between the weeks 3 and 4. Enumerations of IgA plasmocytes in the different groups of gnotoxenic mice were therefore performed 4 to 5 weeks after their inoculation.

TABLE 1. *Comparison between the number of IgA plasmocytes in the duodenum of axenic C3H and BALB/c mice and that of conventional C3H mice*

Mouse strains	No. of IgA plasmocytes[a]		No. of bacteria[b]
	Mean	Range	($\log_{10}$)
Axenic mice			
BALB/c	4	2–6 (5)[c]	0
C3H	2	1–3 (10)	0
Conventional mice,			
C3H	19	14–24 (9)	9.6

[a] Per microscopic field.

[b] $\log_{10}$ total number of colonies grown on medium described by Aranki et al. (1), in anaerobic glove box, and expressed per gram of fresh sample.

[c] Number in parentheses indicates number of animals.

The number of IgA plasmocytes studied in these mice (Table 2) decreased from the duodenum to the ileum as in conventional mice (7). We therefore chose to remove the duodenum. It was noticed that the duodenum contained the smallest number of viable bacteria.

Increase in the population of IgA plasmocytes in the duodenum of axenic mice monoassociated with living bacterial strains of different intestinal origin. (i) Bacterial strains isolated from the digestive tract of conventional mice before and after weaning. The bacterial strains inoculated in the groups of animals 1, 2, and 3 (Table 3) had no effect on the stimulation of IgA IIS. All of these strains were gram positive. The strains inoculated in groups 4 to 7 were only slightly stimulatory. They were either gram positive (*Lactobacillus* s4, *Streptococcus* s1 and s2) or gram negative (*Actinobacillus* s3). The gram-negative strains inoculated in groups 8 to 11 were stimulatory. In all 11 groups, the strains became established in high number in the digestive tract (>10^8 bacteria per g of feces). The

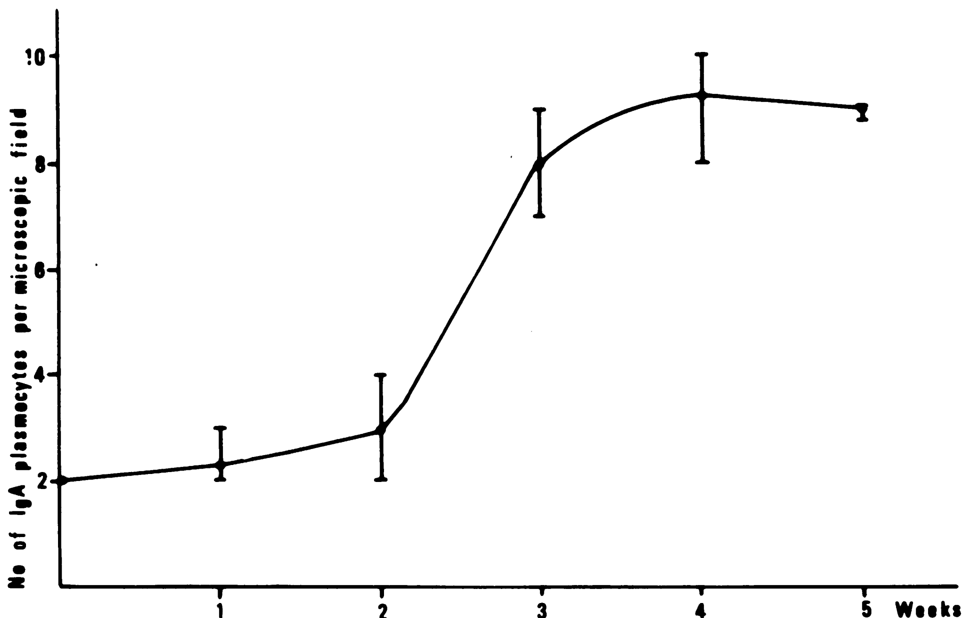

FIG. 1. *Kinetics of appearance of IgA plasmocytes in the duodenum of axenic C3H mice monoassociated with Escherichia coli s7.*

