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New Approaches to Development of Mucosal Vaccine Against Enteric Bacterial Pathogens; Preventing Campylobacteriosis

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A b s t r a c t

Although vaccination, after having been more than 200 years in medical practice, has proven to be the most effective and the cheapest way to prevent infectious diseases, they remain still the main cause of human premature deaths. As many pathogens enter the human body through the mucosal surfaces, the mucosal way of immunization is considered to be the most promising strategy to decrease the number of human infections. Moreover, the oral delivery system eliminates the necessity of injection what is extremely important for pediatric immunization programs. However, most of recently constructed subunit vaccines based on purified bacterial/viral antigens are rather poorly immunogenic. This review presents some novel ways to enhance and modulate host immune responses by combining antigens with specific adjuvants or by employing specific delivery systems. We also discuss some recent technologies, based on mining the genomic sequences of bacterial pathogens, which accelarate and improve identification of new candidates for vaccine construction. As an example, we focus on the progress in the development of vaccine against *Campylobacter* spp. *Campylobacter jejuni* is now recognized as a leading cause of bacterial enteritis in human.

K e y w o r d s: mucosal vaccine, specific adjuvants, antigen carriers, campylobacteriosis

Introduction

Antibiotics and vaccines are one of the most important medical achievements of the 20th century. Their introduction into public medical practice resulted in a sharp decrease in infectious diseases morbidity and mortality, and gave rise to a common belief that infectious diseases can be controlled and prevented. The enormous progress recorded in the area was particularly marked in developed countries doubtlessly due to mass immunisation programs. However, the smallpox is the only infectious disease that has been completely eradicated. Vaccine-preventable diseases are still the number one cause of premature death in the world. According to World Health Organisation data over 17 million people die every year as a result of infectious diseases. A recent literature review indicates the existence of 1415 species of infectious agents pathogenic for human (viruses, bacteria, parasitic helminths, protozoa, prions) (Taylor *et al.*, 2001). Many of them (12%) are regarded by epidemiologists as emerging and re-emerging pathogens. Most of human emerging pathogens are zoonotic. Apart from wild and domestic animals also human population and our environment are a source of new organisms pathogenic for humans. Many physiological and social factors, such as genetic variability of the pathogens (mutations, horizontal gene transfer), ecological changes in human population, progress in medical technologies, and modification of the host-pathogen relationship promote such a huge number of emerging pathogens (Woolhouse, 2002). This fact combined with the emergence of many antibiotic-resistant pathogens makes it necessary to look for new strategies to treat bacterial infections.

The fundamental issue now is how to improve existing vaccines or how to create new ones. The experience of the last century proves that vaccines are the major beneficial factor in medicine of infectious diseases. Our ambition is to introduce new specimens whom would be safe, effective, cheap and easy to deliver. Attempts should be also made to shorten the time of vaccine/drug discovery. Now it takes about 10 years to create new vaccine or new antibacterial drug and it is widely accepted that the time is too long.

The modern era of genomics technologies promises to speed our understanding of the not completely understood field of bacterial pathogenicity. New strategies have been developed for rapid microbes' identification, for new antibacterial drug design and for selecting genes encoding protective antigens.

This review outlines the current trends regarding mucosal vaccination against bacterial enteropathogens. It also presents new achievements concerning anty-*Campylobacter* immunization

1. Mucosal vaccination

The vast majority of bacterial and viral infections originate at mucosal membranes that line interior part of the body. Colonization of these surfaces is the first step for many infectious diseases. For some pathogens mucosal surfaces are their destination point in the host body whereas for others colonization of the mucus membrane is the starting point for spreading in the mammalian organism. For example, among enteric bacteria, *Helicobacter pylori* and *Vibrio cholerea* are extracellular microorganisms existing in the lumen of the gastrointestinal track whereas *Shigella*, *Salmonella* or *Yersenia* cross the epithelium through M or epithelial cells causing localized or systemic infection.

