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Genetic susceptibility to Campylobacter infection

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Abstract

Genetic susceptibility to Campylobacter infection

Genetic factors partially determine the susceptibility of an individual to Campylobacter infection. The genes that are specifically responsible for this have not been identified but probably do play a role in gastric acid production and in the immune response (specific humoral- and cell-mediated immunity). Genetic studies in humans could very well increase our understanding of an individual's susceptibility to Campylobacter infection. Insights gained in this way can contribute to the development of more realistic risk-assessment models and the implementation of effective protective measures. Campylobacter, transmitted through the food chain, is an important cause of bacterial gastro-enteritis. Because approximately 59,000 people in the Netherlands suffer every year from a Campylobacter infection, current government policy is aimed at restricting the spread of Campylobacter, mainly by eliminating the bacterium from the food chain. Extensive intervention strategies have, however, only been partly successful, making it important to define groups in the population that are extra susceptible to this pathogen. Epidemiological research has shown that susceptibility to Campylobacter is in part determined by genetic factors. However, environmental factors (e.g. kitchen hygiene, use of medication) are also clearly important. Appropriate cohorts for studying the role of both genetic and environmental factors in the development of Campylobacter infection are available via the RIVM.

Keywords: Campylobacter, susceptibility to infection, genetic factors, defence, genetic studies

Rapport in het kort

Genetische gevoeligheid voor Campylobacter infectie

De gevoeligheid van personen voor een infectie met de Campylobacter-bacterie wordt voor een deel bepaald door genetische factoren. Welke genen hier specifiek voor verantwoordelijk zijn is nog niet bekend, maar waarschijnlijk spelen zij een rol in de maagzuurproductie en de immuunrespons (specifieke humorale en cellulaire immuniteit). Genetische studies in mensen kunnen inzicht geven in de gevoeligheid van individuen voor Campylobacter-infecties. Dit inzicht draagt bij aan de ontwikkeling van realistische risicoschattingsmodellen en aan het implementeren van beschermingsmaatregelen voor gevoelige groepen in de samenleving.

De Campylobacter-bacterie is een belangrijke veroorzaker van bacteriële gastro-enteritis (een infectie van het maag-darmkanaal). Deze bacterie wordt vooral via voedsel overgebracht. Aangezien jaarlijks ongeveer 59.000 personen een Campylobacter-infectie krijgen, is het overheidsbeleid erop gericht om de verspreiding van de bacterie tegen te gaan. Het beleid richt zich vooral op het weren van de bacterie uit de voedselketen. Omdat de maatregelen op dit gebied tot op heden maar beperkt succesvol bleken, is het van belang risicogroepen te identificeren die extra gevoelig zijn voor de bacterie.

In eerder epidemiologisch onderzoek is vastgesteld dat de gevoeligheid voor de bacterie ook een genetische achtergrond heeft. Uiteraard blijven omgevingsfactoren (hygiëne, gedrag bij voedselbereiding en medicijngebruik) ook belangrijk. Het RIVM beschikt over cohorten waarin de rol van zowel genetische- als omgevingsfactoren op het ontwikkelen van een Campylobacter infectie bestudeerd kan worden.

Trefwoorden: Campylobacter, infectiegevoeligheid, genetische factoren, afweer, genetische studies.

Contents

SAMENVATTING

Campylobacter is de belangrijkste veroorzaker van bacteriële gastro-enteritis in Nederland en naar schatting 100.000 mensen maken jaarlijks een Campylobacter-infectie door. Niet ieder individu is even gevoelig voor infectie met Campylobacter, maar in hoeverre genetische factoren een rol spelen bij het bepalen van zulke verschillen is niet bekend. Epidemiologische bevindingen, bevindingen in patiënten, zeer beperkte bevindingen in diermodellen en in humane vrijwilligersstudies geven duidelijk aan dat maagzuurproductie, humorale immuniteit en cellulaire immuniteit belangrijk zijn voor de afweer tegen Campylobacter. Patiënten met hypo- of agammaglobulinemie, die een defect in humorale immuniteit hebben, en patiënten met HIV of AIDS, die een defect hebben in cellulaire immuniteit zijn zeer gevoelig voor Campylobacter-infectie. Deze patiënten zijn echter niet alleen gevoelig voor Campylobacter maar ook voor een aantal andere pathogenen. Ook een verlaagde maagzuurproductie leidt tot gevoeligheid voor meerdere pathogenen waaronder Campylobacter. Omdat er geen goed ziektemodel is voor Campylobacter in proefdieren is er weinig inzicht in welke gastheerfactoren de gevoeligheid voor Campylobacter-infectie bepalen. Hoewel het duidelijk is dat genetische factoren een rol spelen is het niet bekend of een bepaalde set genen nu juist de gevoeligheid voor dit specifieke pathogeen bepaalt. Daarom is het van belang om meer inzicht te krijgen in de afweer van de gastheer tegen Campylobacter. Humane studies in goed gedefinieerde patiënten populaties die een ernstige Campylobacter-infectie hebben doorlopen kunnen ons inzicht in deze gastheerfactoren vergroten. Genetische studies waarin een beperkt aantal veel voorkomende genetische variaties (polymorfismen) in bovengenoemde routes bestudeerd worden, zullen bijdragen aan een beter begrip van de verschillen in gevoeligheid van individuen voor Campylobacter en aan de ontwikkeling van realistische risicoschattingsmodellen. Het RIVM beschikt over cohorten van vrijwilligers waarmee dergelijke studies kunnen worden uitgevoerd (bijvoorbeeld de CASA studie).

