Urinary tract infections, immunity, and vaccination

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Abstract

Urinary tract infections (UTI) are considered one of the main causes of morbidity worldwide, and uropathogenic *Escherichia coli* (UPEC) is the causative agent associated with these infections. The high morbidity produced by the UTI and the limitation of antibiotic treatments promotes the search for new alternatives against these infections. The knowledge that has been generated regarding the immune response in the urinary tract is important for the development of effective strategies in the UTI prevention, treatment, and control. Molecular biology and bioinformatics tools have allowed the construction of fusion proteins as biomolecules for the development of a viable vaccine against UTI. The fimbrial adhesins (FimH, CsgA, and PapG) of UPEC are virulence factors that contribute to the adhesion, invasion, and formation of intracellular bacterial communities. The generation of recombinant proteins from fimbrial adhesins as a single molecule is obtained by fusion technology. A few *in vivo* and *in vitro* studies have shown that fusion proteins provide an efficient immune response and protection against UTI produced by UPEC. Intranasal immunization of immunogenic molecules has generated a response in the urinary tract mucosa compared with other routes of immunization. The objective of this review was to propose a vaccine designed against UTI caused by UPEC, describing the general scenario of the infection, the mechanism of pathogenicity of bacteria, and the immune response of the host.

Key words: Uropathogenic *Escherichia coli*. Fimbrial adhesion. Fusion proteins. Urinary tract infections. Interleukin.

Infecciones del tracto urinario, inmunidad y vacunación

Resumen

Las infecciones del tracto urinario (ITU) se consideran como una de las principales causas de morbilidad en el mundo, y *Escherichia coli* uropatógena (UPEC, por sus siglas en inglés) es el agente causal asociado a estas infecciones. La alta morbilidad generada por las ITU y la limitación de tratamientos debido al aumento de la resistencia bacteriana a los diversos antibióticos inducen la búsqueda de nuevas alternativas contra estas infecciones. El conocimiento que se ha generado acerca de la respuesta inmunitaria en el tracto urinario (TU) es importante para el desarrollo de estrategias efectivas en la prevención, el tratamiento y el control de las ITU. Los avances en las herramientas de biología molecular y bioinformática

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han permitido generar proteínas de fusión consideradas como biomoléculas potenciales para el desarrollo de una vacuna viable contra las ITU. Las adhesinas fimbriales (FimH, CsgA y PapG) de UPEC son factores de virulencia que contribuyen a la adherencia, la invasión y la formación de comunidades bacterianas intracelulares. Pocos estudios *in vivo* e *in vitro* han mostrado que las proteínas de fusión promueven una respuesta inmunitaria eficiente y de protección contra las ITU causadas por UPEC. Adicionalmente, la vía de inmunización intranasal con moléculas inmunogénicas ha generado una respuesta en la mucosa del TU en comparación contra otras vías de inmunización. El objetivo de esta revisión fue proponer un diseño de vacuna contra las ITU causadas por UPEC, describiendo el panorama general de la infección, el mecanismo de patogenidad de la bacteria y la respuesta inmunitaria del huésped.

Palabras clave: *Escherichia coli* uropatogénica. Adhesina fimbrial. Proteínas de fusión. Infecciones del tracto urinario. Interleucina.

Urinary tract infections

Urinary tract infections (UTI) are caused mainly by pathogens of intestinal origin that contaminate the urethra and ascend to the bladder. Additionally, some factors of the bacteria or the host favor the colonization of the kidney, where the uropathogen ascends through the ureters¹. UTI can be acquired in the community and hospitals, and are associated with high morbidity rates worldwide². These UTIs are classified according to the site of infection: urine (asymptomatic bacteriuria), bladder (cystitis), kidney (pyelonephritis) and blood (bacteremia)³. UTIs are also characterized by general signs and symptoms, such as hematuria, pyuria, dysuria, urinary frequency, urgency, fever, low back and suprapubic pain⁴.