In most cases, the most effective approach to obtain protection against enteric pathogens is the induction of high level of s-IgA. The mucosal route of vaccination, which offers the possibility to induce locally produced and secreted sIgA antibodies, in addition to systemic IgG antibodies may be an effective and particularly convenient way to meet the requirement. Some attenuated strains of enteric bacteria are already approved vaccine against enteric bacteria (attenuated strains of *Salmonella enterica* sv. Typhi and *Vibrio cholerae*) (Dietrich *et al.*, 2003). Despite their efficacy, there is a concern over potential side effects. Some of currently licensed antiviral mucosal vaccines failed and were withdrawn from the market due to serious adverse reactions. On the other hand, orally administrated subunit vaccines composed of purified antigenic components of the microorganisms or inactivated whole bacterial cells are frequently not strong immunogens. Varieties of strategies have been developed to enhance the level of the local immunity induced by subunit vaccines. Among the most commonly studied is the use of bacterial cells as carriers for heterologous antigens, biodegradable microparticles, different formulations of liposomes or the use of specific oral mucosal adjuvants. Also DNA vaccines have been currently developed for administration on mucosal surfaces (McCluskie and Davis, 1999).

1.1. Identification of new potential vaccine candidates

The choice of the antigens is a critical point for effective live subunit vaccine construction. Several new technologies have been recently developed to identify genes required for survival of the pathogen in mammalian host. Most of the strategies allow selection of many genes active during infection in just one experiment. Among them, the most powerful are IVET technology and STM mutagenesis. The first one – positive selection method based on using reporter genes – was originally described to look for *Salmonella* genes expressed *in vivo.* From then on, this strategy has been modified several times and adjusted to study many pathogenic bacterial species and what is more important to allow identification of genes expressed on low level during infection. STM is a negative selection assay based on comparative hybridization and applying specific tag-labelled transposons. The method has been used to fish out many genes needed to establish infection by a variety of pathogens. This strategy which is complementary to IVET, although simple and extremely useful, posses also some disadvantages such as loosing all genes essential for surviving *in vitro* (for review see: Chiang *et al.*, 1999).

A current approach to the identification of new potential candidates for subunit vaccine constructions is reinforced by a recent progress in bacterial genome sequencing. Since the first complete bacterial genome nucleotide sequence was reported in 1995, more than 100 genome nucleotide sequences have been annotated and published and about 200 bacterial genome sequence projects are under way. Unquestionably, any genome nucleotide sequence of bacterial pathogen encodes for many strong protective antigens and the challenge is to find and characterise them. Several types of computational tools have been recently developed to screen bacterial genomes for vaccine candidates. They mainly focus on searching for new virulence factors or membrane-located proteins. Using available data from completely and incompletely sequenced bacterial chromosomes Pallen *et al.* (2001) identified over twenty putative new ADP-ribosylotransferases. Also data-mining of pneumococcal genome identified many surface-exposed proteins (Wizemann *et al.*, 2001). The results of the bioinformatic studies are the starting point for further analysis and need experimental verification. DNA

microarray methods, also based on sequencing achievements, offer an alternative way for identification of genes transcribed under different conditions as well as for studying host response to the infection.

The new strategy of identification of the potential protective antigens based on *in silico* analysis of bacterial genomes, termed "reverse vaccinology", for the fist time was used to search for novel protective antigens in the genome of *Neisseria meningitidis* B serogroup. Out of 570 genes identified as potential vaccine candidates 350 were successfully cloned and expressed in *E. coli.* Finally, after protein purification and immunogenicity and protective efficacy analysis, 22 out of 85 surface-exposed proteins were classified for further analysis. The technology has been currently extended to others pathogens of medical importance (*Streptoccocus pneumoniae*, *Porphyromonas gingivalis*, *Staphylococcus aureus*, *Chlamydia pneumoniae* and *Bacillus anthracis*). This new concept – mining of the bacterial genome *in silico* – has already had a marked impact on vaccine discovery research, mainly due to its rapidity. As every technology it has also several limitations, including the fact that not all protective surface-located antigens can be find by bioinformatic tools. The result of the screening is dependent on the selected criteria. In addition, the second step of reverse vaccinology – cloning of many genes and mainly immunological testing of many proteins- is a time-consuming, rate-limiting step (for review see: Mora *et al.*, 2003).