SUMMARY

Campylobacter is the most common form of bacterial gastro-enteritis in the Netherlands. It was estimated that approximately 100,000 people suffer from this infection each year. Not all individuals display the same susceptibility to Campylobacter infection but to what extent genetic factors contribute to this is unknown. Epidemiological observations, observations in patients, limited data on animal models and human volunteer studies clearly show that acidity of the stomach, humoral immunity and cell-mediated immunity is important in the host-defence against Campylobacter. For instance observations in patients show that patients with hypo- or agammaglobulinemia, who display defects in humoral immunity, patients with HIV/AIDS, who display a defect in cell-mediated immunity, are very susceptible to Campylobacter infection. However, these patient groups are not selectively susceptible to this pathogen en suffer from a variety of infections. Also reduced acidity of the stomach does not lead to selective susceptibility to Campylobacter infection. Due to the lack of an appropriate animal model for Campylobacter-induced disease, knowledge on host-mechanisms involved in the defence against Campylobacter is limited. Although it is clear that genetic factors play a role in determining susceptibility to Campylobacter infection there are no indications that a certain, limited set of genes is involved in this process. Therefore, there is an urgent need to expand our knowledge on host mechanisms necessary for the defence against Campylobacter infection. Genetic studies in well-defined patient populations that suffered from severe Campylobacter disease are a way to expand our knowledge. Small-scale studies aimed at investigating genetic polymorphisms in a limited number of candidate genes in the above mentioned pathways will contribute to our understanding of the human host-defence against Campylobacter and to the development of realistic risk-assessment models.

1. INTRODUCTION

Campylobacter is the most commonly reported cause of acute bacterial gastro-enteritis in the Netherlands and, although the infection is usually self-limiting the total disease burden in the population is high (1). Campylobacter induced gastro-enteritis is mainly caused by *C.* jejuni and C. coli and is transmitted via contaminated food. It was calculated that in 2004 there were approximately 6200 laboratory confirmed cases of Campylobacter enteritis in the Netherlands (2). It is estimated that around 25 people die of Campylobacter infection every year (3). The true incidence of Campylobacter enteritis in the population is estimated to be much higher than suggested by the incidence figures, since most people with Campylobacter gastro-enteritis recover from their disease without consulting a physician. In addition, of patients who do consult their physician, Campylobacter faecal cultures are not always made. The true number of Campylobacter enteritis cases in humans was calculated at approximately 59,000 per year (3;2). The direct health-care costs for Campylobacter induced gastro-enteritis and sequelae of disease, such as Guillain-Barré syndrome and Reactive Arthritis, were calculated to be around 8.5 million euro in 2004 and the total costs of illness were calculated to be around 18 million euro in 2004 (3). The disease burden for 2004 was 875 disease adjusted life years (DALY) (3).

Since the start of Campylobacter registration in 1996, there has been a large variation in the number of Campylobacter cases (Figure 1). Some of these changes in incidence can be explained by changes in chicken consumption. For instance, due to the avian flu outbreak in 2003, chicken sales were lower in that year. Consequently, there were also significantly fewer Campylobacter cases. Most Campylobacter infections occur as a single case and outbreaks are rare. The few reported outbreaks are most commonly associated with raw milk. Although the common belief is that poultry is the major source of infection it is estimated that in the Netherlands 20-40% of all cases are attributable to chicken (4). Other risk factors include, drinking raw milk, travelling abroad and contact with pets. However, a large proportion of all infections (i.e. approximately 50%) cannot be attributed to any of the known risk factors indicating that other sources exist. Extensive intervention strategies have been employed to eliminate Campylobacter from the food-chain and although in Scandinavian countries these measures resulted in a reduction in frequency of Campylobacter in chickens, complete elimination of Campylobacter from poultry is probably not achievable. Since exposure of the population to Campylobacter cannot be prevented, it is crucial to understand the risks involved with exposure and to identify groups in the population that are more at risk.

