

Citrobacter rodentium Infection in Mice Elicits a Mucosal Th1 Cytokine Response and Lesions Similar to Those in Murine Inflammatory Bowel Disease

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Citrobacter rodentium is a classically noninvasive pathogen of mice that is similar to enteropathogenic *Escherichia coli* (EPEC) in man. Following oral infection of young mice, the organism colonizes the distal colon, and within 1 week the colonic mucosa doubles in thickness and there is massive epithelial cell hyperplasia. Since T-cell responses in mouse models of inflammatory bowel disease (IBD) also cause epithelial hyperplasia, we have investigated the possibility that *C. rodentium* promotes similar T-cell responses in the mucosa, thereby increasing epithelial shedding, transmission, and replication of the organism. Beginning 6 days after infection, bacteria were observed to be in close association with the epithelial surface and were also visible scattered throughout the lamina propria and in the submucosa. There was a CD3⁺-cell infiltrate into the colonic lamina propria and epithelium as well as mucosal thickening and crypt hyperplasia. The majority of CD3⁺ cells were CD4⁺ and were not $\gamma\delta$ ⁺. Reverse transcription-PCR analysis of cytokines also revealed a highly polarized Th1 response (interleukin-12, gamma interferon, and tumor necrosis factor alpha) in the mucosa and a large increase in the epithelial cell mitogen keratinocyte growth factor. None of the changes were seen in mice inoculated with bacteria lacking intimin (which is necessary for colonization), but they were seen in mice inoculated with *C. rodentium* complemented with intimin from EPEC. This is the first example of a classically noninvasive bacterial pathogen which elicits a strong mucosal Th1 response and which produces pathology similar to that seen in mouse models of IBD, which is also characterized by a strong Th1 response. These results also suggest that the colonic mucosa responds in a stereotypic way to Th1 responses.

Citrobacter rodentium, formerly *Citrobacter freundii* biotype 4280, causes transmissible murine colonic hyperplasia (6, 45, 46). The lesions produced at the epithelial surface are indistinguishable from those of enteropathogenic *Escherichia coli* (EPEC) infection, a major cause of infantile diarrhea in humans in developing countries (37). Murine colonic hyperplasia is a naturally occurring disease of laboratory mice, and infection is characterized by crypt hyperplasia and dilation, epithelial cell proliferation, mucosal thickening, and an uneven apical enterocyte surface. In experimentally infected mice, large numbers of bacteria colonize the distal colon and are observed adhering to the epithelial surface. The earliest signs of hyperplasia are seen 4 days after oral infection, with mucosal thickening reaching a maximum between 10 and 12 days postinfection (23). An inflammatory response also takes place, but it has not been characterized.

Binding of EPEC or *C. rodentium* to the enterocyte induces a specific attachment and effacement (A/E) lesion. There is dissolution of the brush border and pedestal formation (34). The proteins necessary for the formation of the A/E lesion are delivered to the host cell by a type III secretion system encoded by the chromosomal locus of enterocyte effacement (17, 45). The locus of chromosomal effacement contains 41 open reading frames which include the intimin-encoding *eae* gene (30)

and the *espA*, *espB*, and *espD* genes (14, 32, 36), which encode the secreted proteins that result in the A/E lesion and in host cell cytoskeletal rearrangements and signal transduction. Intimate attachment of the bacteria to the host enterocyte is mediated through the bacterial outer membrane protein intimin, which binds to a bacterially derived receptor, translocated intimin receptor (Tir), that is embedded in the host cell surface (33). The *eae* genes of several different bacterial strains encode intimin proteins that have highly conserved N-terminal regions but show significant heterogeneity at the C termini (24). At least four different intimin proteins have been characterized to date, namely intimins α , β , γ , and δ . All display diversity but contain two stretches of identical amino acid sequence that may be critical to binding. Intimins α and β were found to be expressed largely by strains belonging to EPEC clones 1 and 2, respectively, while intimin γ was determined to be expressed by enterohemorrhagic *E. coli* serotype O157:H7 and intimin δ was found to be expressed by EPEC O86:H34 (1). An intimin mutant of *C. rodentium*, which normally expresses intimin β , does not cause A/E lesions in mice, but when compensated with intimin α derived from EPEC, pathogenicity is restored (23). In addition to participating in the formation of the A/E lesion, intimin has been shown to bind in vitro to β_1 integrins on T cells (25). The function of such an interaction is not understood. The intimin homologue invasion of *Yersinia pseudotuberculosis* costimulates T-cell proliferation in vitro through interaction with the β_1 integrin VLA-4 (18).

In this study, we inoculated mice with wild-type *C. rodentium* or an intimin- β -negative mutant of *C. rodentium*, and the latter was compensated with intimin α from an EPEC isolate. We

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TABLE 1. Bacterial strains used in this study

Strain	Description	Intimin derivative expressed
<i>C. rodentium</i> biotype 4280 DBS255	Wild-type <i>C. rodentium</i>	β
E2348/69	<i>C. rodentium</i> <i>eae</i> mutant	None
DBS255(pCVD438)	Wild-type EPEC (O127ab:H6)	α
	DBS255 complemented with <i>eae</i> from E2348/69	α

have characterized for the first time the host immune response that follows infection with this organism.

MATERIALS AND METHODS

Animals. Female Swiss NIH and C3H mice (21 days old) were obtained from Harlan/Olac, Scunthorpe, United Kingdom. All mice were housed under specific-pathogen-free conditions with unlimited access to food and water.