1.2. Novel promising adjuvants

1.2.1. DNA containing CpG motifs

The first line of immunological action against pathogens is the innate immune system. The innate immune system recognises pathogen-associated molecular patterns (PAMPs) using Toll- like receptors (TLR) and is able to discriminate different microbial components. TLR receptors belong to a large family of PRR receptors (pattern recognition receptors). As far ten classes of mammalian TLRs have been described which distinguish different conserved bacterial structures For example, TLR4 receptors recognize bacterial lipopolisacharides, TLR2 proteins reacted with lipoproteins whereas TLR5 are stimulated by bacterial flagellins. TLR signalling pathways initiated by stimulation of these transmembrane proteins by PAMNs share many similarities with IL-1R signalling and resulted in induction of many cytokine productions. TLR9 receptors recognized bacterial DNA containing unmethylated CpG motifs. The DNA with unmethylated CpG acts directly or indirectly on different kinds of immune cells, such as B cells, dendritic cells, macrophages and monocytes as well. TLR9s, in contrast to others classes of TLRs, are not located on the immune cell surface but are rather directed into cytoplasmic compartments where they are stimulated by intracellularly present bacterial DNA. It was demonstrated that, synthetic oligonucleotides containing CpG motifs (CpG ODN) in a particular sequence context mimic the structure of bacterial DNA and augment immune responses to many antigens. They stimulate both, innate and adaptive immune system. The treatment of infectious diseases with CpG is a relatively new scientific area. However, since the end of XX century when the knowledge concerning CpG motif was put into practice, several bacterial and viral human infections have been experimentally treated in this way (for recent reviews see: Underhill and Ozinsky, 2002; Wagner, 2002; Dittmer and Olbrich, 2003; Klinman *et al.*, 2004).

1.2.2. Enterotoxins and their derivatives (LT and CT)

Other strategy to develop effective mucosal vaccine deliver *via* oral or nasal immunization is to employ the heat-labile *Vibrio cholerea* toxin (CT) and closely related heat-labile enterotoxin of *E. coli* (LT) as mucosal adjuvant. Both toxins are composed of five B subunits responsible for binding to eucaryotic surface exposed receptors (GM1 ganglioside) and one enzymatically active A subunit. A subunit consists of two parts: N-terminal fragment termed A1 which displays ADP-rybosylating activity and COOH terminus termed A2 which binds A subunit with pentamer formed of five identical B subunits. It was documented that CT and LT are the most potent immunogens as far described. In addition they also act as immunoadjuvants towards co-administrated unrelated antigens. The data was obtained from many independent experiments for a variety of microorganisms, as well as their products. The experiments were carried out using animal models and also in human voluntaries trials. The mechanism of mucosal adjuvantcity of CT and LT, which is, to date, not well understood, seems to be extremely complex and involves interactions of several populations of cells, mainly different kinds of APC (antigen presenting cells) (for review see: Freytag and Clements, 1999; Pizza *et al.*, 2001). There is a controversy about the augmentation of Th1 or Th2-dependent immune response by oral administration of LT and CT. Some reports indicate that CT