Currently available risk assessment models do not explicitly take into account that individuals display differential susceptibility to infection. A better understanding of the pathogenic mechanisms of Campylobacter and, importantly, of the host-factors involved in the defence against Campylobacter infection may lead to the identification of risk factors in the population. It is conceivable that the efficacy of some of these host factors in the defence against Campylobacer is genetically determined. Studying these factors could contribute both to novel intervention strategies and to the development of more realistic risk-assessment models which incorporate such host-susceptibility factors.

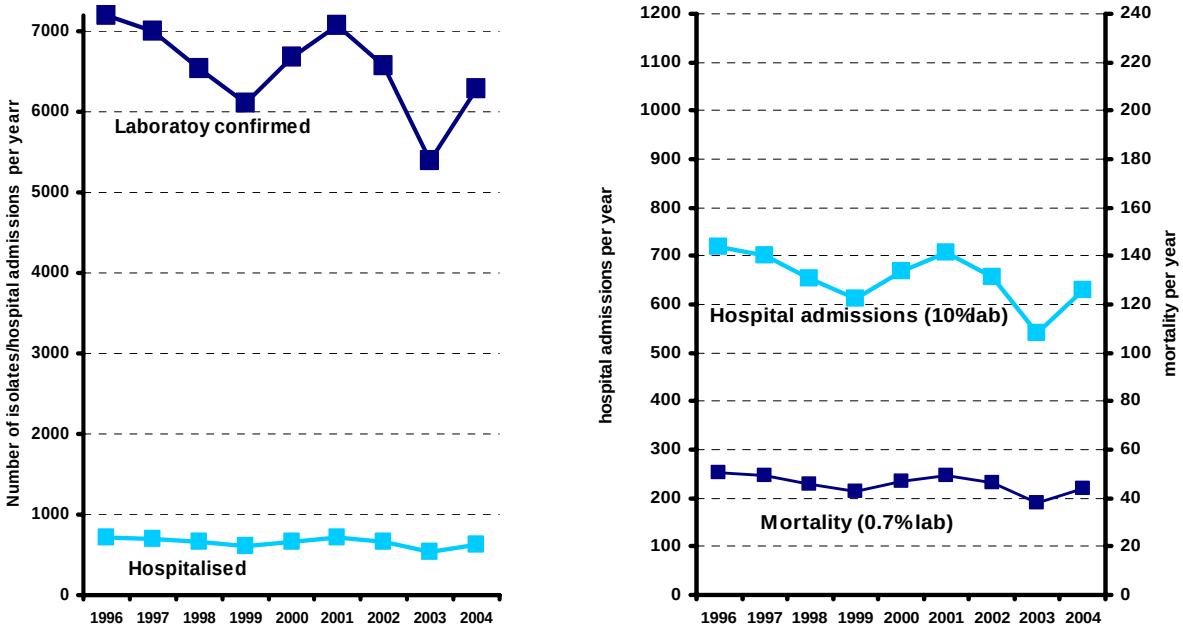

Figure 1. Incidence of laboratory confirmed Campylobacter infection, Campylobacter-associated hospital admissions and Campylobacter-associated deaths. The numbers are corrected to cover 100% of the Netherlands.

The scope of this study was to summarise available data on genetic factors involved in the host-response to Campylobacter. In other diseases, the majority of such factors are elucidated by studying infection in murine models with well-defined genetic mutations in host-defence mechanisms. However, Campylobacter does not induce disease in mice and other appropriate animal models are lacking. The data available are obtained from epidemiological studies and patient research. Therefore, this study was aimed at summarising the current understanding of host mechanisms involved in the defence against Campylobacter by evaluating epidemiological observations and observations in patients, together with limited observations

in animal models and human volunteer studies. These data shed some light on the host-defence against Campylobacter in humans and may contribute to the design of genetic studies in patients with severe Campylobacter infection.

2. DISEASE CAUSED BY CAMPYLOBACTER

2.1 Pathology of Campylobacter infection

Campylobacter enters the host through contaminated food or drink. After passage through the acidic environment of the stomach a large proportion of the inoculum is killed. The remaining bacteria survive and are able to adhere to intestinal epithelial cells or to the mucus overlying these cells and replicate in the intestine. In infected individuals this results either in a colonisation status not accompanied by any clinical symptoms –i.e. bacteria are present in the intestine but they do not induce disease - or in gastro-enteritis. Campylobacter is highly infectious with a probablility of 2% for any colony forming units to establish infection in a volunteer experiment (5;6).