In Mexico, UTIs are a public health problem due to their high morbidity. Approximately 4 million cases have been registered annually⁵. The populations at high risk of suffering from UTI are newborns, preschool girls, women with sexual activity and people of both sexes at advanced ages^{1,2}. UTI during reproductive age represent the second cause of morbidity in women, and during pregnancy, they are the most frequent cause of perinatal complications⁶. In 2016, 3,149,091 cases of UTI were reported in women, of which 1,392,235 cases were in women between 20 and 44 years of age⁶. In males, UTI was the third cause of morbidity, with 957,875 cases per year. The distribution is age-related; however, this infection decreases in adults over 44 years of age⁶. At puberty (15 to 19 years) UTI represent the third cause of morbidity, with 297,831 cases; during the pediatric age (<15 years), 360,220 cases were presented⁶. The UTI prevalence in children under one year of age is 20,300 cases per year; moreover, the frequency of these infections is 0.4-1.0% in females, 0.1% in circumcised males and 0.7% in uncircumcised males^{6,7}.

Uropathogenic *Escherichia coli* (UPEC) is the causal agent in more than 80% of UTI; however, catheter-associated infections presented in hospitals have also been associated with other genres: *Staphylococcus*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Enterococcus*. The complete genome sequencing of the UPEC clinical strains—F11, IAI39, UMN026, UTI89, 536, CFT073, ABU 83972 and VR50—has shown that the acquisition of virulence factors occurs in pathogenicity islands, plasmids, and phages through horizontal gene transfer⁸. These virulence factors are located on the bacterial surface, and some of them are exported to be anchored in the outer membrane and colonize the urinary tract (UT), generating a clinical pathology (Figure 1)^{9,10}. The UPEC mechanism of pathogenicity begins with its adherence through the participation of fimbrial adhesins (FimH, PapG, SfaS, FocH, CsgA, and DrA), located in the distal part of different fimbriae (Type 1, P, S, F1C, curli and Dr, respectively)^{11,12}. The interaction between adhesins and receptors (α -D-mannosylated proteins, glycosphingolipids, neuraminic acid, decay-accelerating factor (Daf), and proteins of the extracellular matrix), located in UT cells, activates different signaling pathways (apoptosis) and contribute to the UT cells colonization^{13,14}.

The expression of α -hemolysin (HlyA), secreted autotransporter toxin (Sat) and cytotoxic necrotizing factor 1 (CNF-1) contributes to the UPEC increase in its cytotoxic capacity in the UT¹⁰. The presence of iron uptake systems (yersiniabactin and aerobactin) is necessary for the UPEC persistence and colonization of an anatomical area with a low iron supply⁹. The UPEC adherence to UT cells is an initial process that promotes invasion, avoiding its drag by the flow of urine, the activity of antibodies and proteins with bactericidal properties, and the antibiotics action¹⁵. The UPEC invasion occurs through a zipper-like mechanism, a process that

Figure 1. Pathogenicity mechanism of uropathogenic *Escherichia coli* (UPEC) in the urinary tract (UT). The main extraintestinal *E. coli* is UPEC, which can colonize the UT by using virulence factors (fimbriae, flagellum, capsule, LPS, autotransporter proteins, toxins, and siderophores). The pathogenicity mechanism begins by the close adherence to the superficial UT cells (1) activating an invasion process (2), which allows the bacteria to remain intracellularly to form IBC (3). The UPEC interaction with the host cell causes an intracellular oxidative state, promoting the bacterial filamentation and its subsequent efflux by the cell lysis (4). The host cell-bacteria interaction stimulates a process of apoptosis and the detachment of protective cells from the UT as a defense mechanism (5). The intermediate epithelium of the UT remains exposed, and UPEC colonizes it again for a new infection cycle (6). After reinfection, UPEC can remain intracellularly in a quiescent state for up to several months (7). The UPEC efflux is stimulated by the same bacteria or by the host cell, the bacteria emerge and colonize the UT. Furthermore, lactoferrin (blue), uromodulin (red), IgA antibodies (black), cathelicidin, and defensins (brown) are secreted by various host cell types to inhibit the binding of UPEC to the UT. The uroepithelium produces IL-6 and IL-8, stimulating the migration of immune response cells, mainly neutrophils (8). The activation of antigen-presenting cells (APC) by the uptake of antigens associated with UPEC (9) allows the peptides presentation to T-cells and the differentiation of B-cells into plasma cells that produce immunoglobulins (10).