TABLE 2. *Distribution of IgA plasmocytes in the small intestine of axenic C3H mice monoassociated with E. coli s7 since 5 weeks*

Intestinal segment[a]	No. of bacteria[b] (log_{10})	No. of IgA plasmocytes[c]	
		Villi adjacent to Peyer's patches	Villi far from Peyer's patches
1	6.7	13, 13[d]	10, 10
2	7.0	11, 10	5, 6
3	7.9		1, 2

[a] Small intestine divided into three equal segments which were designated, beginning proximally, as 1, 2, and 3.
[b] Per gram of fresh sample.
[c] Per microscopic field.
[d] Individual values found in the two mice.

TABLE 3. *Increase in the number of duodenal IgA plasmocytes in axenic C3H mice associated for 4 weeks with several intestinal bacterial strains isolated from conventional mice*

Group	Inoculum	No. of IgA plasmocytes[a]		No. of bacteria[b] (log_{10})
		Mean	Range	
1	*Micrococcus* s6	1	1-1 (2)[c]	8.3
2	*Corynebacterium* s5	1	1-1 (2)	8.0
3	*Eubacterium* S10	2.5	2-3 (4)	9.0
4	*Lactobacillus* s4	3.5	3-4 (2)	8.8
5	*Streptococcus* s1	4	3-5 (3)	9.7
6	*Streptococcus* s2	5	4-6 (4)	8.6
7	*Actinobacillus* s3	5	4-6 (3)	9.5
8	*Escherichia coli* s7	9	6-11 (7)	9.5
9	*Escherichia coli* S11	8.5	8-9 (2)	9.5
10	*Bacteroides* s8	9	6-11 (6)	10.3
11	*Bacteroides* S12	9	6-11 (5)	10.0

[a,b,c] See footnotes to Table 1.

strains belonging to the genera *Lactobacillus, Streptococcus,* and *Bacteroides* did not exhibit the same immunogenicity in the digestive tract although they were all members of the autochthonous flora of mice according to Dubos et al. (10).

(ii) Bacterial strains isolated from the digestive tract of animals other than mice. Table 4 shows that *Bacteroides* R30, H50, P21, and L40 isolated from rats, humans, pigs, and hares do not stimulate IgA IIS more than do *Bacteroides* s8 and S12 isolated from mice. It was also observed that the stimulatory capacity of the human *E. coli* strain (K-12) was almost identical to that of strains s7 and s11, whereas *S. flexneri* did not show any stimulatory capacity. Likewise, the *Lactobacillus* P20 strain isolated from a pig was not more immunogenic in the mouse digestive tract than *Lactobacillus* s4.

Increase in the population of IgA plasmocytes in the duodenum of gnotoxenic mice harboring several bacterial strains. The number of intestinal IgA plasmocytes found in the previous experiments was always lower than that observed in conventional mice. For that reason we decided to associate several bacterial strains with axenic mice to determine whether there is a synergy between the specific effects of each strain on the development of IgA IIS. Table 5 shows that, in the gnotoxenic mice of groups 12, 13, and 14, there was no synergy between the stimulations since the results obtained never exceeded the stimulation observed in axenic mice monoassociated with the most stimulatory strain. On the other hand, the association of slightly or non-stimulatory strains brought about a large stimulation (group 16). However, this synergistic effect was not enhanced by individually stimulatory strains such as *E. coli* s7 and *Bacteroides* s8 (group 17). Considering the size of each of the bacterial populations associated with the axenic mice of groups 16 and 17, the two strains *Corynebacterium* s5 and *Micrococcus* s6 did not play any part in the development of IgA IIS as they were only slightly developed in the feces (about 10^5 bacteria per g of feces). From a histological point of view, the observations made in groups 16 and 17 most closely resemble those made in conventional mice, i.e., distribution of plasmocytes all along the villi and few villi devoid of IgA plasmocytes.

