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Passive Protection of Suckling Infant Mice against F41-Positive Enterotoxigenic *Escherichia coli* Strains by Intravenous Inoculation of the Dams with Monoclonal Antibodies against F41

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Ten monoclonal antibodies (MAbs) against five different epitope clusters of adhesion factor F41 (two MAbs per cluster) were tested for protection of infant mice against an oral challenge with F41-positive enterotoxigenic *Escherichia coli* (ETEC) B2C and B41M. Infant mice suckling dams intravenously inoculated with MAbs were orally challenged, and the survival rates were measured for 12 days after inoculation and challenge. Irrespective of their epitope specificity, all F41 MAbs given in a single dose of 4 mg per dam had a protective effect against both ETEC strains. In contrast, one K99 MAb of the same isotype and given in the same dose as the F41 MAbs did not protect infant mice at all. A reduction in the dose of F41 MAbs to 0.032 mg per dam resulted in a decrease in protection. Two different MAbs against the same epitope cluster were not necessarily equally protective. Combining MAbs two by two, whether the MAbs recognized the same epitope cluster or not, resulted in protective activity essentially similar to that obtained with each MAb separately, without any improvement. Therefore, one MAb against any epitope may be sufficient for protection. Enzyme-linked immunosorbent assay (ELISA) titers of MAbs in the serum of dams were similar, irrespective of the epitope specificity of the MAbs, and gradually decreased from day 1 to day 12 after inoculation. We found a good correlation between colostrum and milk ELISA titers of MAbs and serum ELISA titers of MAbs. Colostrum and milk MAb titers were 10-fold lower than corresponding serum MAb titers and stayed high until day 5 after inoculation. The most protective MAb had the highest ELISA titers in colostrum and milk for the first 5 days after inoculation. ETEC strain B2C colonized the intestines of infant mice suckling MAb-inoculated mothers until day 12 after challenge. Intestinal levels of the challenge strain were high on day 2 but never reached the very high numbers (10^9 to 10^{10}) described previously in a diarrheic infant mouse model. MAbs did not eliminate the challenge ETEC strain from the intestines of infant mice.

Enterotoxigenic *Escherichia coli* (ETEC) induces diarrhea in humans and in domestic animals by colonizing the small intestine and by secreting enterotoxins. Colonization of the gut is facilitated by colonization factors that mediate adhesion and usually are specific for the host species. In bovine ETEC strains, K99 long has been considered the major colonization factor (10, 32). An additional adhesion factor, F41, was found in K99-positive bovine ETEC strains of serogroups O9 and O101 (9, 25). Later, K99 and F41 were described alone or in combination in some porcine ETEC strains (22, 26). K99 and F41 are antigenic, hemagglutinating, and adhesive and have a fimbrial morphology (5, 9, 14, 15, 25, 32, 38).

The fact that K99-mediated colonization can occur independently of F41 makes it difficult to assess the role of F41 in colonization by K99-, F41-positive ETEC. F41 seems to be of minor importance or practical significance in naturally occurring disease. F41 is less prevalent than K88 (18), K99, or 987P and is usually accompanied by K99 (21). There is, however, strong suggestive evidence that F41 can mediate colonization by adhesion. Variants of a K99-, F41-positive porcine ETEC strain that have lost the K99 gene (19) and still carry the gene for and produce F41 are still virulent for newborn pigs (6).

Vaccination of pregnant cows, ewes, and sows with whole cells of K99-positive ETEC strains (1, 2, 16, 20, 24, 28, 29) or with K99 cell extracts (2, 3, 8, 30, 36, 37, 40) protected their suckling offspring against an oral challenge with homologous and heterologous K99-positive ETEC strains. Concomitantly, K99 antibodies appeared in the colostrum of vaccinated dams and supposedly supplied protection. Vaccination of dams with vaccines containing K99 but not F41 protected suckling newborn pigs (33) and calves (2) against a challenge with ETEC strains producing both K99 and F41. Monoclonal antibodies (MAbs) against K99 can protect calves against a challenge with K99-, F41-positive ETEC strains (35). A protective role for the F41 antigen was also demonstrated by Runnels et al. (33). Vaccination with an F41-positive ETEC strain protects against a challenge with F41-positive ETEC strains; however, in contrast to K99 vaccines, such F41 vaccines do not protect against a challenge with a strain producing both K99 and F41 (33). This observation also suggests that F41 may be less important than K99 for colonization. However, theoretically F41 has the potential to become highly important under the selection pressure of K99 vaccines.