Bacterial strains and challenge of mice with bacteria. The bacterial strains used in this study were described previously (23) and are listed in Table 1. Mice (Swiss NIH or C3H) were orally inoculated by gavage with either wild-type *C. rodentium*, *C. rodentium* DBS255, or *C. rodentium* DBS255(pCVD438). For inoculations, bacteria were grown overnight in L broth containing 100 μ g of nalidixic acid per ml or 30 μ g of chloramphenicol per ml. Bacteria were diluted with phosphate-buffered saline, pH 7.2, to an optical density at 600 nm of 1.7 and delivered to mice in a 100- μ l volume containing ca. 1.5×10^7 CFU. Mice were killed at various time points postchallenge, and tissues were snap frozen in liquid nitrogen and stored at -70°C for further analysis.

Immunohistochemistry. Three-step avidin-peroxidase staining was performed on 5- μ m-thick frozen sections of distal colonic tissue as described previously (51), using monoclonal antibodies 145-2C11 (anti-CD3), YTS 191 (anti-CD4), YTS 169 (anti-CD8), and M5-114 (anti-major histocompatibility complex [MHC] class II). Biotin-conjugated rabbit anti-rat immunoglobulin G (IgG; DAKO, High Wycombe, United Kingdom) and goat anti-hamster IgG (Vector Laboratories, Peterborough, United Kingdom) were used at a 1:50 dilution in Tris-buffered saline, pH 7.6, containing 4% (vol/vol) normal mouse serum (Harlan Seralab, Oxon, United Kingdom). Avidin peroxidase (Sigma) was used at a dilution of 1:200 in Tris-buffered saline. A two-step protocol was performed with polyclonal rabbit anti-*E. coli* antibody (Sigma) and rabbit anti-intimin antibody (1) together with horseradish peroxidase-conjugated swine anti-rabbit IgG secondary antibody. Peroxidase activity was detected with 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.5-mg/ml Tris-HCl, pH 7.6, containing 0.01% H_2O_2 . Endogenous-peroxidase-containing cells were visualized by incubation of sections with DAB substrate and H_2O_2 alone. The density of positive cells in the lamina propria was determined by image analysis as described previously (38). Crypt length was measured by micrometry, with 10 measurements being taken in the distal colons of individual mice. Only well-oriented crypts were counted. All measurements were carried out by L.M.H. The measurements were not blinded, but all were independently confirmed by T.T.M.

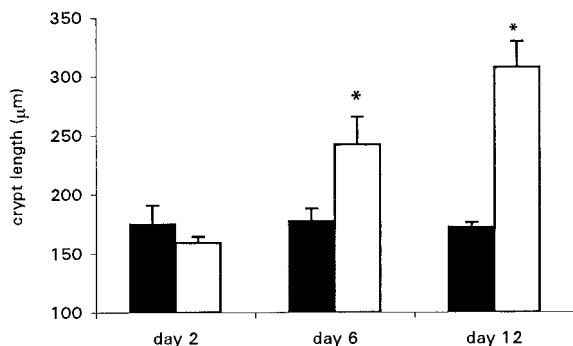


FIG. 1. Swiss NIH mice infected with wild-type *C. rodentium* showed severe colonic hyperplasia compared to mice infected with an intimin mutant strain. Crypt lengths were measured on days 2, 6, and 12 postinfection. Mean crypt lengths in the colons of mice infected with wild-type *C. rodentium* (open bars) were significantly greater than those of mice infected with the control mutant strain (closed bars). Error bars represent standard errors. Each group contained five mice. *, $P < 0.05$.

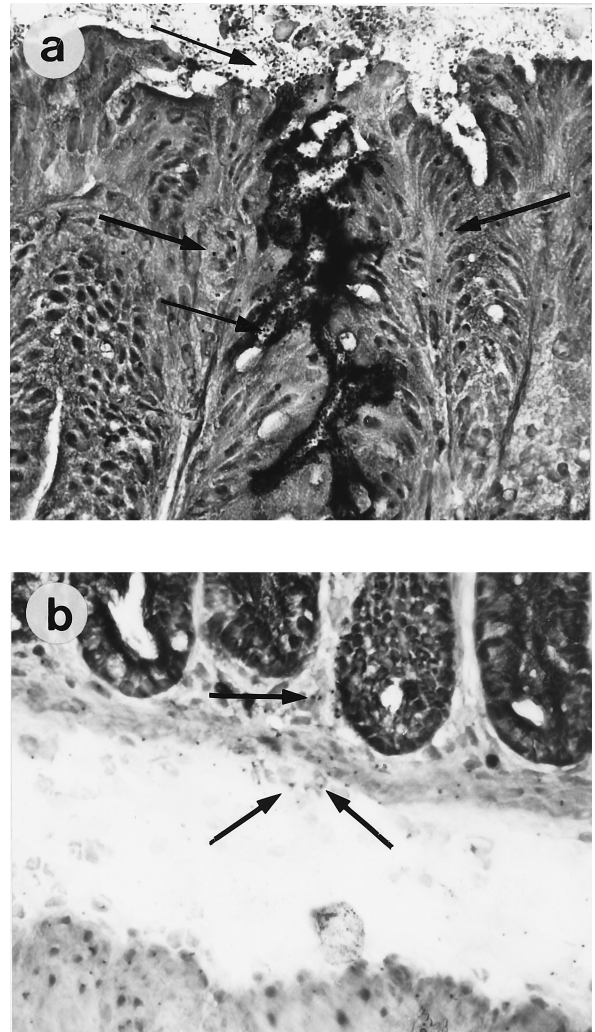


FIG. 2. Bacteria were visualized on the epithelial surface (a) and in the mucosa and submucosa (b) of *C. rodentium*-infected Swiss NIH mice. The clear area between the muscularis mucosa and the external muscle layer is the submucosa, which becomes edematous during *Citrobacter* infection. The arrows indicate bacteria. Immunoperoxidase immunohistochemistry was performed with anti-intimin antibody. Magnification, $\times 400$.