induces strongly polarized Th2 response whereas LT stimulate mixed Th1 and Th2 response (Marinaro *et al.*, 1995; Freytag and Clements, 1999; Petrovska *et al.*, 2003). The high toxicity of both proteins attributed to ADP-ribosyltransferase activity of A_1 subunits precludes their use as adjuvants for human vaccine formulations. Two alternatives have been proposed to overcome the problem. The first one suggests the use of genetically modified toxin constructed by site-specific mutagenesis. As far, more than 50 different site directed mutants of both toxins have been obtained and used to study direct correlation between structure/function of both proteins and their immunomodulatory action. The most profoundly analyzed mutants were those in enzyme active site (Douce *et al.*, 1998; Pizza *et al.*, 2001). The second approach consists of employing native or recombinant B subunits of the proteins which can be co-administrated with unrelated antigens or alternatively genetically or chemically fused to them. However, in both instances (genetically modified toxin or recombinant B subunit) the effectiveness of modified adjuvants has been shown, to be weaker than those of intact toxins. Mucosal immunogenicity has been shown to be retained by the B subunits of both LT and CT. Based on this, the licensed oral anti- *V. cholerea* vaccine is composed of CTB co-administrated with killed whole *V. cholerea* cells. The correct pentameric structure of the protein has been supposed to be crucial for its immunogenicity. Further, the cellular localization of the LT-B influenced the level of its immunogenicity. There are apparently contradictory reports in the literature on the adjuvant effects of CTB and LTB. The reason for the observed discrepancies is not completely understood. This situation is due, in part, to the variables in the model systems used. It is believed that several factors, such as type of antigen, dose, and route of immunization, method of coupling (or lack of coupling) can influence the results of the experiments. The fact that some commercially originated preparations of the B subunits are contaminated by minute amounts of intact toxins should be always taken into account. Some investigators claimed that recombinant B subunits obtained from a nontoxigenic host have no adjuvant activity. On the other hand, others presented results clearly indicating that r-LTB and r-CTB had enhanced immune responses towards intranasally and orally administered antigens (Wu and Russell, 1998; Weltzin *et al.*, 2000). In addition some reports documented distinct differences in immunomodulatory actions of CT and LT, which could resulted from dissimilar binding abilities of CT and LT (Peterson *et al.*, 1999; Millar *et al.*, 2001). Thus, the problem of the CTB or LTB potential adjuvant effect still remains unsolved and still is the critical issue in respect to mucosal immunization.

Recently published data proved strong immunomodulatory effect displayed by genetically modify enterotoxins after intranasal coadministration with different antigens (Jakobsen *et al.*, 1999; Jiang *et al.*, 2003; Periwal *et al.*, 2003). However, at the same time some serious neurological adverse reactions were observed as consequence of immunization with LT as an adjuvant. The significance of both these findings for human LT/CT application seems to have been determined (Green and Baker, 2002).

1.3. Bacterial cells as foreign antigen carriers (attenuated pathogenic bacteria, lactic acid bacteria)

Live attenuated strains of bacterial enteropathogens (*Salmonella*, *Vibrio, Shigella* and *Listeria* spp.) able to stimulate both, innate and adaptive immune responses, are attractive candidates for the development of mucosal multivalent vaccines. Although, many bacterial and viral antigens have been cloned and expressed in attenuated bacteria, only a few have been tested in clinical trials (Kochi *et al.*, 2003).

Attenuated, genetically defined, *Salmonella* strains are particularly promising among bacterial live vectors for at least two reasons. Firstly, because *Salmonella* strains able to immunise *via* mucosal surface induce the broad spectrum of immune responses. Secondly, the genetic manipulation of this genus is well understood. Several attenuated *Salmonella* strains were constructed for using as live vectors to deliver heterologous antigens of various viral and bacterial pathogens. The most profoundly studied strains are those obtained by deleting genes whose products are involved in key biosynthetic pathways (*aro* mutants) or regulatory genes *crp-cya*; *pho*P-*pho*Q). The efficacy of genetically modified *Salmonella enterica* sv. Typhimurium vaccine strains was demonstrated using different animal models. The key problem to overcome in order to obtain effective vaccine based on bacterial strains used as carriers is ensuring an adequate level of the heterologous antigen to allow maximal accumulation of the antigenic protein/s without impairing live vector ability to survive and replicate in host tissues. To solve the problem, genes for foreign antigens are cloned into plasmids of different replication systems. Additionally, foreign genes can be expressed from own promoters or placed under the control of the strong, constitutive host regulatory sequences or alternatively put under the control of environmentally regulated promoters. The subcellular location of the recombinant antigen influences its immunogenicity. Then several systems have been developed

to assure proper assembly of the foreign antigen. Heterologous antigens produced by *Salmonella* vaccine strains can be secreted through cytoplasmic membrane and produced as periplasmic, secreted or outer-membrane anchored proteins. Recent data emerging from studies of the host-pathogen interaction encouraged several investigators to use *E. coli* Hly export apparatus or type III secretion system to target recombinant antigens into appropriate cell compartment (Autenrieth and Schmidt, 2000; Gentschev *et al.*, 2002). Additionally, attenuated *Salmonella* strains can be employed as a carrier for DNA vaccines as well as a system targeting cytokines directly to the host antigen presenting cells (for reviews see: Bumann *et al.*, 2000; Garmory *et al.*, 2002).