More data are needed on the role of F41 in colonization, the relative importance of K99 and F41 in disease caused by

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K99-, F41-positive ETEC, and the protective effects of antibodies against these antigens.

In an infant mouse model, bovine and porcine ETEC strains bearing K99, F41, or both induced fatal diarrhea in the majority of animals (11, 15, 31). F41 was demonstrated to be an important virulence factor in infant mice challenged with a strain bearing both K99 and F41 (4). F41 was also probably largely involved in protecting infant mice by vaccination of the dams against a K99-, F41-positive strain (13). Therefore, this model appears to be a good tool for analyzing anti-F41 immunity and is easier to use than natural hosts.

Recently, the antigenic properties of F41 were extensively studied by use of MAbs (39). These MAbs recognized five different epitope clusters of the F41 antigen. All MAbs against one of these epitope clusters (epitope cluster 1) inhibited the in vitro adhesion of F41 to erythrocytes and intestinal epithelial cells, whereas none of the MAbs against the other epitope clusters inhibited in vitro adhesion (38a). These MAbs are excellent tools for evaluating the role of F41 antibodies, including their epitope specificity, in protecting infant mice or natural hosts against a challenge with F41-positive or K99-, F41-positive ETEC strains.

The aims of the present study were (i) to assess and confirm the role of F41 in protection by use of F41 MAbs in the infant mouse model, (ii) to determine whether MAbs against each of the five epitope clusters were protective and whether combining MAbs against different epitope clusters resulted in better protection, and (iii) to determine whether F41 MAbs protected infant mice from a clinical infection and whether they eliminated the challenge strain from the intestines of infant mice. In addition, we wanted to select the most protective F41 MAb or combination of MAbs for use in the infant mouse challenge model with K99-, F41-positive ETEC strains.

In this paper, we present evidence that F41 MAbs were protective against a challenge with F41-positive ETEC in this model; F41 MAbs probably play a similar role in natural hosts. Moreover, we demonstrate that MAbs against any epitope of F41 were protective in this model and that combining different MAbs did not improve this protection. The duration of protection was related to the MAb enzyme-linked immunosorbent assay (ELISA) titers in the colostrum and milk of dams. MAbs protected infant mice from a clinical infection, but the administration of MAbs did not result in elimination of the challenge strain from the intestinal tract.

MATERIALS AND METHODS

Bacterial strains. Porcine ETEC strain B2C (O9:K35:F41) was described previously (39), and strain B41M (O101:K−:F41) is a mutant of K99 reference strain B41 (25). Both strains secrete heat-stable enterotoxin a but not any of the other known *E. coli* enterotoxins and are thus referred to as "heat-stable enterotoxin a-only" strains.

MAbs. MAbs were prepared and produced as described previously (39). We used purified MAbs (8 or 20 mg of protein per ml in phosphate-buffered saline) that had been stored at −70°C. A previous study demonstrated at least five epitopes of the F41 antigen (39). We used two different sets of MAbs against the same epitope clusters, i.e., 10 F41 MAbs (Table 1). The first set was composed of MAbs CVI F41-4, F41-6, F41-12, F41-20, and F41-21, and the second set was composed of MAbs CVI F41-2, F41-3, F41-5, F41-19, and F41-23. All MAbs were immunoglobulin G1, except for MAb F41-19, which was immunoglobulin G2a. One K99 MAb (CVI K99-12) was used as a control. It was immunoglobulin G1, had the same protein concentration as the F41 MAbs, and was directed against the second of both epitope clusters of the K99 antigen (unpublished data).

TABLE 1. Characteristics of the MAbs (39)

Immunizing antigen	MAb	ELISA titer[a]	Epitope specificity
Purified F41 from B41M[b]	CVI F41-3	5.2	1
	CVI F41-2	4.9	2
	CVI F41-6	5.5	2
	CVI F41-4	5.8	3
	CVI F41-5	5.2	5
Whole B2C cells	CVI F41-12	5.2	1
	CVI F41-19	5.5	3
	CVI F41-20	4.9	5
	CVI F41-21	4.9	4
	CVI F41-23	5.2	4

[a] Titers are expressed as the logarithm of the reciprocal of the highest dilution giving an A_{450} of 50% the maximum obtainable absorbance value; MAb solutions contained 8 mg of protein per ml.
[b] For details, see reference 39.