involves the host cell membrane which envelopes the bacteria by activating several proteins [tyrosine kinases, phosphoinositol-3 (PI-3) kinase and the cell division control protein (Cdc)], which promote complexes between components of the cytoskeleton (such as

actin, microtubules, and vinculin)¹⁶. Interestingly, UPEC can survive within macrophages, an event that contributes to its dissemination in the UT¹⁷. In the cytoplasm, UPEC initiates the formation of biofilm-like structures, called intracellular bacterial communities (IBC), which

are encapsulated in fusiform vesicles RAB27b⁺ (Ras-associated binding protein) and are associated with intermediate filaments of UT cells¹⁸. The IBC formation occurs in three stages: early stage (formation), middle stage (maturation) and late stage (fluxing and release by UT cells)¹⁹. The interaction of lipopolysaccharide (LPS) with the Toll-like receptor 4 (TLR-4) favors the increase of cyclic adenosine monophosphate (cAMP) and the expulsion of UPEC wrapped in RAB27b⁺ vesicles^{18,20,21}. The TLR4 activation by UPEC (LPS, FimH, and PapG) generates an intracellular oxidative state, which promotes UPEC filamentation by inhibiting bacterial division²². The growth of UPEC filaments promotes the host cell lysis, the efflux of the bacteria and the start of a new infection cycle^{23,24}. UPEC can enter a quiescent state for prolonged periods within exosomes, a mechanism that favors the bacteria to go unnoticed by the immune system²⁵. The TRP channel, mucolipin subfamily member 3 (TRPML3) is expressed on the surface of exosomes and can be activated by UPEC, promoting neutralization and exocytosis of exosomes with bacteria in a quiescent state²⁶. The expulsion of the UPEC wrapped in fusiform vesicles is probably promoted by the fusion of the latter with the uroepithelium cellular membranes by using alternate pathways, which are normally intended to increase the cell surface and favor the bladder distension²⁷. The exit of the bacteria from its quiescent state favors the process of UT reinfection by the same bacteria. This process is defined as recurrent UTI, and its complications promote the development of pyelonephritis and urosepsis²⁸. The UPEC pathogenicity through different mechanisms promotes colonization, persistence, and recurrence of infection, although the host also establishes an immune response against UTI (Figure 1).

Immune response in the urinary tract

The bladder is covered by a mucosa constituted of stratified tissue with three to six urothelial layers, classified as basal (5-10 µm in diameter), intermediate (20 µm in diameter) and superficial (25-250 µm in diameter); also, the submucosa contains blood and lymphatic vessels²⁹.

Different cells have been described as part of the mucosal immune system such as antigen-presenting cells (APC), cells expressing major histocompatibility complex class II (MHCII⁺) positive for cellular differentiation protein 11c (CD11c⁺), F480⁺ cells, dendritic cells (DC), macrophages and T-αβ and -γδ cells³⁰. UT lining cells are the first line of defense against uropathogens

and are mainly characterized by secreting soluble proteins, such as uromodulin. This protein stimulates the release of interleukins (IL) -1, IL-6 and IL-8, these are the first molecules detected in the UT after infection and are involved in the myeloid DC maturation and the migration of phagocytes to the bladder and kidney^{31,32}. Uromodulin prevents the adherence of UPEC to UT by the induction of bacteria-uromodulin aggregates, facilitating the elimination of the bacteria by the flow of urine³³⁻³⁵.

