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Experimental campylobacter jejuni infection of scid mice

by *A. Berglöf*[1], *R. Feinstein*[2], *C.I.E. Smith*[1,3], *I. Riesenfeld-Örn*[1] & *K. Sandstedt*[1].
[1]Department of Immunology, Microbiology, Pathology, and Infectious Diseases,
Karolinska Institute, Huddinge Hospital, Huddinge.
[2]National Veterinary Institute, Uppsala, and [3]Center for BioTechnology, Department of Bioscience at Novum,
Karolinska Institute, Huddinge, Sweden.
Correspondance: K. Sandstedt, Department of Immunology, Microbiology, Pathology, and Infectious Diseases,
Karolinska Institute, F 89 Huddinge Hospital, S-141 86 Huddinge, Sweden.

Introduction

Campylobacter jejuni is one of the most frequent enteric pathogens that cause diarrhoeal disease in man all over the world. The bacterium, which is widely spread in the environment, has been isolated from the intestines of a variety of animals, especially birds, and from surface water and sewage. The most important sources of human infection are undercooked poultry, unpasteurised milk and contaminated water (*Altekruse et al.* 1994). The human disease caused by *C. jejuni* is characterised by a watery or bloody-mucoid diarrhoea that usually lasts 2-7 days and is in most cases self-limiting. Bacteriemia and infections outside the gastrointestinal tract seldom occur (*Walker et al.* 1986). However, more severe, prolonged and extraintestinal infections due to *C. jejuni* have also been reported, mostly in patients with hypogammaglobulinemia, suggesting that humoral immunity plays an important role in host defence against this bacterium (*Cover & Blaser* 1989). The pathogenicity of *C. jejuni* is still unclear but attachment of the microorganism to mucosal surface, tissue invasion and toxin production (*Lindblom et al.* 1990, *Klipstein et al.* 1985, *Daikoku et al.* 1990) seem to be the most important factors of its virulence. The lack of a simple animal model that reproduces the human disease has hampered the study of *C. jejuni* pathogenicity, including interactions between this pathogen and the host. During the last years several rodent models have been developed in order to mimic human campylobacteriosis but none of them has gained acceptance as universal model of the infection, since it was impossible to produce an illness similar to that seen in man (*Blaser et al.* 1983, *Blaser et al.* 1984, *Stanfield et al.* 1987, *Yrios & Balish* 1986, *Fauchere et al.* 1985). The reason why animals are more resistant to *C. jejuni* than humans is not known. The role of animal gut flora (*Yrios & Balish* 1986, *Jesudason et al.* 1989, *Field et al.* 1984) and non-specific defence mechanisms such as complement system and mononuclear phagocytes (*Bär* 1988, *Pacorbo et al.* 1994) have been emphasised by some workers.

The aim of this study was to examine the possibility of developing an animal model of the disease caused by *C. jejuni*, with both the clinical and pathological features resembling the human infection. We selected *scid* mice since they have increased susceptibility to a variety of infections, as they lack both B- and T- lymphocytes (Bosma & Carroll 1991). These mice are also suitable for studying the role of acquired immunity in protection against *C. jejuni*.

Materials & Methods

Mice: C.B-17 *scid/scid* and C.B-17 mice, 7 weeks old males, 22-25 g, were obtained from Bommice (Ry, Denmark). During the experiment all mice were housed in a sterilized plastic isolator provided with high efficiency air filters. The environmental conditions in the isolator were as follow: temperature 22°C , relative humidity 60%, 12h light – 12h dark schedule. The mice were fed sterile pellets and water ad libitum. Before the experiment the animals were checked microbiologically and serologically and were found to be free from murine pathogens.

Bacterial strain: *Campylobacter jejuni* strain CCUG 7800 was obtained from Culture Collection, University of Gothenburg. The strain, originally isolated from human faeces, was reported to produce enterotoxin (*Lindblom* 1990). The strain had been stored in -70°C before used.

Culture preparation and inoculation: The strain

was thawed and overnight cultures of *C. jejuni* were harvested and suspended in PBS-buffer to the optical density 1 at 520 nm, which had been checked to correspond with $2x10^8$ CFU/ml. All mice were inoculated orally with 0.1 ml of the bacterial suspension. The dose was controlled retrospectively by viable counts.

Bacterial cultivation: Tissue samples, 0.1g, from liver and spleen were homogenised in 0.2 ml PBS and grown on blood agar. Intestinal contents from jejunum, ileum, cecum and colon (0.05 g from each segment) were serially diluted in PBS and plated on selective blood agar containing vancomycin 20 µg/ml, cefoperazone 6.4 µg/ml and cycloheximide 100 µg/ml. All plates were incubated in microaerophilic environment (BBL Microbiology Systems jar with anaerobic generator sachet and no catalyst) at 37°C over night. The number of colony forming units was counted and the number of viable bacteria per 0.05g intestinal contents was calculated.