Animals. Swiss OF1 virgin mice (IFFA Credo, Saint-Germain-l'Arbresle, France) were raised and mated as described previously (13, 15). The maternal effect before lactation was randomized by pooling at birth mouse pups and randomly distributing eight animals to each mother.

Inoculation of mothers with MAbs and challenge of infant mice. On the first day after delivery, mothers received intravenously 0.2 or 0.5 ml of an undiluted or a 1:5-, 1:25-, or 1:125-diluted MAb solution containing, respectively, 4, 0.8, 0.16, or 0.032 mg of protein. In addition, 1:5-diluted MAbs were also injected in all combinations of two MAbs or of all five MAbs. When combinations were used, the final amount of protein in the mixture was the same as that in a single MAb inoculum. Generally, three mothers were injected with the same MAb or with combinations of MAbs. Controls consisted of mothers intravenously injected with phosphate-buffered saline or with the K99 MAb in the same quantities as the F41 MAbs.

Infant mice were challenged with an ETEC strain within 30 min after inoculation of the dams with MAbs. The challenge was done as described previously (11, 13, 15). In brief, bacterial strains were grown overnight at 37°C on slopes of Trypticase soy agar (bioMérieux, Marcy l'Etoile, Charbonnières-les-Bains, France), and cells were harvested in phosphate-buffered saline (pH 6.8). At 1 day of age, suckling infant mice received orally 10^3 cells of ETEC strain B2C or B41M with a calibrated platinum loop. Infected animals were observed for 12 days after inoculation, and survival was recorded. Because diarrheic episodes may be too transient to be seen at the time of clinical observation or sacrifice, we preferred survival as the decisive criterion for protection. We know from previous studies (4, 11–13, 15) that in infant mice a challenge with F41- or K99-, F41-positive ETEC causes diarrhea followed by dehydration and death and that the mice are not dying of septicemia. When necessary, survival rates for infant mice were compared by the chi-square test, and we compared means of results by variance analysis or by Student's t test. Infant mice were very susceptible to ETEC strains bearing F41 (4, 15). Therefore, we used the terms partial protection and high protection when survival rates were significantly ($P < 0.001$ to

TABLE 2. Protection of suckling infant mice against challenge with ETEC strain B2C after intravenous inoculation of dams with the first set of F41 MAbs

Inoculated MAb (epitope)	Total no. of mice	Survival[a] (%) after the following no. of days:				
		1	2	5	8	12
Undiluted						
None	56[b]	96 ± 6	31 ± 14	13 ± 13	10 ± 9	10 ± 9
K99-12 (2)	48[c]	83 ± 12	29 ± 30	4 ± 0	2 ± 3	2 ± 3
F41-6 (2)	24	100	96	75	17	8
F41-4 (3)	22	100	100	82	23	9
F41-21 (4)	24	100	100	87	25	21
F41-12 (1)	23	100	100	96	57	39
F41-20 (5)	24	100	100	100	75	50
1:5 diluted						
None	128[d]	98 ± 4	42 ± 21	5 ± 10	4 ± 7	4 ± 7
F41-6 (2)	87[e]	100 ± 0	100 ± 0	43 ± 11	16 ± 14	12 ± 8
F41-4 (3)	96[f]	100 ± 0	100 ± 0	58 ± 25	18 ± 10	10 ± 11
F41-21 (4)	104[g]	100 ± 0	99 ± 2	36 ± 13	12 ± 5	10 ± 11
F41-12 (1)	72[h]	100 ± 0	100 ± 0	44 ± 11	7 ± 6	1 ± 2
F41-20 (5)	104[g]	100 ± 0	100 ± 0	66 ± 19	31 ± 14	19 ± 12

[a] Mean ± standard deviation of results for different groups.
[b] Two groups, one of 24 and one of 32.
[c] Two groups of 24 each.
[d] Five groups, four of 24 and one of 32.
[e] Four groups, three of 24 and one of 15.
[f] Four groups of 24 each.
[g] Four groups, three of 24 and one of 32.
[h] Three groups of 24 each.