RNA extraction and quantitative RT-PCR. Total cellular RNA was isolated from frozen distal colonic tissue by homogenization of the tissue in TRIzol (Gibco Life Technologies, Paisley, United Kingdom) and incubation at room temperature for 5 min. RNA was extracted with chloroform (Sigma) and then centrifuged for 15 min at $12,000 \times g$ and 4°C . The aqueous phase was precipitated with an equal volume of isopropanol (Sigma) and subsequently centrifuged for 15 min at $12,000 \times g$ and 4°C . The pellet was washed with 70% ethanol and resuspended in 50 μ l of water. Total RNA was determined by spectrometric analysis. Constructs encoding standard RNAs (pMCQ1, pMCQ2, pMCQ3, and pMCQ4), kindly provided by M. F. Kagnoff, Department of Medicine, University of California, San Diego (16), and a construct encoding keratinocyte growth factor (KGF) RNA, generated by M. Bajaj-Elliott, Department of Paediatric Gastroenterology, St. Bartholomew's Hospital, London, United Kingdom, (3), were used for quantitative competitive reverse transcription (RT)-PCR. To generate standard RNA, plasmids were linearized with *SalI* (pMCQ1), *NotI* (pMCQ2, -3, and -4), or *HindIII* (pKGF) and transcribed in vitro with T7 RNA polymerase under conditions recommended by the supplier (Promega, Southampton, United Kingdom). Serial 10-fold dilutions of standard RNA (1 pg to 1 fg) were co-reverse transcribed with total cellular RNA (2 μ g) at 42°C for 50 min in a 20- μ l reaction volume containing 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM MgCl_2 , 3 mM dithiothreitol, 10 mM deoxynucleoside triphosphate mix, and 0.5 μ g oligo(dT) (Pharmacia Biotech, St. Albans, Hertfordshire, United Kingdom), using 100 U of reverse transcriptase (Superscript II; RNase H negative; Gibco). The reaction was terminated by heat inactivation at 70°C for 10 min. PCR

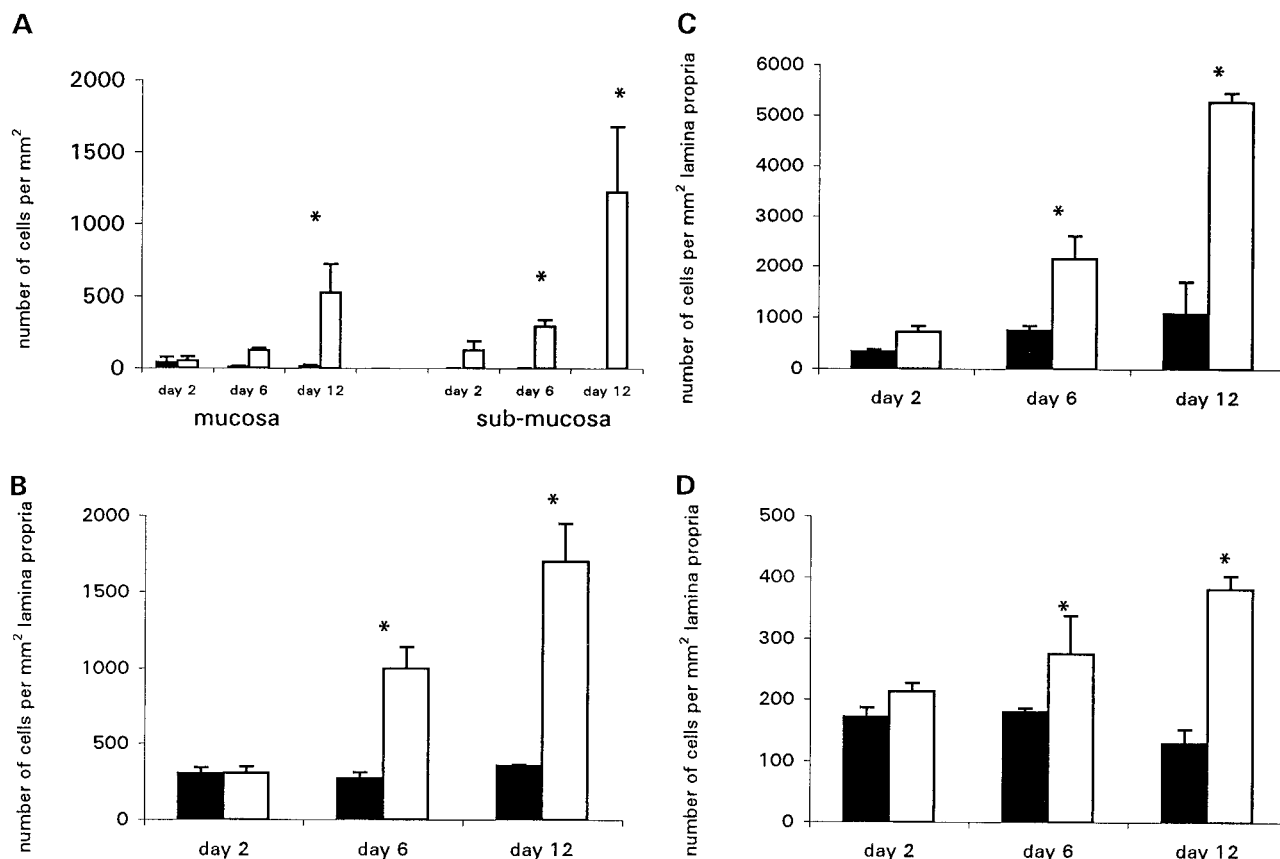


FIG. 3. Mean cell counts for peroxidase-containing cells in the mucosa and submucosa (A) and for CD3⁺ (B), CD4⁺ (C), and CD8⁺ (D) cells infiltrating the lamina propria of Swiss NIH mice were determined on days 2, 6, and 12 postinfection. In addition to the massive increase in numbers of CD3⁺ and CD4⁺ cells in the mucosa of *C. rodentium*-infected mice, there was also a slight increase in numbers of these cells in control mice. This probably reflected the increase which is seen in normal mice around this time (21). Closed bars represent mice infected with an intimin mutant strain; open bars represent mice infected with wild-type *C. rodentium*. Error bars indicate standard errors. Each group contained five mice. *, $P < 0.05$.