Both extremely oxygen-sensitive strains and

TABLE 4. *Increase in the number of duodenal IgA plasmocytes in axenic C3H mice monoassociated for 4 weeks with intestinal bacterial strains isolated from other animal species*

Origin	Inoculum	No. of IgA plasmocytes[a]		No. of bacteria[b] (log_{10})
		Mean	Range	
Human	*Escherichia coli* K-12	6	5-7 (5)[c]	10.0
	Bacteroides H50	10	6-14 (5)	9.5
	Shigella flexneri	2.5	1-3 (4)	9.7
Hare	*Bacteroides* L40	11	9-14 (7)	10.0
Pig	*Bacteroides* P21	12	12 (1)	10.0
	Lactobacillus P20	5	2-7 (4)	8.3
Rat	*Bacteroides* R30	7	6-8 (4)	10.0

[a,b,c] See footnotes to Table 1.

TABLE 5. *Increase in the number of duodenal IgA plasmocytes in axenic C3H mice associated for 4 weeks with several intestinal bacterial strains isolated from conventional mice*

Group	Inoculum[a]	No. of IgA plasmocytes[b]	
		Mean	Range
12	*Actinobacillus* s3 (9.3)[c] + *Streptococcus* s1 (9.5)	5.5	5–6 (2)[d]
13	*Bacteroides* s8 (9.6) + *Actinobacillus* s3 (8.3)	8	6–11 (3)
14	*Bacteroides* s8 (9.0) + *Actinobacillus* s3 (<6.0) + *Escherichia coli* s7 (9.7)	9	7–11 (4)
15	*Eubacterium* S10 (9.3) + *Micrococcus* s6 (8.0)	4	3–5 (4)
16	*Actinobacillus* s3 (9.0) + *Streptococcus* s1 (10.0) + *Lactobacillus* s4 (8.9) + *Corynebacterium* s5 (4.7) + *Micrococcus* s6 (5.3) + *Streptococcus* s2 (9.5)	10.5	8–13 (2)
17	*Actinobacillus* s3 (6.5) + *Streptococcus* s1 (<9.0) + *Lactobacillus* s4 (8.5) + *Corynebacterium* s5 (5.7) + *Micrococcus* s6 (5.3) + *Streptococcus* s2 (9.5) + *Bacteroides* s8 (10.3) + *Escherichia coli* s7 (9.8)	12	9–15 (4)

[a] In groups 12, 13, 14, and 15, the bacterial strains were inoculated simultaneously into the digestive tract of axenic mice. In groups 16 and 17, the strains were inoculated according to the order indicated in the table.
[b] See footnote *b*, Table 1.
[c] Number in parentheses indicates $\log_{10}$ of bacteria per g of fresh feces.
[d] See footnote *c*, Table 1.

those not extremely oxygen sensitive belonging to the genus *Clostridium* are dominant in the digestive tract of conventional mice (14). It was therefore of interest to study the effect of these strains on the development of IgA IIS. Table 6 indicates that, when a variable number of *Clostridium* strains were inoculated into axenic mice monoassociated with a "helper" strain such as *S. flexneri* or *Clostridium* C1, they were only slightly or non-stimulatory regardless of the number of associated strains. If nine *Clostridium* strains (group 28) were added to the strains present in the animals of group 17, the stimulation was not significantly enhanced and remained lower than the mean stimulation observed in conventional mice.

Increase in the population of IgA plasmocytes in the duodenum of axenic mice receiving dead bacteria in their drinking water. The purpose of this trial was to examine whether killed microflora are as immunogenic as living ones, whether the treatment used to kill the bacteria affects their immunogenic capacity, and at which concentration the killed bacteria are immunogenic.