TABLE 3. Protection of suckling infant mice against challenge with ETEC strain B41M after intravenous inoculation of dams with the first set of F41 MAbs

Inoculated MAb (epitope)	Total no. of mice	Survival[a] (%) after the following no. of days:				
		1	2	5	8	12
Undiluted						
None	24	100	100	25	12	12
K99-12 (2)	24	100	100	33	25	25
F41-6 (2)	16	100	100	100	56	56
F41-4 (3)	16	100	100	100	56	50
F41-21 (4)	24	100	100	100	67	54
F41-12 (1)	24	100	100	100	67	33
F41-20 (5)	24	100	100	96	67	42
1:5 diluted						
None	48[b]	100 ± 0	92 ± 12	23 ± 3	12 ± 0	12 ± 0
K99-12 (2)	24	100	100	37	25	25
F41-6 (2)	48[b]	100 ± 0	100 ± 0	92 ± 12	39 ± 51	35 ± 15
F41-4 (3)	48[b]	100 ± 0	98 ± 3	81 ± 3	31 ± 3	27 ± 9
F41-21 (4)	24	100	100	83	54	54
F41-12 (1)	24	100	100	67	33	25
F41-20 (5)	24	100	100	100	42	37

[a] Mean ± standard deviation of results for different groups.
[b] Two groups of 24 each.

0.05) higher than those for respective controls on days 5 and 8, respectively.

Titration of MAbs in serum, colostrum, and milk. Serum, colostrum, and milk samples from four or five mothers suckling nonchallenged infant mice per MAb were collected just before and at days 1, 2, 5, 8, and 12 after intravenous inoculation. Litters were removed from the dams 4 h before colostrum and milk samples were collected. Lactating mice were milked by the technique of Chardès et al. (7). Blood samples were collected by retro-orbital puncture. All samples were stored at −70°C.

MAb titers in serum, colostrum, and milk samples were determined by an indirect double-antibody sandwich ELISA described before (39). In brief, microtiter plates were coated with rabbit anti-F41 immunoglobulins, the plates were washed, and a crude F41 antigen preparation was added to the wells. After incubation and washing of the plates, serial twofold dilutions of the samples were added. Peroxidase-conjugated rabbit anti-mouse immunoglobulins were used as a conjugate, and a solution of 5-aminosalicylic acid containing hydrogen peroxide was used as a substrate. Titers were expressed as the logarithm of the reciprocal of the highest dilution giving an A_{450} of 50% the maximum obtainable absorbance value. The correlation coefficient between MAb titers in serum and those in colostrum was calculated at the same time. Means of MAb titers were compared by variance analysis and by Student's t test.

Autopsy. Intestines were removed aseptically from infant mice sacrificed at days 2, 5, and 12 after challenge with ETEC strain B2C. Five animals per group were randomly chosen to be autopsied at these times. Each intestine was weighed and ground in 2 ml of phosphate-buffered saline (pH 6.8). The number of lactose-fermenting colonies in the intestinal suspensions was determined by plating of serial dilutions on Drigalski medium (Institut Pasteur Production, Ville d'Avray, France). Ten lactose-fermenting colonies were isolated and serologically identified (15). The total number of lactose-fermenting colonies serologically identical to the challenge strain in the intestines of infant mice was then calculated as described previously (15). Intestinal levels of the challenge ETEC strain were compared by Student's t test and variance analysis.

RESULTS

Protection of infant mice by intravenous inoculation of mothers with the first set of MAbs. ETEC strain B2C was highly pathogenic for infant mice (Table 2). Inoculation of the mothers with each of the undiluted F41 MAbs increased the survival rates for infant mice challenged with strain B2C (Table 2). In contrast, inoculation with the K99 MAb did not raise the survival rates for challenged infant mice (Table 2). The different F41 MAbs did not protect equally: survival rates for challenged infant mice suckling mothers inoculated with F41 MAbs differed significantly at day 8 ($P < 0.001$) and at day 12 ($P < 0.01$). MAbs F41-12 and F41-20 provided high protection (Table 2). MAbs F41-6, F41-4, and F41-21 provided only partial protection. MAbs diluted 1:5 still provided protection, but only partially, except for MAb F41-20, which still increased survival rates significantly ($P < 0.01$) compared with the survival rates for the respective controls 8 days after inoculation (Table 2). Further dilution of MAbs, i.e., 1:25 and 1:125, decreased but did not cancel protection, except for that with MAb F41-12, which provided no more protection at a dilution of 1:125. At dilutions of 1:25 and 1:125, differences in the degree of protection provided by the different MAbs were not marked (data not shown). Further experiments were done with 1:5-diluted MAbs.