The colonisation status in humans is reminiscent of that found in various rodents, mammals and birds. Colonised animals can shed large amounts of Campylobacter in their faeces. The difference between humans and animals is that in the latter Campylobacter fails to cause gastro-enteritis, indicating either that animals lack specific factors, e.g. receptors, necessary for Campylobacter to cause disease, that efficient immune mechanisms are operative in animals that prevent the development of clinical disease, or that disease-causing host-responses are absent.

After colonisation of the intestine clinical disease can occur. Based on the clinical syndromes found in patients three mechanisms were postulated by which Campylobacter can induce disease (7):

1. adherence of Campylobacter to the intestine and production of enterotoxins, which alter the fluid resorption capacity of the intestine resulting in secretory diarrhoea;
2. bacterial invasion and replication within the intestinal mucosa accompanied by an inflammatory response resulting in blood-containing, inflammatory diarrhoea;
3. passage of the intestinal mucosa and migration to extra-intestinal sites via the lymphatic system resulting in systemic disease It is important to note that systemic disease is very rare in immunocompetent individuals and that Campylobacter is not believed to be transmitted from person to person.

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Clinical disease is characterised by acute diarrhoea accompanied by intense abdominal pain. Campylobacteriosis is an inflammatory enteritis which commonly extends down the intestine to affect the colon and the rectum. The incubation time is 1-7 days (mean: 3 days), which is longer than most other intestinal pathogens. The diarrhoea can either be watery or bloody (15-25% of cases) (1;8) indicating that the extent of intestinal inflammation varies between individuals. Usually diarrhoea begins to ease after 3 to 4 days but Campylobacter can be found in the faeces for several weeks. Although a lot of patients feel nauseous, only about 15% of patients vomit (8).

In 30% of patients the disease does not start with diarrhoea but with a prodrome of influenza-like symptoms such as fever, headache, dizziness and myalgia. Patients that suffer from such a prodrome tend to have more serious disease than patients without the prodrome but the reasons for this are currently unknown.

In most immunocompetent individuals campylobacteriosis is a self-limiting disease and treatment with antibiotics will only reduce the period of faecal shedding and is therefore not necessary (9;10). When patients suffer from recurrent or systemic infections with Campylobacter, antibiotic treatment helps resolve the infection. However, an increase in the occurrence of antibiotic resistance both in human isolates and in isolates from poultry has been observed over the last decade (2).

2.2 Sequelae of Campylobacter infection

Campylobacter enteritis is usually self-limiting and in the majority of cases the disease is resolved within one week. However, some individuals develop sequelae after Campylobacter infection. Approximately 1:1000 infected individuals develops Guillain-Barré syndrome (GBS), a serious autoimmune-mediated neurological disease which can cause symptoms ranging from weakness of extremities to complete paralysis and respiratory insufficiency. Mortality rates due to GBS in de developed world are 2-3% although the majority of patients recover completely within 6-12 months (11). The health burden for GBS was 164 DALY in 2004 (3).

GBS is thought to occur because of molecular mimicry between cell-wall components of Campylobacter and sugar moieties on nerve-gangliosides (12). Antibodies that are raised against Campylobacter during infection can therefore in some individuals cross-react with nerve gangliosides leading to demyelinisation of nerves. Miller-Fisher syndrome is a sub-variant of GBS which predominantly affects the nerves that govern eye movement. Genetic

factors involved in determining susceptibility to GBS are now being studied extensively and HLA, FAS, and Fc receptors (receptors for immunoglobins) are believed to be involved (13-15). However, GBS can also be induced by infection with other pathogens and genes that determine susceptibility to GBS may not be involved in determining susceptibility to gastro-enteritis.

Other immune-mediated sequelae of Campylobacter infection include Reactive Arthritis (16;17) and Reiter syndrome, an inflammatory disease with either conjunctival or urethral inflammation (18). Symptoms of Reactive Arthritis usually occur around 14 days after infection (range: 3 days-6 weeks) and the estimated incidence of Reactive Arthritis in community outbreaks ranges from 0 to 7% (3;19-21). Usually these joint symptoms resolve completely. There are also a few case reports of Hemolytic-Uremic Syndrome after Campylobacter infection (8) and although Campylobacter has been isolated from patients with inflammatory bowel syndromes (IBD) such as Crohn’s disease and has been associated with flare-ups of IBD, a causal link between the two is still under debate (22-24).