The results (Table 7) show that the killed *E.*

TABLE 6. *Increase in the number of duodenal IgA plasmocytes in axenic C3H mice associated for 4 weeks with several intestinal Clostridium strains isolated from the dominant flora of adult conventional mice*

Group	Inoculum	No. of IgA plasmocytes[a]	
		Mean	Range
18	C1 + C2	2	1–3 (2)[b]
19	C1 to C4	5	5–5 (2)
20	*Shigella flexneri* + C5 + C6	5.5	5–6 (2)
21	C1 to C6	8	8 (1)
22	*S. flexneri* + C3 to C6	7	4–11 (3)
23	*S. flexneri* + C5 to C9	4.5	4–5 (2)
24	C1 to C9	4	3–5 (4)
25	*S. flexneri* + C3 to C9	5	5–5 (2)
26	C1 to C13	5	4–6 (2)
27	C1 to C15	6	6–6 (2)
28	Strains of group 17[c] + C1 to C9	14	12–15 (3)

[a,b] See footnotes *a* and *c*, Table 1.
[c] Of Table 5.

TABLE 7. *Increase in the number of duodenal IgA plasmocytes in axenic C3H mice receiving dead bacterial strains per os during 4 weeks*

Inoculum	Sterilization technique	$\log_{10}$ of dead bacteria[a]	No. of IgA plasmocytes[b]	
			Mean	Range
Total flora from conventional mice (dilution 1:100)	Heating	7.6	2	1–3 (4)[c]
	Irradiation	7.6	2	1–3 (2)
Escherichia coli s7	Heating	9.0	11	8–14 (4)
	Irradiation	9.0	12	9–17 (6)
	Irradiation	8.0	6	5–7 (2)
	Irradiation	7.0	4.5	3–6 (2)
	Irradiation	6.0	2.5	2–3 (2)
Bacteroides s8	Heating	9.0	7.5	7–8 (2)
	Irradiation	9.0	5.5	4–7 (5)
	Irradiation	6.0	1	1–2 (2)

[a] Per milliliter of drinking water.
[b] Per microscopic field.
[c] Number in parentheses indicates number of animals.

coli s7 and *Bacteroides* s8 strains led to the development of almost the same number of IgA plasmocytes in the duodenum as did the living strains, regardless of the treatment used to kill the cells. Nevertheless, this immune response depended on the concentration of killed bacteria. No response was obtained when the concentration of dead cells was lower than 10^7/ml. A killed, 1:100 diluted conventional mouse flora was never immunogenic.

DISCUSSION

Our study on gnotoxenic mice shows that the bacterial strains present in the digestive tract of conventional mice or in that of other animal species are not all able to stimulate the development of IgA IIS although they have all become largely established in the intestine. In particular, the gram-positive strains belonging to the genera *Lactobacillus, Streptococcus, Micrococcus, Corynebacterium,* and *Eubacterium* have very poor action or no action at all when they are monoassociated with axenic mice. On the contrary, the gram-negative strains belonging to the genera *Bacteroides* and *Escherichia* have a stimulatory effect leading to a fivefold increase in the number of IgA plasmocytes found in axenic mice. Accordingly, it seems that only gram-negative bacteria are stimulatory for the mouse strain C3H. However, this finding cannot be generalized, as it was observed that neither *S. flexneri* nor *Actinobacillus* species were stimulatory. The absence of immune response observed by Pollard and Sharon (21) in the Peyer's patches of axenic mice monoassociated with *Streptococcus faecalis* might thus be due to the absence of a stimulatory capacity in this bacterial strain and not, as assumed by these authors, to a lack of susceptibility in gnotoxenic mice. The study of axenic mice monoassociated with *E. coli,* a stimulatory strain, clearly shows that the kinetics of appearance of IgA plasmocytes and their distribution in the mucosa of the small intestine is absolutely comparable with that observed in conventional animals (8).

None of the bacterial strains monoassociated with axenic mice has succeeded in increasing the number of IgA plasmocytes up to the level found in conventional mice. It therefore seemed likely that an association of strains would be more efficient. Our results show that the simultaneous presence of two stimulatory strains (*E. coli* s7 and *Bacteroides* s8) did not bring about an increase in the number of IgA plasmocytes as compared with that found with each of the strains separately. This fact might be due to cross-reactions between the specific antigenic determinants of each strain. Inversely, the rather high number of IgA plasmocytes obtained in gnotoxenic mice harboring four strains that are non-stimulatory separately (*Lactobacillus* s4, *Streptococcus* s1 and s2, and *Actinobacillus* s3) suggests the existence of synergistic effects between bacterial strains. These effects might be explained either by the absence of cross-reactions between the antigenic determinants of these four strains or by the adjuvant effect of one or several strains towards other antigens. A study of the specificity of the antibody activity in the secreted IgA plasmocytes might be a means for choosing between these two hypothesis. However, the maximum amount of IgA plasmocytes obtained with this association of four strains represents only two-thirds of that obtained with conventional microflora of mice. Consequently, other bacterial strains of the dominant flora of mice are involved in the development of IgA IIS, either alone or in synergy.