amplification was routinely carried out in 50- μ l reaction volumes (10 mM Tris [pH 9], 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each deoxynucleoside triphosphate, and 10 pmol each of 5' and 3' primers) as described elsewhere (19, 20), using 1 U of *Taq* polymerase (Pharmacia Biotech). Forty amplification cycles consisting of a 45-s denaturation at 94°C, a 45-s annealing at 58°C, and a 75-s extension at 72°C were used. After amplification, PCR products were analyzed on 1% agarose gels and bands were visualized by ethidium bromide staining. Band intensities were quantified by densitometry (Seescan, Cambridge, United Kingdom). The sensitivity of this technique enables the detection of $>10^3$ mRNA transcripts per μ g of total RNA.

Statistics. The significance of differences between means was determined by using the Mann-Whitney U test.

RESULTS

Colonic hyperplasia and bacterial colonization caused by infection with *C. rodentium*. Swiss NIH mice were killed on days 2, 6, and 12 postinfection, and their colons were examined for signs of bacterial colonization and mucosal hyperplasia by immunohistochemistry. The colons from wild-type-bacterium- and mutant-bacterium-infected mice were indistinguishable on day 2. Macroscopic thickening of the distal colon was observed in approximately 60% of the mice infected with wild-type bacteria by day 6 and in all such mice by day 12. Microscopic examination showed massive epithelial cell hyperplasia with a two- to fourfold increase in mucosal thickness and crypt length; the latter is quantified in Fig. 1. In preliminary experiments it was observed that male and female mice were equally suscep-

tible to infection and displayed similar pathologies. All results presented here represent infection of female mice.

The presence of bacteria adhering to the epithelial surface was visualized by immunohistochemistry with an anti-*E. coli* antibody that cross-reacts with *C. rodentium* or with anti-intimin antibody. The two types of immunostaining revealed identical patterns of expression. In mice infected with intimin mutants, no bacteria were present at any time point, while in wild-type-*C. rodentium*-infected mice, bacteria were observed in close association with enterocytes both at the tips of the villi and deep down in crypts (Fig. 2a). Colonization of the epithelium was directly associated with mucosal thickening and crypt hyperplasia on days 6 and 12, and no bacteria were observed on day 2 postinfection. In addition to the bacteria at the enterocyte surface, organisms were seen scattered throughout the lamina propria and in the edematous submucosa (Fig. 2b). Viable bacteria were occasionally isolated from the mesenteric lymph nodes (results not shown). Other features of pathogenesis included loss of goblet cells, increased shedding of epithelial cells into the lumen, and an uneven apical surface.

T-cell infiltrate resembling inflammatory bowel disease (IBD). Infiltrating T cells and macrophages were visualized by immunohistochemistry analysis for CD3⁺, CD4⁺, and CD8⁺ cells on frozen sections. Once colonization had taken place, a striking inflammatory infiltrate was observed. Numbers of endogenous-peroxidase-containing cells were increased, particu-