TLR2, TLR4, TLR5, and TLR11 are pattern recognition receptors (PRRs) that are expressed in UT cells and are capable of initiating a strong pro-inflammatory immune response^{30,36}. The early activation (2 hours post infection) produced by UPEC of the PRRs in UT cells, favors the release of IL-8, a chemoattractant that promotes the neutrophils migration to the bladder for the bacterium clearance³². Resident macrophages that do not express the lymphocyte antigen 6 complex (LY6C⁻ locus C1) in the UT submucosa act as uropathogenic monitor cells; once activated, they secrete C-X-C motif chemokine ligand 1 (CXCL1) and macrophage migration factor (MIF), which recruit a higher number of neutrophils. Meanwhile, C-C motif chemokine ligand 2 (CCL2) is secreted by LY6C⁻ macrophages for its own recruitment³⁷. Below the uroepithelium mast cells are located, which act as immunomodulators during UTI, inducing the release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and histamine³⁸. From 6 to 12 hours post infection, mast cells produce IL-10, which suppresses the immune response³⁹. There are other immune system cells known as natural killers (NK), DC and T-γδ; however, their function in the UT has been poorly studied. *In vivo* models using NK-cells deficient mice showed that they are more susceptible to a UPEC infection, probably due to the deficient release of TNF⁴⁰. Similarly, mice deficient in the T-γδ cell receptor are more susceptible to UTI compared with wild-type mice since these cells are a source of IL-17⁴¹. DC identified in the UT have shown significant activity during UTI, but their contribution to the immune response has not been precisely defined yet⁴². The UT adaptive immunity in the bladder is limited; however, it has been suggested that it can produce specific antibodies against UPEC in the kidney⁴³.

Multidrug- and extensively drug-resistant UPEC clinical strains

The increase in strains of multidrug-resistant (MDR) and extensively drug-resistant (XDR) UPEC has

complicated the UTI treatment, which has had a direct impact on costs and hospital stay⁴⁴. Recently, the characterization (resistance profile, integrons and extended-spectrum beta-lactamases (ESBL)) and typing (virulence genes and phylogenetic groups) of MDR and XDR-UPEC clinical strains isolated from children with complicated UTI have been described. Briefly, the MDR-UPEC clinical strains were grouped within the phylogenetic D group and were associated with the presence of classes 1 and 2 integrons. The XDR-UPEC strains were grouped mainly in the phylogenetic B2 group and showed an ESBL phenotype. The distribution of genes encoding fimbrial adhesins FimH, CsgA and PapG variant II were identified in both groups of UPEC clinical strains⁴⁵. It is important to highlight that a significant decrease in UTI requires the contribution of new viable vaccines that generate efficient protection against MDR and XDR-UPEC strains.

Vaccination

Currently available vaccines are mainly directed to promote a systemic immune response due to the difficulty to specifically stimulate mucosal immunity⁴⁶. Different UPEC antigens [O antigen, FimCH (FimH attached to chaperone FimC), PapDG (PapG bound to chaperone PapD), HlyA, and IroN] have been evaluated after parenteral immunization in animal models, generating a specific response at systemic level but not at the mucosal one⁴⁷⁻⁵⁰. The administration of antigens effectively induces the immunogenicity in the mucosa via intranasal (IN), intravaginal, and oral, while with parenteral administration a non-efficient response is obtained⁵¹. The SolcoUrovac® vaccine (uropathogenic lysates) administered vaginally significantly reduces recurrent UTI in phase II clinical studies⁵². However, the vaginal administration of the vaccine has shown adverse reactions manifested as pain and irritation of the vaginal epithelium⁵³. The oral administration of the immunomodulator Urostim stimulates a cellular and humoral response, but without generating protection against infection⁵⁴. Daily oral administration of OM-89/Uro-Vaxom® results in a reduction in recurrent UTI; however, this immunization route generates immunological tolerance and manifestations at gastrointestinal level⁵⁵. Transurethral immunization with UPEC strains attenuated in mice is not persistent in the UT and favors nonspecific protection⁵⁶. The IN immunization of different UPEC antigens (ChuA, Hma, Iha, IreA, IroN, IutA, and FimH) activates the release of high concentrations