Our results obtained with killed flora show that the dead cells of a stimulatory strain (*E. coli* or *Bacteroides* sp.) still possess an immunogenic capacity. However, for the same concentration of total cells (10^8 cells per ml), killed *E. coli* is always stimulatory, whereas a 1:100 dilution of the fecal flora of conventional mice (also killed by irradiation) has no action. It may be assumed either that the number of cells of the stimulatory strains present in the feces of conventional mice is lower or perhaps equal to 10^9 bacteria per g of feces or that these strains, that we have not yet isolated, are nonimmunogenic when they are killed. The absence of stimulation observed in axenic mice receiving a concentration equal or inferior to 10^7 killed *E. coli* per ml of drinking water raises another question. In conventional mice, like in axenic mice monoassociated with living *E. coli,* the number of viable bacteria in the duodenum does not exceed 10^7 bacteria per g of content, whereas the number of IgA plasmocytes is the most abundant. The stimulatory action of the bacteria can therefore only be exerted on the precursor cells of the Peyer's patches (9) located in the ileum and the cecum where the number of viable bacteria is sufficient, whereas, for reasons which are still unknown, the populating of the lamina propria of the small intestine takes preferential place in the duodenum, after the gut cell IgA cycle (15).

According to Dubos et al. (10), the bacterial strains belonging to the genera *Lactobacillus, Streptococcus,* and *Bacteroides* are members of the autochthonous flora of conventional mice. These bacteria are present in the dominant flora of mice during their whole life. Foo and Lee (13) have shown that suspensions of a *Bacteroides* sp. isolated from mouse intestine and given par-

enterally do not initiate any antibody formation in mice. They conclude that the bacteria of the autochthonous flora are nonimmunogenic for the host, whereas homologous strains isolated from other animal species would be immunogenic. This conclusion, based on the study of antibodies after parenteral injection of killed bacteria as well as on results obtained with the *Bacteroides* strain only, is somewhat astonishing. As a matter of fact, Foo and Lee observed a positive immune response towards *Lactobacillus* and *Streptococcus* although these strains are also members of the autochthonous flora of mice. Our results show that three strains of autochthonous flora of mice, belonging to the genera *Lactobacillus* and *Streptococcus,* have a poor effect on the development of IgA IIS in the intestinal mucosa, a fact that would be in favor of an immunotolerance of these strains at the level of the intestine. However, a different result is obtained with *Bacteroides* s8 and S12. The *Bacteroides* strains s8 and S12 were isolated from the dominant flora of a young unweaned mouse and from the dominant flora of an adult mouse. So the genus *Bacteroides* belongs to the autochthonous flora according to the definition of Dubos et al. (10). Nevertheless, the action of these *Bacteroides* strains on the development of IgA IIS is as large as that of *E. coli* s7, which does not belong to the autochthonous flora according to Dubos et al. (10). On the other hand, various bacterial strains of the genus *Bacteroides* isolated from the microflora of animal species very different from mice (pigs, rats, humans, hares) do not induce a larger stimulation of IgA IIS than that observed in mice monoassociated with a *Bacteroides* strain of their autochthonous flora.

It may be concluded from this work than an increase in the number of IgA plasmocytes may be obtained in axenic mice associated with certain living or dead bacterial strains whether they belong to the autochthonous flora or not. Accordingly, the gnotoxenic animal represents a valuable tool for studying the interactions between the intestinal immune system of the host and its microflora.

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