3. VIRULENCE OF CAMPYLOBACTER

Although Campylobacter species play a leading role in bacterial diarrhoeal diseases, detailed knowledge on the bacterial virulence factors is still very limited (25). Genome analysis of *C. jejuni* NCTC11168 did not directly support the discovery of classical virulence mechanisms. The genome seemed to lack information encoding homologues of bacterial toxins, adhesions, invasions, protein secretion systems or pathogenicity islands (26). Further investigation of the Campylobacter genome however revealed hidden virulence genes required for adaptation to specific conditions in the vertebrate gut. Currently, identified bacterial factors involved in host cell invasion are capsule synthesis, polysaccharides, motility, adhesion and protein secretion systems (27-30). The cytolethal distending toxin also has been identified to contribute to pathogenesis. It is present in culture supernatants and is required for full invasiveness in immunodeficient mice (1).

Adaptation of Campylobacter to the host plays a key role in pathogenesis, a view that is supported by pronounced genetic intra-species variability, which is thought to be driven by clonal selection. Genetic variation in surface structures of the bacteria is due to the presence of hypervariable sequences in genes involved in the synthesis of such surface structures (31). But extensive intra-species variability in genes for surface structures is also caused by insertion or deletion of complete sets of genes. (32). Moreover, expression of genes encoding a major outer membrane protein in C. jejuni responds to temperature shift (33), one of the factors involved in regulating virulence gene expression in pathogenic bacteria.

4. THE ROLE OF HUMAN IMMUNITY IN CAMPYLOBACTER DISEASE

4.1 Epidemiological observations

There is a lot of epidemiological evidence to suggest that not every individual has the same susceptibility to infection with Campylobacter. When outbreak data are analysed it is clear that not every person exposed to a certain dose of Campylobacter will be colonised or will develop disease. These differences can be associated with non-specific factors such as stomach content and, related to this, the acidity of the stomach; indeed the use of proton-pump-inhibitors in the month prior to Campylobacter infection was shown to increase the risk of clinical disease by as much as 10-fold (34). However, also innate and specific immune factors play a role in determining the susceptibility of an individual to Campylobacter infection.

In developing countries only children appear to be susceptible to Campylobacter enteritis whereas in developed countries Campylobacter disease occurs in all age-groups and the course of disease is generally more severe; i.e. infection is more often accompanied by bloody diarrhoea (35;36). In addition, it is thought that in the developing world asymptomatic infections are more common than in the Western world. In the developing world children are exposed to Campylobacter infection early in life, as exemplified by an early rise in Campylobacter-specific antibodies and higher antibody levels than in children in the USA (37-39). In Thailand, bloody diarrhoea was most often associated with disease in the first year of life suggesting that bloody diarrhoea is associated with primary infection and that frequent exposure to Campylobacter results in protection against disease (40). However, frequent exposure does probably not result in protection against colonisation (36).

Observations in abattoir workers in Sweden support the idea that frequent exposure to Campylobacter induces protection against disease. Recently employed and, presumably immunologically naïve workers, suffered many more episodes of Campylobacter diarrhoea than workers who were employed for many years. Consistent with the observation in the developing world, the latter group of workers did regularly succumb to asymptomatic infection with Campylobacter (41;42) These data indicate that humans can develop immunity

to Campylobacter disease, but probably not to colonisation, although this immunity seems to be short-lived and frequent exposure is necessary to boost this immunity.
In conclusion these epidemiological observations indicate that differences in immune response are observed in various individuals but to what extent they are genetically determined remains to be established. It is also clear that acidity of the stomach is a crucial early defence mechanism against Campylobacter. Although this is not specific for Campylobacter and has also been observed for other pathogens such as Salmonella, the genes encoding the proton-pumps involved in acid production could be further explored as possible candidate genes for human studies on Campylobacter susceptibility.

4.2 Observations in patients

Certain groups of patients are more susceptible to Campylobacter disease than the general population. The two main groups of patients that are very susceptible are patients with hypo- or agammablobulinemia, who suffer from a defect in humoral immunity and patients with HIV or AIDS, who suffer from a defect in cell-mediated immunity (8;43). These patients often experience more severe clinical disease that is more frequently accompanied by bacteraemia, i.e. presence of bacteria in the blood. The incidence of Campylobacter disease in AIDS patients was shown to be 40-fold higher than in the general population (44). Chronic carriage and recurrent infection is also more frequently found in these populations of highly susceptible patients and repeated courses of antibiotic treatment are often needed to resolve the infection. Severe Campylobacter infection is found in patients with HIV or AIDS both in the Western world and in developing countries (45).
The (genetic) causes of the above mentioned immunoglobulin deficiencies can be a result of a whole range of primary or acquired immune deficiencies and these patients are not only susceptible to Campylobacter, but to a whole range of other pathogens. The most frequent cause of hypogammaglobulinemia is Common Variable Immunodeficiency, a heterogeneous disease which occurs in approximately 1:50,000-100,000 Caucasians. Mutations in the gene encoding ICOS, a co-stimulatory molecule essential for proper B-cell activation is one genetic cause of Common Variable Immunodeficiency (46). Agammaglobulinemia is a very rare but serious recessive X-linked disease which is usually caused by mutation in Bruton tyrosine kinase and enzyme essential for B-cell maturation.
From these observations it can be concluded that genetic factors are involved in determining