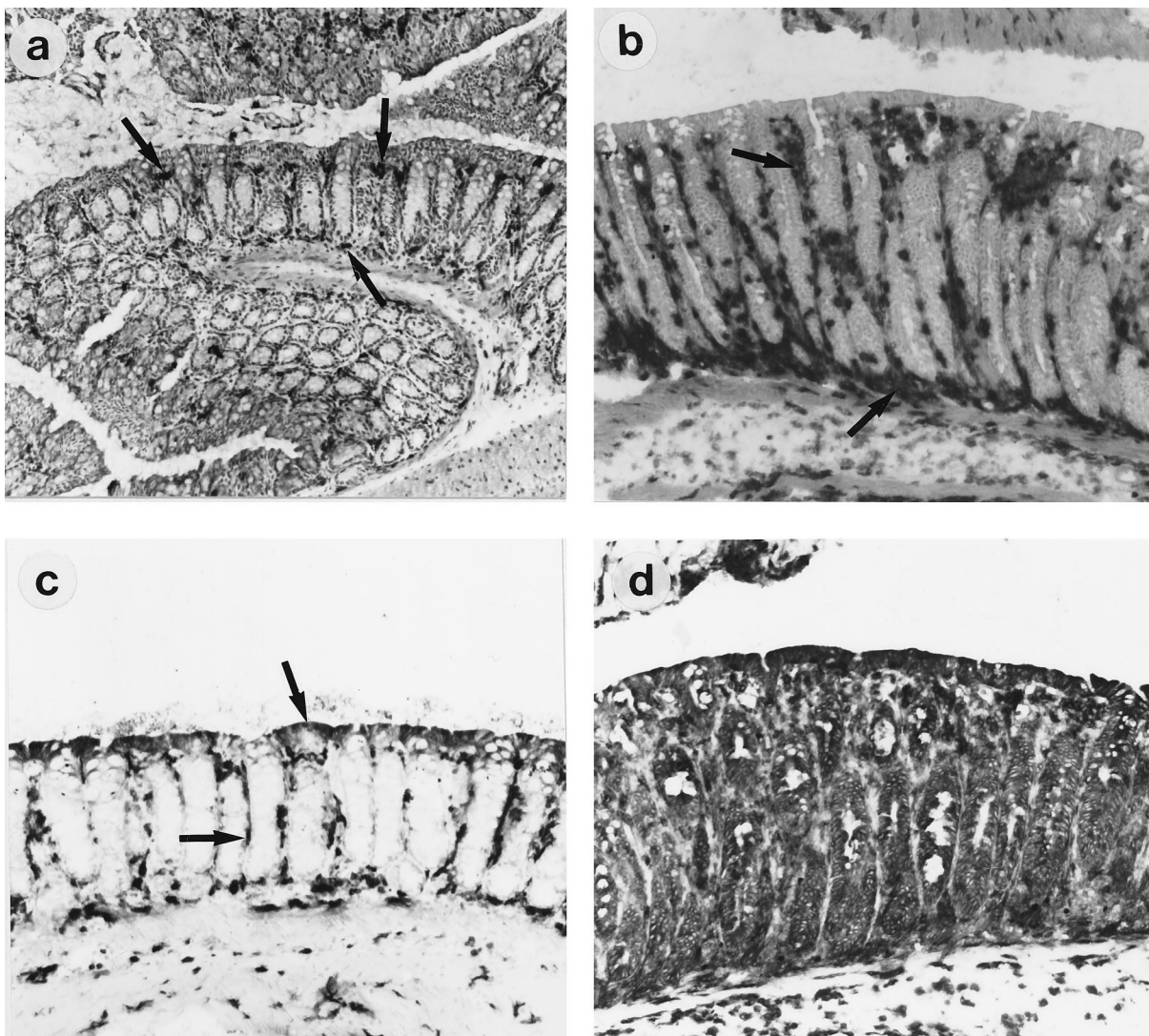


FIG. 4. Immunoperoxidase immunohistochemistry of colon tissue of infected Swiss NIH mice. (a and b) $CD4^+$ cells in mice infected with an intimin mutant strain (a) or wild-type *C. rodentium* (b). In colon tissues of control mice, only a few $CD4^+$ cells are visible in the lamina propria (arrows). In *Citrobacter*-infected mice, there is a massive accumulation of $CD4^+$ cells at the crypt bases and in the lamina propria (arrows). (c and d) MHC class II-positive cells in the lamina propria of intimin-mutant-infected mice (c) and wild-type-*C. rodentium*-infected mice (d). In control mice, MHC class II-positive cells can be seen in the lamina propria, and the surface epithelium is also positive (arrows). In *Citrobacter*-infected mice, every surface and crypt epithelial cell is strongly class II positive, as are all cells in the lamina propria. Serial sections stained with control antibodies showed only a few cells expressing endogenous peroxidase in the mucosa, although these cells were more abundant in the submucosa (see Fig. 3A). Original magnification, $\times 100$.

larly in the submucosa (Fig. 3A). The predominant cell types infiltrating the mucosa were $CD4^+$ and $CD3^+$ T cells and $CD4^+$ macrophages. Since the density of $CD4^+$ cells exceeded that of $CD3^+$ cells, we assume, but have not formally shown, that the excess $CD4^+$ cells are macrophages. There was also a slight increase in numbers of $CD3^+$ cells and $CD4^+$ cells in mice given intimin-negative *C. rodentium* over the course of the experiment (Fig. 3B and C). Rather than this being a weak inflammatory response to the bacteria, we feel that this reflects the normal age-associated increase in numbers of mucosal T cells and macrophages which occurs around this time in all mice (21). $CD8^+$ cells also increased in number, as quantified in Fig. 3D ($CD4^+$ cells are visualized in Fig. 4a and b). The infiltrate was located predominantly in the lamina propria at the base of the crypts. There was only a marginal increase in the number of intraepithelial lymphocytes, and these were

almost exclusively of the $CD8^+$ phenotype (Fig. 5). An increase in $\gamma\delta$ intraepithelial lymphocytes was not seen (data not shown). No significant difference between the wild type and controls was observed on day 2. In addition, in mice infected with intimin-negative *C. rodentium*, only a few MHC class II-positive cells were observed scattered throughout the lamina propria, and surface epithelial cells were also evident. However, in mice infected with intimin-expressing *C. rodentium* there was strong MHC class II expression on crypt and surface epithelial cells as well as on most of the cells in the lamina propria, suggesting that gamma interferon ($IFN-\gamma$) is being produced by mucosal T cells (Fig. 4c and d).

Infiltrating T cells are of a Th1 phenotype. The cytokine profile in the distal colons of infected mice was determined by competitive quantitative RT-PCR. Mice infected with wild-type *C. rodentium* showed a striking increase in mRNA tran-