However, in the case of using genetically engineered, attenuated pathogenic microorganisms the attention should be paid to genetic stability, host-and food-independence of the vector's attenuated phenotype as well as on the impact of vector priming on the immunogenicity of foreign antigens. Using the probiotics [LAB (lactic acid bacteria) strains with GRAS- (Generally Recognized As Safe) status] which are autochthonous microorganisms, instead of attenuated pathogenic strains to deliver foreign antigens will allow overcoming these concerns. Moreover, this LAB-based approach seems to be very promising because most strains from *Lactobacillus* and *Lactococcus* genera are acid resistant and survive in the stomach. Many genetic tools required for foreign gene cloning and manipulation of the protein locations have been developed recently (for reviews see: Pouwels *et al.*, 1998; Steidler, 2003).

2. Preventing campylobacteriosis

Food poisoning and diarrhoeal diseases in Europe as well as in USA continues to be a serious health care problem and *Campylobacter* spp, gram-negative microorganisms, are one of the leading causes of bacterial gastroenteritis in human worldwide. The clinical spectrum of enteric disease due to *Campylobacter* infection ranges from generally mild non-inflammatory diarrhoea to severe inflammatory diarrhoea with faecal blood and leukocytes. The former is the most common clinical manifestation of the disease observed in patients from developing countries, while the latter is typical for the patients living in industrialised regions of the world. *C. jejuni* is also considered to be, after *E. coli* ETEC strains, the second most common cause of traveller's diarrhoea (Altekruse *et al.*, 1999; Skirrow and Blaser, 2000; Coker *et al.*, 2002). In addition to acute gastrointestinal disease, infection with *C. jejuni* has been shown to be associated with GBS (Guillan-Barre syndrome), a neurological disease that may lead to respiratory muscle compromise and death. It was documented that about 30% of GBS cases is preceded by *C. jejuni* infection (Nachamkin *et al.*, 1998). Additionally, systemic infections do occur specially in patients at the extremes of age or those who are immunocompromised such as HIV-infected individuals.

Although the mortality associated with *Campylobacter* infections is relatively low and no specific treatment is required for the great majority of patients with *Campylobacter* infection it constitutes a serious problem because of high number of cases and neurological symptoms which are a consequences of *Campylobacter* infection as well as because of high social and economic costs of disease. As the average lifetime of Europeans has been increasing consistently, one can expect more serious complications of *Campylobacter* infections particularly in cases involving old patients. C*ampylobacter* bacteraemia has a high mortality rate mainly due to increase in resistance to two commonly used antibiotics (fluoroquinolones and macrolides) among *Campylobacter* isolates (Aarestrup and Wegener, 1999; Engberg *et al.*, 2001).

2.1. Attempts to construct human anty- *Campylobacter* vaccine

C. jejuni was first isolated from human diarrhoeal stools in 1972 by a filtration technique. Despite more than thirty years of investigations, the molecular mechanisms involved in *Campylobacter* virulence and pathogenesis are far from being completely understood. Relatively little is also known about immune responses during *Campylobacter* infection. Epidemiological and human challenge studies proved that protective immunity develops after infection; thus the prevention of *Campylobacter* disease seems to be feasible. It is suggested that the human vaccine will be used not for global immunisation but to immunise the selected high-risk populations such as children living in the developing countries where the disease is endemic, medical personnel or individuals travelling to highly endemic areas. Several approaches to develop an oral human vaccine have been undertaken. The investigations mainly concentrate on the evaluation of the efficacy of killed whole-cell vaccine administrated with mucosal adjuvant or prepared utilizing NST (Nutriment Signal Transduction) technology (Scott *et al.*, 1997). However, the application of the whole

cells (killed or attenuated) becomes questionable in the light of tremendous antigenic and genetic diversity of the *Campylobacter* species, the lack of complete understanding of the mechanism of *Campylobacter*-associated polyneuropathy (GBS) and the fact that *Campylobacter* spp. are naturally transformable (Duim *et al.*, 2000; Dorrell *et al.*, 2001). Publishing of the nucleotide sequence of the *Campylobacter jejuni* NCTC 11168 genome greatly facilitates analysis of the mechanisms responsible for genetic diversity and colonization process (Parkhill *et al.*, 2000; Wren *et al.*, 2001; Gaynor *et al.*, 2004).