susceptibility to Campylobacter although it has to be taken into account that the above mentioned diseases all lead to severe immune defects resulting in susceptibility to a whole range of pathogens. Thus, although these patient groups shed some light on host-responses involved in the defence against Campylobacter, they do not explain susceptibility to this specific pathogen.

4.3. Humoral immunity to Campylobacter

After infection with Campylobacter antibody responses appear from day 5 of illness and peak within 2 to 4 weeks. IgA levels decline within one month after onset of illness whereas IgM and especially IgG remain high for a longer time (47-49). Intestinal antibodies are also produced and human volunteer studies have shown that infection induces short-term immunity against the homologous strain. Like in serum, IgA in saliva declines rapidly after infection whereas IgG remains higher for a longer time (49).

A large proportion of the antibody response against Campylobacter is directed against LPS, outer membrane proteins and flagellin antigens FlaA and FlaB. Cytolethal Distending Toxin (CDT), the toxin produced by Campylobacter, is also an important target of the human antibody response. Interestingly, chickens do not develop neutralizing antibodies against CTD indicating that there is host-specificity in the immune response to Campylobacter (50).

4.4 The role of humoral immunity in protection

As described above epidemiological data indicate that humoral immunity is crucial for the development of protection against Campylobacter disease. Consistent with this, patients with defects in immunoglobin production are more susceptible to infection.

The first humoral immune mechanism encountered by Campylobacter during infection is secretory IgA (sIgA) and various studies have shown that the presence of Campylobacter-specific sIgA and serum IgA correlates with protection against disease (51;52). Also studies in breast-fed infants point to a protective role of sIgA against infection. In a Mexican study, where children were followed from birth to the age of 2 years, breast feeding decreased the incidence of diarrhoea caused by C. jejuni and this decrease was associated with the presence of Campylobacter-specific sIgA in breast-milk (51). Breast-milk containing sIgA against Campylobacter flagellin proteins also decreased the incidence of Campylobacter induced

diarrhoea in babies. In addition, there is also a description of one immunocompromised patient in which oral sIgA administration resolved a recurrent Campylobacter infection (53).

Even though all these data point to an important role for (s)IgA in protection against Campylobacter disease it is surprising that there are no studies to suggest that patients with IgA deficiency are more susceptible to Campylobacter infection than the general population (54). IgA deficiency is the most common primary immunodeficiency found in humans and it is estimated to occur at a frequency of 1:333-1:700 in Caucasians. The genetic cause underlying IgA deficiency is unknown but from these data it can be concluded that in the absence of IgA other compensatory mechanisms are activated and that IgA is important but not crucial for the host-defence against Campylobacter.

A protective role of IgM against Campylobacter infection was suggested by the observation that in hypo- or agammaglobulenemic patients who suffered from severe Campylobacter infection, infusion of a pentaglobin preparation, which contained Campylobacter-specific IgM, completely resolved the infection whereas immunoglobin preparations that only contained IgG did not (55). Although this observation was made in a small number of patients, it does point to a role of IgM in protection. This also fits with the assumption that increased IgM production is one of the general immune-compensation mechanisms in patients with IgA deficiency. In addition, there is an active secretion mechanism for IgM at mucosal surfaces (56), and IgM antibodies can fix complement almost 200 times more efficiently than IgG (57). In contrast to Campylobacter-specific IgG, IgM can also enhance reactive oxygen intermediate production and bactericidal activity of PMN (58).

From the finding that patients with hypo- or agammaglobulinemia are more susceptible to Campylobacter infection, it is clear that IgG also plays an important role in protection against disease. IgG remains high for a longer time than IgA and IgM after infection (47;49). Chronic raw milk consumers have high IgG and seem to be protected against disease (59). Similarly, children in developing countries develop IgG responses very early in life and are then protected against bloody diarrhoea (37;38), indicating that IgG is also involved in protection against disease.