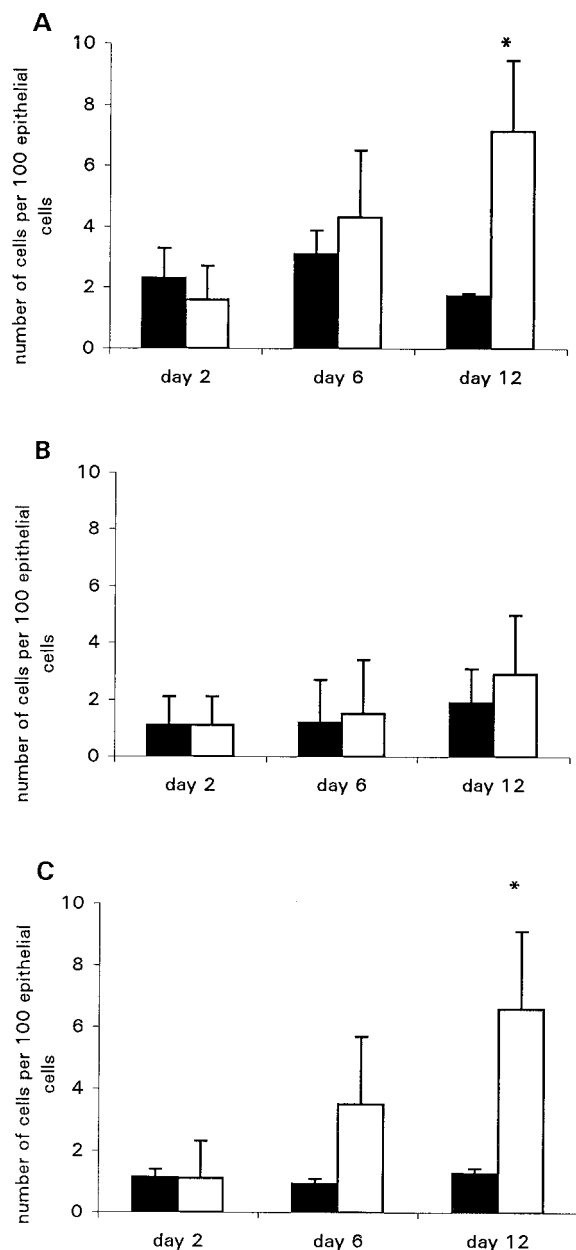


FIG. 5. Quantitation of intraepithelial cells. Counts are expressed as the number of positive intraepithelial lymphocytes per 100 epithelial cells. Each group contained five Swiss NIH mice. Closed bars represent mice infected with the intimin mutant strain; open bars represent mice infected with wild-type *C. rodentium*. Values are means \pm standard errors. (A) CD3⁺ cells; (B) CD4⁺ cells; (C) CD8⁺ cells. *, $P < 0.05$.

scripts for interleukin-1 (IL-1), tumor necrosis factor alpha (TNF- α), IL-12, and IFN- γ . Results for mice killed on days 6 and 12 are shown in Fig. 6. Transcript numbers for the Th2 type cytokine IL-4 in the colons of wild-type-infected mice were not significantly different from those of control mice (Fig. 6A to E).

Elevated production of the epithelial cell growth factor KGF. In response to proinflammatory cytokines, stromal cells produce KGF, which interacts with the KGF receptor on epithelial cells and increases epithelial cell proliferation (4). Be-

cause of the overwhelming increase in epithelial cell proliferation in wild-type-infected mice, levels of mRNA transcripts for KGF in colonic tissue were measured by quantitative RT-PCR. A dramatic increase in transcript levels was observed in mice displaying hyperplasia. Results for mice killed on days 6 and 12 are shown in Fig. 6F.

Infection of different strains of mice and infection with intimin- α -expressing *C. rodentium*. All of the results presented in this article so far represent infection of Swiss NIH mice with wild-type *C. rodentium* expressing intimin β . C3H mice were also infected with this organism as well as with a strain of *C. rodentium* expressing intimin α . Colonic hyperplasia was seen when either strain of bacterium was used, with bacteria being evident on the epithelial surface upon immunostaining (data not shown). In addition, immunohistochemistry was performed on the colonic tissues from these mice, and CD3⁺, CD4⁺, and CD8⁺ cells were counted. When C3H mice were infected with either intimin- α - or intimin- β -expressing *C. rodentium*, a significant increase in CD3⁺ and CD4⁺ cells in the lamina propria was seen, although this increase was smaller than that observed in Swiss NIH mice (Fig. 7A). There was also a significant increase in crypt length in C3H mice infected with wild-type *C. rodentium* expressing intimin β and in mice infected with *C. rodentium* expressing human intimin α (Fig. 7B).

DISCUSSION

In the present study, we have characterized the inflammatory infiltrate in the colon following oral infection of young mice with *C. rodentium*. First, we demonstrated that infection with *C. rodentium* elicited a mucosal T-cell infiltrate associated with colonic hyperplasia. Second, we demonstrated that the infiltrate consisted predominantly of CD4⁺ T cells with a Th1 phenotype. Third, the epithelial cell hyperplasia was found to be associated with KGF production. Finally, we showed that *C. rodentium* expressing either wild-type intimin β or EPEC intimin α produced a similar response following infection.

Murine colonic hyperplasia caused by *C. rodentium* was first identified more than 2 decades ago (5), but the role of inflammatory mediators in this response has not been reported. Studies of EPEC in humans tend to concentrate on the resulting diarrhea rather than the extent of mucosal damage owing to the ethical problem of taking biopsy specimens during infections. However, there have been occasional reports of small-intestinal mucosal injury in children with chronic diarrhea caused by EPEC. In one such study, biopsy samples from five of six children showed villus atrophy and crypt hyperplasia (28). Others have also reported a high incidence of villus atrophy in jejunal specimens, as well as colitis in EPEC-infected children (20). Extensive mucosal damage, including villus atrophy and crypt hyperplasia, was also seen in piglets infected with EPEC, even after the diarrhea had subsided (50). In addition, although mucosal damage was present when the large intestine was infected, diarrhea occurred only when the small bowel was affected. These studies highlight the fact that different strains of EPEC are capable of infecting both the small and large intestine, although infection of the jejunum and duodenum has been more closely associated with human disease.

The A/E epithelial lesion formed by *C. rodentium* and EPEC involves an intimate, noninvasive association of the bacteria with the host cell (14, 30, 32, 33, 36). Despite this highly characterized A/E lesion, EPEC can also enter the mucosa, a feature we visualized with *C. rodentium*. Donnenberg et al. (13) examined the invasiveness of EPEC compared to those of enteroinvasive *E. coli*, enterotoxigenic *E. coli*, and enterohem-