2.2. Chicken anty-*Campylobacter* vaccine

In the developed countries campylobacteriosis is, a food born disease. Outbreaks of *Campylobacter* enteritis are frequently traced to contaminated milk or water, whereas the most common cause of sporadic cases is eating of undercooked poultry meat. The contaminated chickens are, by far, the principal vehicles of infection. The epidemiology of *C. jejuni* in broiler flocks is still unclear. Generally, birds become infected at about 3 weeks of age The sources and routes of transmission of the microorganism to the broilers on the farms remain undetermined. Recently obtained data have indicated several sources of infection, including water, wild birds and farm's personnel. Young chickens (0–3 weeks) are protected against infection by maternally derived specific antibodies (Sahin *et al.*, 2003). Although, the reported level of *Campylobacter* organisms in the chicken intestine, especially in the ceca, varies between 10^5–10^{10} per g of cecael contents, the massive colonisation does not induce any signs of the disease. A large amount of *C. jejuni* in the bird faeces causes further cross-contamination of *Campylobacter*-negative chicken carcasses in the processing plants. As a result, *Campylobacter* contaminates 50–80% of the raw chicken carcasses, depending on the geographical region where the study was conducted out and the method used. This fact, in combination with the relatively low human infection dose can explain why eating undercooked poultry causes majority of sporadic cases of the campylobacteriosis (for review see: Corry and Atabay, 2001). Efforts to reduce the level of contamination by a variety of intervention programs such as improvement of the biosecurity in the hatchery, a competetive exclusion technology or using chlorinated water gave, as far, variable results when introduce at the farm level and in most cases have been unsuccessful (Newell and Wagenaar, 2000). An alternative, more realistic approach for the control of *Campylobacter* contamination is active immunisation of the birds. To date, there is limited data on chicken immune system functioning. Furthermore, the relationship between the host and microorganism is comensal, so the elimination of *Campylobacter* from bird intestinal tract is not an easy task.

In recent years some attempts have been undertaken to develop an effective chicken vaccine against *Campylobacter.* The immunogenicity and efficacy of several vaccine regimens have been evaluated in a chicken model. Rice *et al.* (1997) demonstrated some but not significant reduction of *Campylobacter* colonization of chicks orally vaccinated with formalin-killed whole bacterial cells including *E. coli* heat-labile toxin when compared to non-vaccinated control. In contrast, Baqar *et al.* (1995a; 1995b) immunized non-human primates and mice using the same vaccine prototype and observed stimulation of the immune response by LT. A different approach involves a subunit vaccine, which requires the choice of an appropriate protective antigen and a way to deliver it to the host immune system. Recent data documented that multiple, immunogenic *Campylobacter* proteins are post-translationaly modified by glycosylation. In addition, glycosylation process affects ability of the pathogen to colonize chicken intestinal track (Karlyshev *et al.*, 2004). Genetic and structural analysis of the O- and N-linked glycosylation systems of *Campylobacter* as well as cloning and expression of *Campylobacter pgl* gene cluster in *E. coli* facilitates selection of proper antigen for immunization (Wacker *et al.*, 2002; Szymanski *et al.*, 2003). So far only flagellin has been evaluated in the chicken model (Khoury and Meinersmann, 1995; Lee *et al.*, 1999). Variability of the surface-exposed domains of the FlaA and its O-linked glycosylation complicate the use of flagellin for vaccination. Live and genetically engineered bacterial strains are considered to be most effective as vaccines against many enteropathogens. To be used as chicken vaccine a *Campylobacter* strain needs not only to be attenuated for humans but also to be immunogenic for birds. This means that it needs to persist long enough in the birds' gut-associated tissues to induce the protective immune responses. Despite many efforts such a strain has not yet been developed. Ziprin *et al.* (1999; 2001) have shown that genetic knockout of four *C. jejuni* genes (*ciaB*, *dnaJ*, *pldA* and *cadF*), which code for proteins involved in different stage of pathogenesis renders strains incapable of colonizing the chicken intestinal tract, although they are able to colonize the crop (Ziprin *et al.*, 2002). The post-genomic era results in new instruments for analyzing virulence-related mechanisms. Several new *Campylobacter* virulence factors have been identified by bioinformatics tools. One of them is new IM-located thiol-oxidoreductases of *C. jejuni* encoded by gene denoted *dsbI. Campylobacter* strains lacking DsbI activity will be examined as potential vaccine candidate (Raczko *et al.*, 2004).