These observations point to several candidate genes which may be involved in determining susceptibility to Campylbacter. For instance genes involved in secretion of IgA or IgM could be studied. In addition, complement factors or receptors for immunoglobins (Fc Receptors), which are important for clearance of bacteria could be studied.

4.5 Cellular immunity

Serious and recurrent Campylobacter infections in patients with HIV or AIDS, who have a strong reduction in CD4+ T-cells, point to an important role of cell-mediated immunity in the defence against Campylobacter infection although B-cell responses and antibody production can also be impaired in AIDS patients. There is one report on cellular immunity of a patient who suffered from severe Campylobacter infection. Peripheral blood mononuclear cells of this patient proliferated in response to the homologous strain (60). In addition rapid induction of pro-inflammatory cytokine production was observed in the serum of this patient indicating that Campylobacter induces cell-mediated immune responses.

There are also indications that Campylobacter extracts cause in vitro expansion of γ/δ T-cells obtained from healthy controls. This cell-type has been implicated in mucosal immune responses. These cells respond to non-protein components in the Campylobacter extract (61). Since it is not known whether γ/δ T-cell expansion also occurs in vivo, the significance of this observation in relation to protection against Campylobacter infection is unknown.

Although cell-mediated immunity appears to be important in the defence against Campylobacter, the available data do not point to specific candidate genes which could be studied in humans. Cell-mediated immunity against (bacterial) pathogens is usually initiated by activation of the innate immune response. For instance, Toll-like receptors, which are important in initiating the innate defence and form the bridge between innate and adaptive immunity, are possible candidate genes for genetic studies in humans.

5. LESSONS FROM EXPERIMENTAL INFECTION

5.1 In vitro models of infection

Studying the mechanisms of Campylobacter infection and pathogenesis is complicated by the lack of simple animal models that mimic the in vivo situation in humans. In vitro cell culture methods provide a useful alternative to investigate the interactions between Campylobacter and the host epithelium that occur during infection. In the genomics era there is an increasing use of in vitro cell culture techniques to interrogate the potential role of different genes in infection and pathogenesis. In vitro studies on host-pathogen interactions often use cells of epithelial origin. These can be non-polarised (Hela, HEp-2, INT407) or polarised (Caco-2, HT29, T84) cells. Polarised cell lines have an apical surface facing the luminal side and a basolateral side interfacing with the lamina propia and mimic the in vivo situation. Both sides differ biochemically with respect to transport functions and cellular localization of surface components such as Toll-like receptors (26;62;63). The use of polarised models is useful for studying microbial effects on transport, transcytosis mechanisms and cell invasion (64). Non-polarised models can also be used for studying bacterial virulence. Such studies have elucidated receptors, signalling pathways and internalization mechanisms (65;66).

Invasion assays using in vitro cell culture models allow many parameters to be independently amended to achieve optimal results. Incubation time and assay volume which can affect the results, are standard variables, while the number of internalised bacteria strongly depends on type of cell line and Campylobacter strain used, the number of bacteria added per cell, and concentration of antibiotics used to kill non-internalised bacteria (67). Taken together these data indicate that the invasive properties of various Campylobacter strains are not fully understood.

5.2 Animal models of infection

Murine models with defined deletions in components of innate or adaptive immunity are crucial in identifying genetic factors involved in the host-defence against infection. Progress in our understanding of Campylobacter infection and disease have however been seriously

hampered by the lack of an appropriate animal model which makes studies in above mentioned gene-deleted mice impossible. Whereas most animals can be colonized with Campylobacter, gastro-enteritis does not occur. Mice are not normally colonised with Campylobacter but in an experimental setting colonisation can be established. Campylobacter vaccination experiments have also been performed using such models and protection against colonisation with a homologous strain could be induced. Some authors have been able to induce gastro-intestinal disease in infant mice (68). In these mice intra-peritoneal injection with C. jejuni produced a self-limiting diarrhoea but since infant mice do not have a fully developed immune system they are not suitable for studying “normal” Campylobacter disease or vaccine induced protection. Also in a-thymic, germ-free, nude mice transient diarrhoea has been observed (69) and recently it was shown that NF-κB deficient mice, which have a defect in the induction of pro-inflammatory cytokine production such as TNFα, IL12, IL1 and IL6 develop gastro-enteritis when infected with Campylobacter (70).

Because the above mentioned models all display very severe defects in the capacity to raise innate and adaptive immunity they are not suitable for measuring immune responses to Campylobacter. Therefore, an intranasal challenge model has been developed in mice (71). Although this is not the natural infection route, intranasal infection of mice with Campylobacter results in systemic disease and death of a high proportion of mice. Various clinical isolates were differentially virulent in this model and also vaccine induced protection could be measured. Extensive follow up studies have however not been performed.