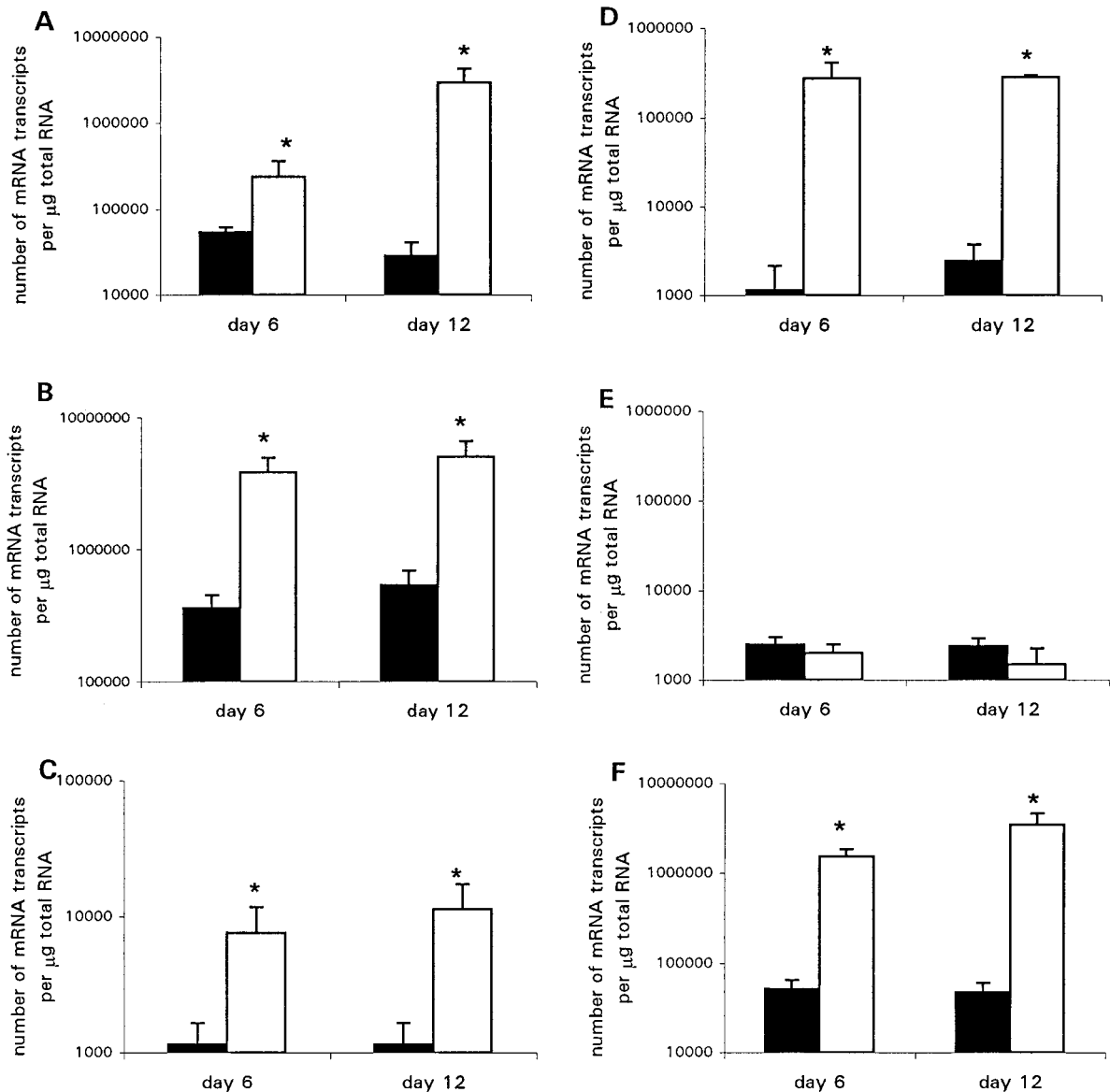


FIG. 6. Cytokine and KGF mRNA transcripts in distal colonic tissue as measured by RT-PCR on days 6 and 12 postinfection. Closed bars represent mice infected with the intimin mutant strain; open bars represent mice infected with wild-type *C. rodentium*. Each group contained five Swiss NIH mice. Values are means \pm standard errors. (A) IL-1; (B) TNF- α ; (C) IL-12; (D) IFN- γ ; (E) IL-4; (F) KGF. *, $P < 0.05$.

orrhagic *E. coli* and found, for the particular strains studied, that EPEC was actually more efficient at invading Hep-2 cell lines than was enteroinvasive *E. coli*. In infected piglets, EPEC was shown to translocate into the lamina propria, causing mucosal injury but not diarrhea (50), and it has been found within the epithelium in a small-bowel biopsy specimen from a child with acute diarrhea (19). Translocation of bacteria into the lamina propria may occur through leaky tight junctions or a damaged epithelium. Increased epithelial permeability following coinoculation of EPEC with T84 monolayers has been reported, and it has also been shown that EPEC adherence disrupts T84 barrier function, resulting in increased paracellular conductance (47). The reduction in the junctional integrity may be triggered by the activation of sodium ion transporters (12). Colonization and invasion of intestinal epithelial cell lines at different stages of differentiation have also been examined.

EPEC efficiently colonizes only fully differentiated cells, while invasion occurs only in recently differentiated, but not fully differentiated, cell lines (26).

In *C. rodentium*-infected mice, we also observed via immunohistochemistry that bacteria do transverse the epithelial barrier and enter the colonic mucosa, where interaction with host immune cells is highly likely. It is possible that these bacteria are nonviable and are passively leaking into the lamina propria. Although we have detected viable *C. rodentium* in the mesenteric lymph nodes of orally infected mice, we have no way of telling how many of the bacteria that we visualized in the mucosa and draining lymphatics are alive. A similar process is believed to occur in IBD; enteric bacteria have been isolated from the mesenteric lymph nodes of patients with Crohn's disease (2).