The poultry are a major source of human *Salmonella* as well as *Campylobacter* infections. Since the attenuated *Salmonella* strains are highly effective chicken anti- *Salmonella* vaccine than one may suppose that avirulent *Salmonella* producing *Campylobacter* antigens may be attractive option for chicken dual vaccine (Curtiss and Hassan, 1996). Several *C. jejuni* genes coding immunodominant proteins have been identified. Three of them have been cloned, sequenced, characterized and expressed. in vaccine *Salmonella* strains. CjaA (Cj0982c – 30 kDa) and CjaC (Cj0734c – 28 kDa) proteins exhibit relevant overall homology to several prokaryotic solute-binding proteins (family 3) components of the ABC transport system (Pawelec *et al.*, 1997; Pawelec *et al.*, 1998). CjaD (Cj0113 – 18 kDa) protein exhibits homology to PAL (peptidoglican-associated lipoprotein) of gram-negative bacteria. *Campylobacter* genes *cjaA, cjaC and cjaD* were cloned into Asd^+ cloning vector and introduced into avirulent *S. enterica* sv. Typhimurium D*asd* D*crp* D*cya* c3987. It was documented that chicken orally immunized with avirulent *Salmonella* strain expressing *Campylobacter* antigen developed serum IgG and mucosal IgA responses against *Campylobacter* membrane proteins. Moreover, this strategy greatly reduced the ability of heterologous wild-type strain to colonize the bird cecum (Wyszynska *et al.*, 2004). In order to augment the efficacy of vaccine prototype several fusions of two *Campylobacter* genes (*cjaA* and *cjaD)* with *etxB,* which encodes B subunit of *E. coli* LT toxin, have been constructed (Wyszynska *et al.*, 2002). Recombinant plasmids expressing hybrid proteins introduced into avirulent *Salmonella enterica* sv. Typhimurium strain will be utilized to study the LTB adjuvant effect on the immunogenicity of the *C. jejuni* co-administrated antigens on two animal models (chicken vs mice).

Conclusion

The post-genomic era, started in 1995, brings many new technologies useful for studying bacterial pathogenesis, specifically the host-pathogen interaction processes. Recent strategies, such as reversed vaccinology or DNA miccroarrays, doubtlessly decrease the time and cost required for identification of bacterial antigens. However, to put the knowledge into medical practice all new proteins have to be tested in animal models to prove their efficacy and safety. Moreover, determination of gene function, being sometimes hard or even impossible to accomplish, ensure for reasonable choice of proper antigen. Another limitation of the vaccinology development derives from the lack of understanding of mammalian immune system functioning. The recent discovery of TLR receptors, the better understanding of APCs (antigen presenting cells) role in prevention of the infectious diseases as well as cross-talk between innate and adaptive immune systems are the milestones of the vaccinology. The gained knowledge facilitates modulation of the immune responses by using appropriate delivery system or/and adjuvant, which are crucial to differentiate immune response into Th1 or Th2 type. Then, the recent achievements in bacterial genomics and proteomics as well as in human immunology and genomics radically changed vaccine development. Novel approaches towards vaccination should result in modern effective and easy to deliver vaccines against emerging or re-emerging pathogens. Also, there are many "old" infectious diseases for which conventional ways to discover vaccines failed. However, taken into account knowledge about mechanisms responsible for genetic diversity of microorganisms and recent vaccinology failures (oral vaccine against rotavirus diarrhea and nasal vaccine against influenza) one has to be more realistic. The future will show us to what extent the optimism is justified.

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