of IgA in saliva, vagina, and urine^{57,58}. Recently, it was demonstrated that antibodies against fimbrial adhesins induce a decrease in bacterial adhesion and invasion and, therefore, yield protection in human bladder cells against UPEC colonization⁵⁹. IN vaccination with fimbrial adhesins may be the best way to generate a humoral immune response with IgA antibodies in the UT mucosa, protecting the host from UTI produced by UPEC.

Intranasal vaccination

The mucosa-associated lymphoid tissue (MALT) includes NALT (nasal-associated lymphoid tissue), BALT (bronchus-associated lymphoid tissue), GALT (gut-associated lymphoid tissue, including Peyer's patches and lymphoid follicles), genitourinary organs, and mammary and salivary glands as well. These mucous membranes are functionally connected as a common mucosal immune system, with an inductive site (inactive T and B cells) and an effector site (effector T and B cells)^{60,61}. The intranasal administration of vaccines with low dose antigen induces a higher immune response in the nasal, oral and urogenital mucosa compared to other immunization routes⁶². IN immunization in mice with a fusion protein based on bacterial adhesins of UPEC and *Proteus* stimulates the production of IgG and IgA antibodies in serum samples, nasal lavage, vaginal lavage, and urine⁶³. Moreover, the intranasal administration of six proteins related to UPEC iron uptake in CBA/J mice generated a systemic and mucosal response with high levels of IgM, IgG, and IgA antibodies, as well as a cellular response characterized by the induction of interferon gamma (IFN γ) and IL-17⁵⁸. The adjuvant type is important to obtain an effective immunization; however, in this review, our focus is only on describing the monophosphoryl lipid A (MPL) compound derived from *Salmonella*-LPS⁶⁴. MPL is an effective adjuvant with immunostimulatory properties such as the LPS but without its toxicity⁶⁵. Vaccines formulated with hepatitis B surface proteins, tetanus toxoid, influenza antigens and supplemented with MPL adjuvant have shown high IgA antibody titers in vaginal mucosal wash samples⁶⁶. Moreover, high IgG1 and IgG2 antibody titers have been observed in sera from mice intranasally immunized; IgA antibody titers have also been located at the effector site (tracheal lavage) and distal mucosa (vaginal lavage). Vaccines formulated with MPL induce a characteristic Th1 type systemic immune response⁶⁶.

Fimbrial adhesins and immune response

Several studies have described the pathogenicity of the bacteria, the immune response at the UT level and the vaccines assessed against UTI. These data have been used for the development of effective strategies directed at the prevention, treatment, and management of UTI caused by the emergence of MDR- and XDR-UPEC strains. UTI vaccines are generated from UPEC proteins (fimbrial adhesins, autotransporters, toxins, siderophores, flagellum and external membrane proteins) which are located on the bacterial surface, involved in the pathogenicity mechanisms, expressed during infection and considered as stimulators of the host immune response⁶⁷. UPEC produces the adhesin FimH, located in the distal part of the type 1 fimbriae, PapG in the P fimbriae, SfaS in the S fimbriae, FocI in the F1C fimbriae, DraA in the Dr fimbriae, and CsgA in the curli fimbriae. These fimbrial adhesins participate significantly in the adherence and colonization of UT cells¹¹. Type 1 fimbriae are widely distributed (80-90%) in UPEC strains and are related to processes of adhesion, invasion, and IBC formation in the UT⁶⁸. The *csgA* gene, which encodes the CsgA protein of the curli fimbriae, has been identified in the majority ($\geq 95\%$) of UPEC strains and is characterized by participating in cystitis and urosepsis⁶⁹⁻⁷¹. The P fimbriae have a distribution in UPEC strains between 35-45% and participate in the kidney colonization through the interaction with globosides, which are expressed on the surface of the renal cells. The globosides diversity has favored the appearance of three allelic variants of the *papG* gene: *papG*_{J96} (variant I), *papG*_{AD/IIA2} (variant II) and *prsG*_{J96} (variant III)⁷². It is important to mention that adhesins FimH, CsgA, and PapG (variant II) are relevant in the UPEC pathogenesis besides their wide distribution when compared to other fimbrial adhesins. Therefore, these adhesins can be considered viable biomolecules for the generation of an effective vaccine that stimulates an immune response.