Gastro-enteritis can be induced in young weanling ferrets and in some non-human primates and a RITARD model has been developed in rabbits (72). Although these models can shed light on virulence of Campylobacter and the pathogenesis of the disease they do not contribute to our understanding of host-genetic factors involved in determining susceptibility to infection.

Consistent with observations in patients these studies show that severe immune defects in mice also lead to enhanced susceptibility to infection. Genetic factors may be involved in this although these studies do not point to genetic factors that specifically determine susceptibility to Campylobacter.

5.3 Human volunteer studies

With the lack of an appropriate animal model for Campylobacter infection, infection of human volunteers has been important in increasing our understanding of colonization and disease induction. These studies have shown that there is a clear dose-response relation between the number of ingested bacteria and colonisation of the patients and that Campylobacter is highly infectious (6;73). Surprisingly, no clear dose-response relation between the number of ingested bacteria and the development of clinical disease could be found in these studies. The volunteers in this study were however not screened for pre-existing immunity to Campylobacter and this, together with the small study groups, may (partially) explain this finding. The two Campylobacter strains used in these studies induced disease with different severity indicating that not all Campylobacter strains have similar disease inducing properties. After recovery of the volunteers some of them were challenged with the homologous strain and it appeared that primary infection resulted in protection against disease but not against colonization. These data indicate that vaccination against Campylobacter may be feasible although the high level of variability between Campylobacter strains may hamper this approach.

6. CONCLUSIONS

The aim of this study was to identify genetic factors involved in the host-defence against Campylobacter induced gastro-enteritis. Due to the limited information available on this subject, first (immune) mechanisms involved in the host-defence against campylobacteriosis were identified from epidemiological observations and observations in patients. These studies indicate that the first defence mechanism encountered by Campylobacter, the acidity of the stomach is very important; they show that humoral immunity is crucial for the host-defence against Campylobacter; and they reveal that there may also be a role for cell-mediated immunity, although the latter statement is only based on the susceptibility of HIV and AIDS patients. This study also reveals that there are large gaps in our knowledge on host-factors involved in determining an individual’s susceptibility to Campylobacter infection.

7. DESIGN OF FUTURE STUDIES

7.1 Genetic studies in humans

How can these observations now lead to a comprehensive study of genetic factors in patients who suffered from severe campylobacteriosis? Previously studies on the genetic susceptibility to Salmonella resulted in the selection of a number of candidate genes involved in cell-mediated immunity (74). For Campylobacter the picture is, however, much less clear. Analysis of large amounts of polymorphisms in a case-control study is therefore probably not warranted. Participants in the Campylobacter-Salmonella patient control study (CASA), where high and low risk groups were formed based on a questionnaire and proven infection, are now included in a genetic study where the involvement of functional genetic polymorphisms in candidate genes known to be important for the defence against Salmonella infection will be studied. This study focuses on genes involved in cell-mediated immunity. Since the Campylobacter cases will be analysed as a control group suffering from gastro-enteritis caused by a pathogen other than Salmonella, this study may also reveal candidate genes that are relevant for the susceptibility to Campylobacter infection. This CASA study includes confirmed Campylobacter and Salmonella cases, and a control group. The risk-factors for developing these infections were already evaluated. Analysis of genetic factors in this population will give insight into the relative contribution of genetic variation of host genes to infection susceptibility.

7.2 Candidate genes for host-susceptibility to Campylobacter

The current study points to two other pathways/mechanisms in which susceptibility genes could be found: firstly, acidity of the stomach and secondly, humoral immunity. Decreased acidity of the stomach is also a clear risk-factor for the development of Salmonella infection and therefore the proton-pumps that are involved in this process are good candidate genes for study. This will be further evaluated.

The humoral immune response encompasses a very large set of candidate genes including genes involved in B-cell activation, genes encoding receptors for immunoglobins (Fc-Receptors) and a large number of genes encoding complement factors. Genes involved in secretion of IgA and IgM are obvious candidates . The genes to study could also include two genes that are involved in determining susceptibility of patients to GBS, i.e. FcγRIII, FAS and mannose-binding lectin (MBL). However, these genes could be GBS specific and may not be involved in determining susceptibility to gastro-enteritis. Because of the uncertainty about the role of all selected candidate genes in determining susceptibility to infection with Campylobacter it is of paramount importance to select polymorphisms that occur at high frequencies in the population, and to select only those polymorphisms that are known to affect the function of the gene-product. Analysis of a limited number of polymorphism in this case-control cohort may increase our understanding of host-susceptibility to campylobacteriosis.

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