The protective effect against ETEC strain B41M was also tested, since some MAbs were prepared against it. Strain B41M was less pathogenic than strain B2C (Table 3). As for strain B2C, MAb K99-12 did not induce any protection of infant mice against strain B41M. Every undiluted F41 MAb supplied approximately similar high protection. When they were diluted 1:5, MAbs F41-21 and F41-2 still provided high

TABLE 4. Protection of suckling infant mice against challenge with ETEC strain B2C after inoculation of dams with the second set of F41 MAbs

Inoculated MAb[a] (epitope)	Survival[b] (%) after the following no. of days:				
	1	2	5	8	12
None	96	96	4	0	0
F41-2 (2)	100	100	79	8	4
F41-19 (3)	100	100	87	37	8
F41-23 (4)	100	100	83	29	11
F41-3 (1)	100	100	96	58	17
F41-5 (5)	100	100	71	33	12

[a] Undiluted.
[b] Each group consisted of 24 infant mice.

TABLE 5. Protection of suckling infant mice against challenge with ETEC strain B2C after intravenous inoculation of dams with combinations of two MAbs[a]

MAb (epitope)	Survival (%) after inoculation of MAb (epitope)					
	None	F41-12 (1)	F41-6 (2)	F41-4 (3)	F41-21 (4)	F41-20 (5)
None	0	33	54	37	25	50
F41-12 (1)	33	33	33	62	79	50
F41-6 (2)	54	33	54	37	58	62
F41-4 (3)	37	62	37	37	67	62
F41-21 (4)	25	79	58	67	25	75
F41-20 (5)	50	50	62	62	75	50

[a] Twenty-four animals were used for each combination. Survival was recorded for 5 days after challenge.

protection of infant mice, whereas MAbs F41-6, F41-4, and F41-12 provided partial protection. These results confirmed the fact that MAb F41-20 was highly protective. MAb F41-4 was more protective against strain B41M than against strain B2C, perhaps because it was prepared against the former strain. However, MAb F41-20, prepared against strain B2C, was equally protective against both challenge strains. For further experiments, we preferred strain B2C because protection could be analyzed earlier than with strain B41M.

Protection of infant mice by intravenous inoculation of mothers with the second set of MAbs. Another set of five F41 MAbs raised against epitope clusters 1 to 5 was tested for protection against ETEC strain B2C (Table 4). As before, the MAb against epitope cluster 2 provided partial protection, whereas the MAbs against epitope clusters 1 and 5 provided high protection. However, infant mice suckling mothers inoculated with MAbs against epitope cluster 3 or 4 were protected better than those suckling mothers that received MAbs against the same epitope cluster but from the first set (Tables 2 and 4). Combining two MAbs directed against the same epitope from both sets led to protection similar to that provided by each MAb separately (data not shown).

Protection of infant mice by intravenous inoculation of mothers with all combinations of two MAbs. Combining F41 MAbs two by two induced either similar or improved protection, compared with that provided by each MAb separately (Table 5). Protection was improved when the mortality of infant mice 5 days after challenge significantly differed from that of infant mice suckling mothers inoculated with one of the MAbs separately. Most combinations of F41 MAbs had effects similar to that of each MAb alone. Combining MAbs F41-12 and F41-4, F41-12 and F41-21, or F41-4 and F41-21 resulted in better protection than using each MAb separately (Table 5). Combining all five MAbs together resulted in no improvement of the protection induced by each MAb separately (data not shown).