Immunogenicity of FimH

The adhesin FimH of type 1 fimbriae has been widely used as a vaccine in animal models. Serum from C3H/HeJ mice immunized with FimH and type 1 fimbriae inhibits UPEC adherence to human bladder cells⁷³. The FimH mannose-binding domain, the complete protein and in association with FimC (chaperone) significantly reduce the adhesion in bladder and

kidney of mice and cynomolgus monkeys. These data suggest that anti-FimH specific antibodies inhibit UPEC colonization^{49,74-76}. Multivalent vaccines generated with various virulence factors of the bacterial surface are considered functional biomolecules due to their immunogenic capacity. The recombinant fusion protein FimH/FliC induces an increase of the cellular and humoral immune response against UTI in a murine model. Furthermore, high levels of immunoglobulins (IgG1 and IgG2a) and the release of cytokines (IFN γ and IL-4) by T cells (Th1 and Th2) have been identified after subcutaneous immunization⁷⁷. The fusion protein of FimH (UPEC) and MrpH (*Proteus mirabilis*) significantly induces the release of IgG and IgA antibodies in samples obtained from mice (serum, urine, nasal, and vaginal lavage) after intranasal immunization. Th1 and Th2 type cellular immunity, generated by the FimH/MrpH proteins with or without MPL adjuvant, suggests that the function of these proteins as adjuvant molecules⁶³. The FimH protein interacts with TLR4 through an α -mannosylated co-receptor that favors the activation of CD4- epithelial cells via Tirap-MyD88 pathway to produce the neutrophils recruitment into the mucosa^{78,79}.

Immunogenicity of CsgA

The CsgA protein has been considered as an adhesin involved in the UPEC adherence to bladder cells⁷¹. High levels of anti-CsgA antibodies identified in serum from convalescent sepsis patients have suggested that the CsgA protein is expressed *in vivo*. However, the immunogenicity of this protein in UPEC has not been studied, although the curli fimbriae of other *E. coli* significantly induce the release of proinflammatory cytokines such as TNF α , IL-6, and IL-8, in macrophages⁸⁰. The recombinant protein CsgA of *Salmonella* and the curli fimbriae of *E. coli* MC4100 participate in the IL-8 release in human THP-1 macrophages by the cooperative interaction of TLR1 and TLR2⁸¹. The CsgA protein has also been considered a PAMP (pathogen-associated molecular pattern), responsible for generating an IL-6 and IL-1 β response through the inflammasome pathway (NLRP3)^{82,83}.

Immunogenicity of PapG

UPEC adhesin PapG has been implicated in pyelonephritis processes in humans⁸⁴. The interaction of PapG with a TLR4 co-receptor with sphingolipids characteristics activates the secretion of IL-6 and IL-8^{78,85}.

Figure 2. Fimbriae visualization in UPEC U52 strain by transmission electron microscopy (MET). A micrograph of the UPEC U52 shows the presence of short and long fimbriae, corresponding to fimbriae type 1 and P, respectively (**A**). Both fimbriae are morphologically straight and thin. Another micrograph shows fine fibers in the form of flexible curls that form amyloid structures named curli (**B**). Micrographs were generated in the Central de Instrumentación de Microscopía of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, under the advice of Dr. Edgar Oliver López Villegas. Scale bar = 500 nm.