Peyer's patches and lymphoid follicles of the large intestine

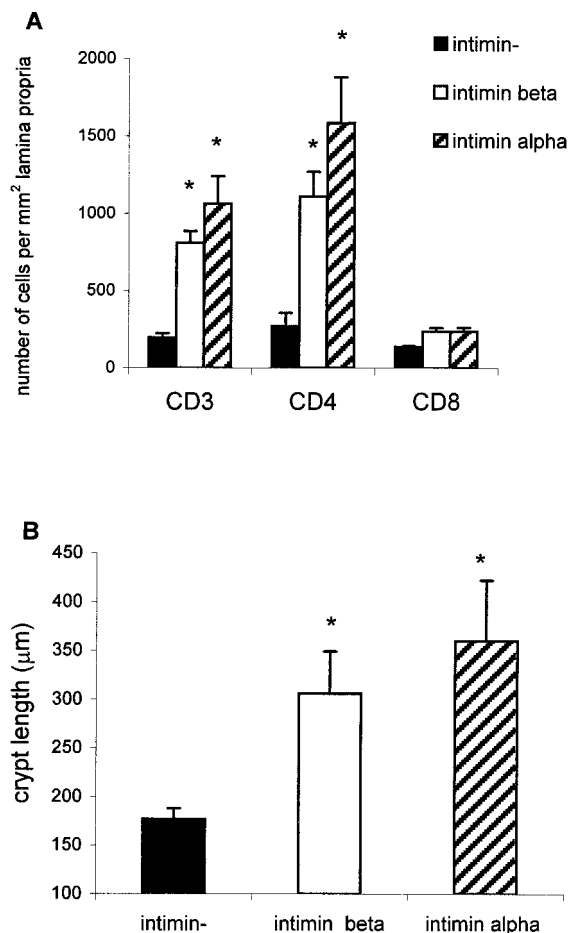


FIG. 7. (A) Cell counts on day 12 postinfection of C3H mice with wild-type intimin- β -expressing *C. rodentium* (open bars), intimin- α -expressing DBS255 (pCVD438) (hatched bars), and intimin mutant (closed bars) strains. (B) Crypt lengths (day 12) in the colons of wild-type-*C. rodentium*-infected mice (open bars) or mice infected with *C. rodentium* expressing human intimin α were significantly greater than those of mice infected with the control mutant strain (closed bars). Each group contained five mice. Values are means \pm standard errors. *, $P < 0.05$.

are the primary sites of antigen uptake from the lumen. Antigen is taken up by M cells in the follicle-associated epithelium overlying the organized lymphoid follicles (40). Many invasive pathogens use M cells as a way of transiting across the mucosal barrier (27, 31). *Yersinia enterocolitica* infects M cells by using invasin, the intimin homologue, to bind to β_1 integrins on the apical surface of these cells (11). It may also cause systemic infection by a Peyer's patch-independent mechanism (41). It is well established that rabbit EPEC adheres to M cells via either plasmid-encoded pili, AF/R1, or, in *afrA*-negative strains, some other unknown ligand (52). The M-cell receptors, however, are unknown. It is highly likely that *C. rodentium* and EPEC also interact with β_1 integrins on M cells through intimin, although this has not been formally shown.

The striking T-cell response which accompanies A/E lesion formation and crypt hyperplasia indicates that *C. rodentium* is capable of activating mucosal T cells, which under normal conditions are hyporesponsive and require strong costimulatory signals before cytokine production occurs (49). This lack of responsiveness is essential for the prevention of inappropriate immune activation to innocuous gut antigens, including the

normal flora. However, it is now well established that a breakdown in tolerance is a feature of IBD, as demonstrated by Duchmann and colleagues, who found that both peripheral blood and lamina propria T cells isolated from patients with Crohn's disease were activated by autologous gut microflora antigens (15).

The T-cell infiltration, cytokine production, and epithelial cell proliferation seen in *C. rodentium*-infected mice resemble those seen in murine models of IBD. These include mice with a variety of T-cell defects, including mice homozygous for null mutations in the genes encoding IL-2 (44), IL-10 (35), T-cell receptor alpha (TcR α) or TcR β (39), and G α_{i2} (43). In addition, T-cell-reconstituted tge26 mice transgenic for the human CD3 ϵ gene (29) and mice transgenic for IL-7 (53) develop chronic IBD. In these models, disease is a result of immune dysregulation and is mediated principally by CD4 $^+$ T cells of a Th1 phenotype and in certain cases, including TcR α knockout mice, of a Th2 phenotype (48). As in models of IBD, infection of mice with *C. rodentium* is dependent on host genetic background and gut microflora (7).

We have observed an increase in mRNA for KGF in *C. rodentium*-infected mice. Increasing epithelial cell proliferation is advantageous for the colonizing organism since it results in increased substrate surface area for formation of A/E lesions. Following T-cell activation, cytokine production triggers a cascade of events, including production of KGF (4). KGF is produced by mesenchymal cells and interacts with the KGF receptor on epithelial cells, resulting in proliferation (42). Elevated KGF mRNA levels have been detected in gastrointestinal tissue isolated from patients with Crohn's disease, ulcerative colitis, and celiac disease (9, 22, 54). In addition, the role of KGF in repair of mucosal injury has been examined in several animal models of IBD (54). In agreement with the concomitant KGF and cytokine production detected in this study, others have shown that proinflammatory cytokines, including IL-1, IL-6, and TNF- α , upregulate KGF production by fibroblasts (8, 10).

The interaction of VLA (very late activation antigen) integrins on T cells with intimin has been demonstrated previously (25), and although it is widely believed that intimin does not interact with β_1 integrins on epithelial cells (33), the function of intimin-integrin interaction in infection remains unknown. It is conceivable that costimulation of T cells by intimin expressed on the outer surface of *C. rodentium* results in mucosal T-cell proliferation.

In conclusion, we have demonstrated for the first time that *C. rodentium*, the mouse equivalent of human EPEC, induces a T-cell response that is similar to that seen in IBD. We do not, however, suggest that IBD is a result of a specific pathogenic agent but rather imply that the gut behaves in a stereotypic fashion to Th1 responses.

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