MAb ELISA titers in serum, colostrum, and milk of mothers. After MAb administration, MAb titers increased in the serum and colostrum of the mothers and were maximal at day 1, but colostrum MAb titers were approximately 10-fold lower than corresponding serum MAb titers (Fig. 1). From day 2 to day 12 serum MAb titers decreased gradually and significantly, whereas colostrum or milk MAb titers remained at approximately the same levels until day 5, after which they began to decrease (Fig. 1). There was a very good correlation ($0.98 < r < 0.99$) between mean serum MAb titers and mean colostrum MAb titers. On the same day, variance analysis of the titers of all five MAbs revealed no significant differences in serum or colostrum and milk. However, when titers of MAbs were compared two by two, MAb F41-4 and F41-20 titers were higher than the other MAb titers until at least day 5. In serum these differences were not significant. In contrast, in milk, the titer of MAb

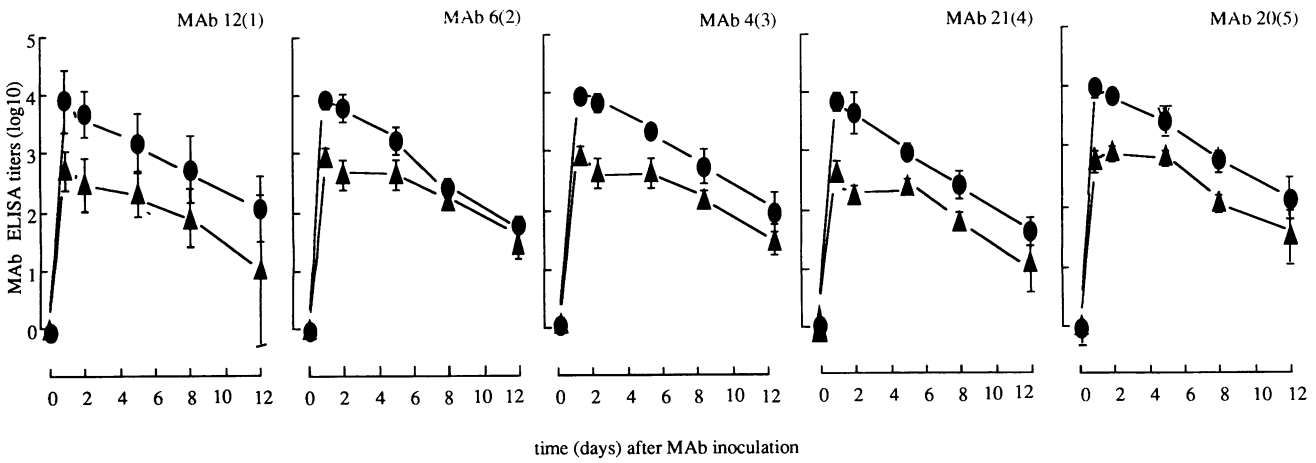

FIG. 1. MAbs titers in serum (●) and in colostrum or milk (▲) of mothers inoculated on day 0 with undiluted MAbs. Numbers in parentheses after MAbs indicate epitopes.

TABLE 6. Intestinal colonization by challenge ETEC strain B2C in infant mice suckling MAb-inoculated dams

Inoculated MAb (epitope)	Intestinal levels[a] of challenge strain B2C after the following no. of days:		
	2	5	12
None	6.32 ± 2.31 (5/5)		
F41-12 (1)	6.78 ± 1.13 (4/5)	9.60 (1/5)	6.74 ± 3.17 (2/5)
F41-6 (2)	6.56 ± 2.04 (5/5)	6.92 ± 2.11 (4/5)	7.30 ± 1.28 (4/5)
F41-4 (3)	7.41 ± 0.73 (5/5)	7.89 ± 0.66 (4/5)	8.32 ± 0.68 (4/5)
F41-21 (4)	6.83 ± 0.19 (2/5)	7.70 (1/5)	(0/5)
F41-20 (5)	8.29 ± 0.53 (4/5)	7.90 ± 0.62 (4/5)	8.80 ± 0.27 (3/5)

[a] Mean ± standard deviation of the numbers ($\log_{10}$) of lactose-fermenting colonies serologically identical to the challenge strain per gram of intestine (number of animals harboring the challenge ETEC strain in the lactose-fermenting colonies tested/number of animals examined at autopsy).