Intraperitoneal immunization by the complete fimbria and a PapDG protein complex induces the production of specific antibodies in mouse and cynomolgus monkey sera; also, histological sections show protection against kidney inflammation^{47,86}. However, non-significant differences were observed between the number of bacteria recovered in urine and the control group probably due to the expression of other fimbriae that contributed to bacterial colonization⁴⁷.

Multimeric fusion proteins

Recombinant proteins generated by fusion technology incorporating antigens from one or more pathogens have conferred a higher immune response and protection in animal models against UTI^{63,87}. These antigens or biomolecules are known as chimeras or fusion proteins. Recently, the importance and distribution of fimbria type 1 (FimH), curli (CsgA) and P (PapG) in UPEC strains isolated from pediatric patients have been demonstrated⁴⁵. These MDR- and XDR UPEC strains contain resistance factors to different groups of antibiotics, which reduces treatment options⁴⁵.

Our research group has designed, in the *Laboratorio de Investigación de Bacteriología Intestinal* (Intestinal Bacteriology Research Laboratory), a functional

biomolecule for the generation of a viable vaccine with the aim of reducing UTI in Mexico. We constructed the fusion biomolecule from the adhesins FimH, CsgA, and PapG variant II of the fimbria type 1, curli, and P of UPEC, respectively (Figure 2). The theoretical data of the fusion proteins indicated a stable conformation, with a high number of linear, conformational epitopes and peptides with MHC class II affinity (Table 1). The bioactivity assays of these proteins showed an increase in the release of IL-6 and IL-8 in human bladder HTB5 cells, with a maximum of 521.24 pg/ml for FCP (FimH-CsgA-PapG) and 450.4 pg/ml for FC (FimH-CsgA). Assays of fusion proteins antigenicity studied in serum and urine samples of patients with UTI detected high levels of IgA in comparison with samples obtained from patients with a negative urine culture (without the presence of leukocytes or nitrites in urine and UTI symptoms). Polyclonal rabbit antibodies against the fusion proteins reduced the UPEC adherence to HTB5 bladder cells, showing a 73% inhibition for the FC dimeric protein⁵⁹.

Data generated by our research group showed that the use of two or more molecules involved in the UPEC pathogenesis could be considered a new way to enhance the immune response against UTI (Figure 3A). Multimeric vaccines against UTI can be

Table 1. Bioinformatics analysis of the physicochemical and immunological properties of fusion proteins.

Fusion proteins	Number of amino acids	Molecular weight	ΔG (kcal/mol)	Linear epitopes	Conformational epitopes	MHC class II peptides
FC	417	43.09	-11,912.82	18	13	86
CP	450	48.83	-7,040.78	27	12	68
PF	617	66.4	-7,140.54	32	8	147
FCP	751	79.85	-13,866.68	32	17	149
CPF	751	79.85	-13,343.83	33	14	152
PFC	751	79.85	-13,577.65	33	13	159

F, FimH; C, CsgA; P, PapG; ΔG , Gibbs free energy; MHC, major histocompatibility complex

designed from the various antigens that are expressed by UPEC strains. Other studies have shown that the protein fusion of FimH from UPEC and MrpH from *Proteus mirabilis* (MrpH/FimH) generates an immune response and protection against both bacteria. Interestingly, IN immunization with the FimH/MrpH fusion protein in BALB/c mice generates the same immunogenic activity⁶³. Fusion proteins that include PAMP induce a specific, potent, and rapid response in the absence of an adjuvant⁸⁸. The specific recognition of PAMP is mainly mediated by TLR, a PRR that stimulates changes in antigen presentation and cell activation³⁶.