Antibiotic treatment: Enrofloxacin (Baytril vet, Bayer) was used in this study in attempt to disrupt the intestinal flora. Antibiotic treated animals were injected s.c. with 0.2 ml Baytril vet in dose 5 mg/kg body weight. The mice received the antibiotic for 3 days and the treatment was suspended 24 h before the bacterial administration.

Experimental design: Fifteen *scid* and 15 C.B-17 mice were infected per os with *C. jejuni* on day 0. Out of these 30 animals 8 *scid* and 8 C.B-17 were pre-treated with enrofloxacin. Groups of 4 mice (1 *scid*, 1 C.B-17 and 1 antibiotic treated mouse from each strain) were sacrificed at postinfection day 1, 3, 5, 7, 14, 21 and 28 resp. Two mice were sacrificed on day 42. Additionally 4 uninfected mice were killed as controls on day 0. All mice were necropsied immediately after euthanasia by CO_2 inhalation. At each time point samples for *C. jejuni* isolation and histopathological studies were taken. Intestinal contents were sampled for bacteriological examination. Using sterile technique the liver and spleen were removed and samples consisting of tissue blocks were investigated to examine the possibility of extraintestinal infection. Specimens of both the small and the large intestine, liver and spleen were investigated histopathologically.

Histological methods: Tissue samples were fixed by immersion in 10% buffered formalin pH 7.4. The specimens embedded in paraffin were processed routinely, sectioned at 4 µm, and stained with haematoxylin and eosin. Selected sections were stained with the Warthin-Starry (silver) method.

Results

C. jejuni was not recovered from any of the uninfected control mice. The findings in mice infected with *C. jejuni* are summarised in Table 1. During the experiment the mice did not show any clinical signs of illness, such as diarrhoea, loss of the body weight or behavioural changes. The result from bacteriological examination showed that all infected mice, except one *scid* sacrificed on day 28 p.i., were heavily colonised after oral inoculation with *C. jejuni* and the highest numbers of the organisms were recovered from the large intestine. Differences in the colonisation pattern between immunocompetent C.B-17 and immunodeficient *scid* mice, or between antibiotic treated and untreated animals within each strain were not observed. The intestines remained colonised for 49 days (as long as the experiment lasted), but the bacteria could not be recovered from liver or spleen of any of the animals.

Neither the uninfected mice, *scid* as well as C.B-17, nor the infected animals of both strains showed significant macro- or microscopical changes attributable to an infection with *C. jejuni*. Histologically, the architecture of the intestine was well preserved and differences in the appearance of the intestine, or other organs examined, between the uninfected and the infected animals were not observed. In selected sections stained with W. Starry (silver) method the campylobacters could not be found.

Discussion

Our results showed that the degree of gut colonisation by *C. jejuni* was similar in *scid* and C.B-17 mice. However, although the mice were heavily colonised, no clinical signs were observed. This was consistent with the absence of intestinal damage. The fact that *scid* mice, lacking mature B and T lymphocytes, did not develop disease suggests that specific humoral and cellular immunity are not directly involved in protection against *C. jejuni* in our model. This is in contrast to the results found in previous studies. *Lane et al.* (1987) and *McSweegan et al.* (1987) reported about the importance of

Table 1. Experimental infection with *C. jejuni* in C.B-17 and *scid* mice.

			Bacteriological examination. [CFU/0.05g intestinal contents]			
mice	**body weight^**	**days^^**	**jejunum**	**ileum**	**cecum**	**colon**
C.B-17	24.8	1	0	0	10^6	10^5
C.B-17*	25.5	1	0	0	10^6	10^6
scid	22.3	1	10^3	10^3	10^5	10^5
*scid**	22.5	1	10^4	10^4	10^6	10^6
C.B-17	25	3	10^4	10^5	10^7	10^7
C.B-17*	26.5	3	0	10^5	10^5	10^7
scid	22.1	3	0	0	10^7	10^7
*scid**	24.2	3	10^1	10^4	10^7	10^7
C.B-17	27.5	5	10^3	10^4	10^5	10^5
C.B-17*	24.5	5	10^4	10^5	10^6	10^6
scid	22	5	10^4	10^5	10^7	10^7
*scid**	23.3	5	0	10^3	10^6	10^5
C.B-17	27.2	7	10^3	10^4	10^7	10^7
C.B-17*	21.6	7	10^4	10^3	10^7	10^7
scid	23.6	7	10^2	10^3	10^5	10^5
*scid**	20.5	7	10^4	10^5	10^6	10^6
C.B-17	26.4	14	10^5	10^5	10^7	10^7
C.B-17*	25.1	14	10^3	0	10^7	10^7
scid	24.7	14	0	10^4	10^7	10^7
*scid**	23	14	10^1	10^5	10^7	10^7
C.B-17	25.4	21	10^4	10^4	10^7	10^6
C.B-17*	23.3	21	10^3	10^4	10^6	10^5
scid	26.4	21	10^4	10^4	10^7	10^7
*scid**	26.8	21	10^7	10^7	10^7	10^7
C.B-17	26.5	28	10^4	10^4	10^7	10^6
C.B-17*	31.3	28	0	0	10^5	10^5
scid	22.6	28	0	0	0	0
*scid**	24.1	28	10^1	10^4	10^7	10^7
C.B-17*	28.9	49	10^5	10^5	10^5	10^5
*scid**	27.6	49	10^4	10^5	10^7	10^7
Controls						
C.B-17	22	0	0	0	0	0
C.B-17	23.6	0	0	0	0	0
scid	20.1	0	0	0	0	0
scid	24.9	0	0	0	0	0