F41-4 significantly ($P < 0.01$) differed from that of MAb 6 on day 8 and the titers of MAb F41-20 were significantly higher on day 2 than the MAb F41-12 titer ($P < 0.05$), the MAb F41-6 titer ($P < 0.05$), or the MAb F41-21 titer ($P < 0.01$) and on day 5 than every other MAb titer ($P < 0.001$ to 0.05). These results suggest that some relationship existed between clinical protection and MAb titers in colostrum and milk.

Intestinal colonization of infant mice by ETEC strain B2C. Autopsied animals did not show any symptoms of diarrhea at the time of sacrifice. The few controls still alive on day 2 after challenge had not developed diarrhea yet. In most cases, infant mice had intestinal lactose-fermenting colonies consisting only of the challenge ETEC strain. Two days after challenge, intestinal levels of ETEC strain B2C were between 5×10^6 and 5×10^8 per g (Table 6). At this time, there were no significant differences between intestinal levels in controls available for autopsy and those in infant mice suckling mothers inoculated with MAbs. A low mean level of B2C in controls at day 2 and a high standard deviation were explained by high levels of strain B2C in the intestines of some animals (7.56, 7.38, and 8.90) and lower levels in the others (4.00 and 3.75). All controls were dead at day 5. On days 5 and 12, the intestinal levels of the ETEC challenge strain in most animals increased slightly but not significantly. Moreover, no differences were observed in the intestinal levels of the ETEC challenge strain in infant mice from mothers inoculated with different MAbs. Animals suckling mothers inoculated with MAb F41-12 or F41-21 seemed to eliminate the challenge ETEC strain earlier than the others, but the differences were not significant.

DISCUSSION

In the present study, we demonstrated that intravenous injection of dams with F41 MAbs resulted in a protective effect in suckling infant mice against oral challenge with ETEC strains B2C and B41M, both bearing colonization factor F41 (Tables 2 and 3). MAbs against all five epitope clusters were protective; the degree of protection was not similar among the MAbs. MAb titers in the colostrum and milk of the dams correlated with serum MAb titers and were consistent with the protection of infant mice. Protection was not accompanied by elimination of the challenge ETEC strain from the intestine.

Initially, the F41 antigen was described as a second colonization factor in addition to K99 in K99-positive ETEC strains of serogroups O9 and O101 (9, 25). Later, K99 and F41 were found alone or in combination in porcine ETEC strains (22, 26). F41 is antigenic, hemagglutinating, adhesive, and fimbrialike (9, 14, 25). Vaccination of dams with whole-cell vaccines containing F41 protects infant mice (13) and piglets (33) against a challenge with F41-positive ETEC. This protection is probably mediate by colostrum antibodies (13, 33). To simulate the natural route, we preferred intravenous inoculation of the dams with MAbs to oral administration of MAbs to infant mice; moreover, treatment of infant mice with an oral gift of rabbit polyclonal antibodies proved to be less protective than intravenous inoculation of the dams (unpublished data).

The results of the present study confirm the protective role of F41 reported earlier (13) and prove the protective role of F41 antibodies against a challenge with F41-positive ETEC in infant mice; the protection provided by the F41 MAbs was specific, because an MAb against the K99 antigen, belonging to the same isotype and given in the same amount as the F41 MAbs, did not protect infant mice from a challenge with F41-positive ETEC strains.

The serum ELISA titers of the different MAbs were similar and gradually decreased from day 1 to day 12. Only 10% of the inoculated MAbs passed through the colostrum; thus, there was no concentration of MAbs from the serum to the colostrum. Vaccination of dams with ETEC strains or purified colonization factors resulted in the appearance of antibodies against colonization factors in the serum and colostrum of natural hosts (1–3, 17, 33, 36). Very often, colostrum MAb titers were higher than serum MAb titers (2, 3, 17). This result was also observed for vaccinated mice (13). In the present study, MAbs were passively transferred and not actively produced by the dams, possibly explaining why colostrum MAb titers were lower than serum MAb titers. In addition, MAbs may have properties different from those of polyclonal antibodies. In contrast to the serum MAb titers, approximately the same level of colostrum MAb titers was maintained until day 5 after inoculation and then ELISA titers began to decrease gradually, but at day 12, MAbs were still present in the milk and the ELISA titers were between 10 and 10^2.