TLR4 is activated by its interaction with the FimH protein through an α -mannosylated co-receptor that stimulates the MyD88-NF κ B pathway, allowing the IL-6 and IL-8 release. TLR4 is also activated by the interaction of the adhesin PapG with the glycosphingolipid co-receptor (GLS), which promotes the Tram/Trif-NF κ B pathway, inducing the release of IL-6, IL-8 and the migration of neutrophils to the uroepithelium⁷⁸. Both adhesins increase the release of cytokines, as was recently demonstrated by this research group⁵⁹. Moreover, the adhesin CsgA, another protein with adhesive properties, can interact with the TLR1/TLR2 complex, generating a greater stimulation of the IL-6 and IL-8 release via the MyD88-NF κ B pathway (Figure 3B)^{81,83}. The interaction of PRR with fusion proteins generated by different adhesins (FimH, CsgA, and PapG) may produce a rapid activation and presentation of the different antigens in the APC. In specific cases, some of the PAMPs can serve as an effective and safe adjuvant. The B cells can act as APCs and present peptides to induce the helper T-cell activation⁸⁹. Also, the B cell can activate itself and

become a plasma cell that secretes specific antibodies against the different epitopes of the adhesins; the presence of these antibodies has been demonstrated in serum and urine of patients with UTI (Figure 3C)⁵⁹. The purpose is the generation of specific mucosal IgA antibodies against the main fimbriae and with the capacity to block the first pathogenicity step of the UPEC in UTI, as was recently demonstrated in inhibition assays⁵⁹.

Perspectives

UTI produced by UPEC are associated with the expression of multiple virulence factors assembled on the bacterial surface. The prevention of adherence, which represents the initial and fundamental step in UT colonization, may be the way to prevent UTI. Adherence is mediated by fimbrial adhesins with antigenic and immunogenic properties. However, the variety and regulation in the expression of the fimbriae have limited the efficacy of vaccines with simple formulations. The use of biomolecules as multimeric formulations is viable due to advances in bioinformatics, molecular biology, and protein purification protocols. The increase in the number of epitopes and peptides in the immunological synapse can generate antibodies with protective capacity against UPEC adherence to the UT. The advantage of using multimeric components in the generation of a fusion protein is the possibility that one of these proteins can function as an alternative adjuvant, avoiding the use of synthetic adjuvants in the formulation. IN immunization has generated significant benefits in the induction of an immune response in mucous membranes distant from the nasal mucosa. Under this immunization scheme, it is

Figure 3. The proposed mechanism of interaction of fusion proteins with the immune system. A. Fimbrial adhesins FimH, PapG, and CsgA linked with the repeated sequence EAAAK can potentiate the immune response against UTI. B. The FimH protein activates the TLR4 through an α -mannosylated co-receptor via the MyD88-NF κ B pathway, as well as the PapG adhesin by the interaction with a GLS co-receptor that promotes the Tram/Trif-NF κ B pathway. Both cell signaling pathways promote the release of IL-6 and IL-8 followed by the migration of neutrophils to the uroepithelium. The adhesin CsgA interacts with the TLR1/TLR2 complex generating a greater stimulation of the IL-6 and IL-8 release via the MyD88-NF κ B pathway. C. The interaction of the adhesins FimH, CsgA, and PapG with PRR can increase the rapid activation and the epitopes presentation in the APC cell. Some of these adhesins can function as an effective and safe adjuvant. The B-cell can act as an APC, presenting the peptides for activation of T helper cells and self-activation. Subsequently, plasma cells that secrete specific antibodies against the different epitopes of the adhesins are generated in sera and urine of patients with UTI.

considered that the administration of a multimeric fusion protein can activate the immune system cells in the NALT, migrate to its effector site in the UT and produce IgA antibodies to protect the host against UPEC infection.

Conclusions

The antigens located on the bacterial surface, expressed *in vivo* and involved in pathogenicity mechanisms, are viable for the generation of multimeric fusion proteins, which can be used for the development of new vaccines against UTI. The new data generated from fusion proteins suggest that these biomolecules can be considered as functional vaccines without the use of adjuvants in the formulations against UTI. IN immunization has been the best route of administration for its ability to generate an immune response in the UT mucosa. However, more studies should be considered in the future, focusing on the cellular and humoral immune response in the UT.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Conflicts of interest

The authors declare no conflicts of interest.

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