* mice treated with antibiotic
^ body weight at euthanasia
^^ time elapsed between infection and euthanasia

secretory IgA in preventing colonisation with campylobacters. *Ueki et al.* (1987) found reduced colonisation of the intestinal tract when the strain of *C. jejuni* was treated with IgM class monoclonal antibody before inoculation into suckling mice. Results presented by *Abimiku & Dolby* (1987) showed the possibility of protecting infant mice against *C. jejuni* colonisation when dams were vaccinated i.p. with heat-killed bacteria before mating. High prevalence of *C. jejuni* infections in patients with congenital or acquired hypogammaglobulinemia as well as rising titers of specific antibodies in patients with campylobacteriosis also indicate the protective role of immunoglobulins (*Blaser & Duncan* 1984, *Johnson et al.* 1984). Nevertheless, the cell-mediated immunity appears to be less important in defence against campylobacters. Infections in patients with acquired immune deficiency syndrome seem to be associated with defective humoral response to this organism (*Perlman et al.* 1988, *Chui & Owen* 1994). T cells can still play a role in controlling the spread of *C. jejuni* to internal organs since dissemination occurred more often in experimentally infected athymic than in euthymic mice (*Yrios & Balish* 1986). In our experiment a great number of *C. jejuni* were microbiologically isolated from mucus and intestinal contents. Interestingly, no bacteria were seen on histological sections stained with the W. Starry (silver) method. The bacteria, which are mucus colonisers (*Lee et al.* 1986), probably did not adhere to epithelial cells and that could explain their absence in histologically stained sections. The campylobacters were not found outside the intestinal tract neither in C.B-17 nor in *scid* mice. This may be due to the fact that the strain used in the experiment did not produce cytotoxin. It has been suggested that this toxin may be involved in tissue invasion and spread of the bacteria from intestinal lumen (*Pang et al.* 1987, *Mizuno et al.* 1994). The protective role of the indigenous microflora in preventing gut colonisation with different intestinal pathogens, including *C. jejuni*, has been shown by many authors. There are results suggesting that commensal flora may impede luminal colonisation with pathogens by inhibiting their growth, competing with them about nutrition or by decreasing their pathogenesis in different ways. Antibiotic administration, which results in disruption of the intestinal flora, is known to enhance gut colonisation with pathogens (*Yrios & Balish* 1986, *Field et al.* 1984). In our experiment the treatment with enrofloxacin did not result in increased colonisation with *C. jejuni*. *Scid* and C.B-17 mice, whether treated or untreated with antibiotic showed the same colonisation pattern, suggesting that normal flora does not affect colonisation with *C. jejuni*. On the other hand this study shows that *scid* mice can become inapparent carriers of *C. jejuni* infections. Finally, our results indicate that *scid* mice orally inoculated with *C. jejuni* do not develop a disease comparable to human campylobacteriosis. Still more work remains to be done before a suitable mouse model is developed.

Summary

Campylobacter jejuni was inoculated orally to C.B-17 *scid/scid* (severe combined immunodeficient) and C.B-17 immunocompetent mice. Groups of mice from each strain were pretreated with enrofloxacin in an attempt to increase colonisation. All mice were heavily colonised but neither clinical signs of illness nor histological lesions were observed. Results from our experiment indicate that specific humoral and cellular immunity are not directly involved in the protection against *C. jejuni*, since *scid* mice were not more sensitive to the infection than C.B-17 mice. Animals, treated as well as untreated with antibiotic, showed the same colonisation pattern which suggests that normal flora does not hamper establishing of this bacterium in the intestine. On the other hand this study shows that both C.B-17 and *scid* mice may become inapparent carriers of *C. jejuni*.

Sammanfatning

C.B-17 *scid/scid* (severe combined immunodeficient) och C.B-17 immunokompetenta möss inokulerades peroralt med *Campylobacter jejuni*. En grupp av möss från varje stam förbehandlades med enrofloxacin för att främja kolonisation. Alla möss blev koloniserade med stora mängder av *Campylobacter*. Dock kunde varken kliniska eller histologiska tecken på sjukdom observeras. Eftersom *scid* möss inte visade större känslighet för infektionen än immunokompetenta möss antyder resultaten att specifik cellulär och humoral immunitet inte deltar i det direkta skyddet mot *C. jejuni*. Såväl antibiotika-behandlade djur som obehand-

lade visade samma kolonisationsmönster, vilket tyder på att normalfloran inte hindrar etablering av bakterier i tarmen. Emellertid ger studien belägg för att både C.B-17 och *scid* möss kan bli symtomlösa bärare av *C. jejuni*.

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