The duration of the protection provided by the F41 MAbs was limited. We believe that this limitation was caused by the decrease in the colostrum MAb titers after day 5, and the inability of the MAbs to eliminate the ETEC challenge strain from the intestinal tract during the first days of life. The relationship between colostrum MAb titers and the duration of protection was obvious. Most infant mice suckling MAb-inoculated dams survived the first 5 days after challenge, i.e., the same period in which colostrum MAb titers were stable and high.

MAbs were not able to eliminate the challenge ETEC strain from the intestines of infant mice; ETEC strain B2C (Table 6) persisted for at least 12 days after challenge. In every autopsied animal, the intestinal levels of the challenge strain were abnormally high on day 2 and similar on days 5 and 12, compared with intestinal levels of the normal coliform flora (23) in nonchallenged animals. Intestinal levels of strain B2C of autopsied animals were never high enough to induce diarrhea; previous studies (12, 15) demonstrated that diarrheic mice harbored a significantly higher level of the challenge strain (10^9 to 10^{10} cells per g of intestine). In this study, no significant differences in intestinal levels of the challenge strain were found between autopsied controls and MAb-fed infant mice at day 2 after challenge (Table 6). The reason for this observation is the high virulence of strain B2C for unprotected mice: almost all controls died within the first 2 days after challenge; the few controls left for autopsy

probably had not developed diarrhea yet. Because in this model animals died of diarrhea and dehydration and not of septicemia, the autopsied controls should have developed diarrhea later if they were not sacrificed and would have had high numbers of the challenge strain in the intestinal tract. Because the intestinal levels of the challenge strain in mice suckling MAb-inoculated dams were lower than those in diarrheic animals, MAbs probably act by decreasing the intestinal colonization of the challenge strain, as described earlier for polyclonal antibodies in infant mice suckling vaccinated mothers (12). The late mortalities after day 5 can be explained by the low milk MAb titers, which result in an increase in intestinal levels of the challenge strain, followed by diarrhea and death.

Five known epitope clusters of the F41 antigen could be involved in the specific protection against both ETEC strains B41M and B2C, which were used to produce the MAbs (Table 1) (39). The results of this study demonstrated that one MAb against any epitope was sufficient for protection. The origin of the antigen used to produce the MAbs did not influence the protection. Cross-protection occurred when challenge was done with strains B2C and B41M. The better protection provided by the F41 MAbs against epitope clusters 1, 2, and 5 suggested that some epitopes are more involved in inducing protection than others. However, MAbs against epitope clusters 3 and 4 were more or less protective, depending on the set used. Combining MAbs two by two usually induced a protective effect similar to that provided by each MAb separately, whether the MAbs were directed against the same epitope cluster or not. The results were the same when MAbs for all five epitopes were combined. Therefore, the intensity and duration appeared linked more to the colostrum MAb titers than the epitope specificity. MAb affinities may also be involved in the effectiveness of protection.

It is assumed that antibodies against colonization factors protect by reacting with the receptor combining sites on fimbriae and thus directly block the adhesion of ETEC strains to intestinal epithelial cells (8, 27, 34, 40). The antiadhesion properties of our MAbs were tested in vitro, with the same MAb solutions, in brush border inhibition and hemagglutination inhibition assays with four F41-positive ETEC strains (38a). All 7 MAbs against epitope cluster 1 inhibited the in vitro adhesion of F41 in both assays, whereas none of the 16 MAbs against the other four epitope clusters inhibited F41 adhesion. Because MAbs against all epitope clusters had a similar protective effect in the infant mouse model, the results of the present study indicate that protection provided by antibodies may not depend on direct blocking of the receptor combining sites on fimbriae. Moon (21) suggested that other, less highly specific mechanisms, such as agglutination, changes in surface charge, binding of bacteria to antibody in mucus rather than to receptors on the brush borders, and opsonization factors, may be involved in protection mediated by antibodies against colonization factors. All F41 MAbs used in our study agglutinated strains B41M and B2C in vitro.

The role of F41 in protection against a challenge with ETEC strains bearing both K99 and F41 by vaccination of dams has been demonstrated for infant mice (4). The protective effect of MAb F41-20 and K99 MAbs against a challenge with K99- and K99-, F41-positive ETEC strains in the infant mouse model will be reported in a separate article (15a). In addition, we will examine the protective effect of F41 MAbs in naturally infected species, such as neonatal piglets.

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