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REVIEW

Modulation of connexin signaling by bacterial pathogens and their toxins

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Abstract Inherent to their pivotal tasks in the maintenance of cellular homeostasis, gap junctions, connexin hemichannels, and pannexin hemichannels are frequently involved in the dysregulation of this critical balance. The present paper specifically focuses on their roles in bacterial infection and disease. In particular, the reported biological outcome of clinically important bacteria including *Escherichia coli, Shigella flexneri*, *Yersinia enterocolitica*, *Helicobacter pylori*, *Bordetella pertussis*, *Aggregatibacter actinomycetemcomitans*, *Pseudomonas aeruginosa, Citrobacter rodentium*, *Clostridium* species, *Streptococcus pneumoniae*, and *Staphylococcus aureus* and their toxic products on connexin- and pannexin-related signaling in host cells is reviewed. Particular attention is paid to the underlying molecular mechanisms of these effects as well as to the actual biological relevance of these findings.

Keywords Connexin · Pannexin · Hemichannel · Gap junction · Bacteria · Toxin

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Abbreviations

ADP	Adenosine diphosphate
A/E	Attaching and effacing
ATP	Adenosine triphosphate
CagA	Cytotoxin-associated antigen A
cAMP	Cyclic adenosine monophosphate
(CA-) MRSA	(Community-associated) methicillin-resistant *Staphylococcus aureus*
CL	Cytoplasmic loop
CNF1	Cytotoxic necrotizing factor 1
CT	Cytoplasmic carboxy tail
Cx	Connexin
DAEC	Diffusely adherent *Escherichia coli*
DNT	Dermonecrotic toxin
EAEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EL	Extracellular loop
EPEC	Enteropathogenic *Escherichia coli*
ERK1/2	Extracellular signal-regulated kinase 1/2
ETEC	Enterotoxigenic *Escherichia coli*
GJIC	Gap junctional intercellular communication
GTPase(s)	Guanosine triphosphate hydrolyzing enzyme(s)
HC	Hemichannel
IFN	Interferon
IL	Interleukin
ITX	Iota toxin
LJP	Localized juvenile periodontitis
LPS(s)	Lipopolysaccharide(s)
MALT	Mucosa-associated lymphoid tissue
MAPK	Mitogen-activated protein kinase
MRSA	Methicillin-resistant *Staphylococcus aureus*

NO	Nitric oxide
NT	Cytoplasmic amino tail
OMP(s)	Outer membrane protein(s)
Panx	Pannexin
PKA	Protein kinase A
PLC	Phospholipase C
PTX	Pertussin toxin
TLR	Toll-like receptor
TM	Membrane-spanning domain
ZO-1	Zonula occludens 1

Introduction

As a part of the innate immune system, epithelial cells form a first line of defense against infectious agents, such as bacteria and their toxins, by forming a physical barrier and by mediating inflammatory responses. Clearly, the integrity of the epithelium is heavily challenged upon infection, a process that might also affect the junctional complex [1] comprising an elaborated morpho-functional machinery that consists of anchoring junctions (i.e., adherens junctions and desmosomes), occluding junctions (or tight junctions) and communicating junctions [2]. The latter, also known as gap junctions, are composed of connexin proteins and mediate direct intercellular communication. In recent years, however, it has become clear that connexin-related signaling is not only restricted to gap junctions but also involves a number of other players, including connexin hemichannels and pannexins [3, 4]. The current paper discusses the involvement of connexins and their channels in bacterial infection and disease. In the first part, a concise overview of gap junction biology is provided, including their structural, functional, and regulatory properties. In the second part, the documented biological effects of prominent bacterial pathogens and their toxic products on connexin channels are reviewed.

Connexins channels: general properties

Structure

Morphologically, gap junctions appear as plaques at the cell plasma membrane surface and arise from the docking of two hemichannels (connexons) of adjacent cells, which on their turn are composed of six connexin (Cx) units. The connexin family comprises as many as 20 isoforms in mammals. They all share an identical molecular architecture, consisting of four membrane-spanning domains, two extracellular loops, one intracellular loop, one cytoplasmic N-terminal tail, and one cytoplasmic C-terminal tail (Fig. 1). Differences between connexins are mainly due to structural variety within the cytoplasmic regions. Connexins are named after their molecular weight and are expressed in a tissue-specific and even in a cell-specific manner. Thus, the most abundant connexin species in the human body has a predicted weight of 43 kDa and is therefore designated Cx43 [5–9]. Connexins interact with a number of other cellular proteins, including scaffolding proteins, junctional proteins, cytoskeletal proteins, trafficking regulators, posttranslational modifiers, and growth regulators, all of which may affect connexin metabolism and functionality [5, 10]. In the last few years, a second set of gap junction-related proteins has been characterized, the pannexin (Panx) family, which are structurally similar to connexins. At present, three pannexins have been identified in humans and rodents, namely Panx1-3, and they mainly occur in a hemichannel configuration [3, 11].

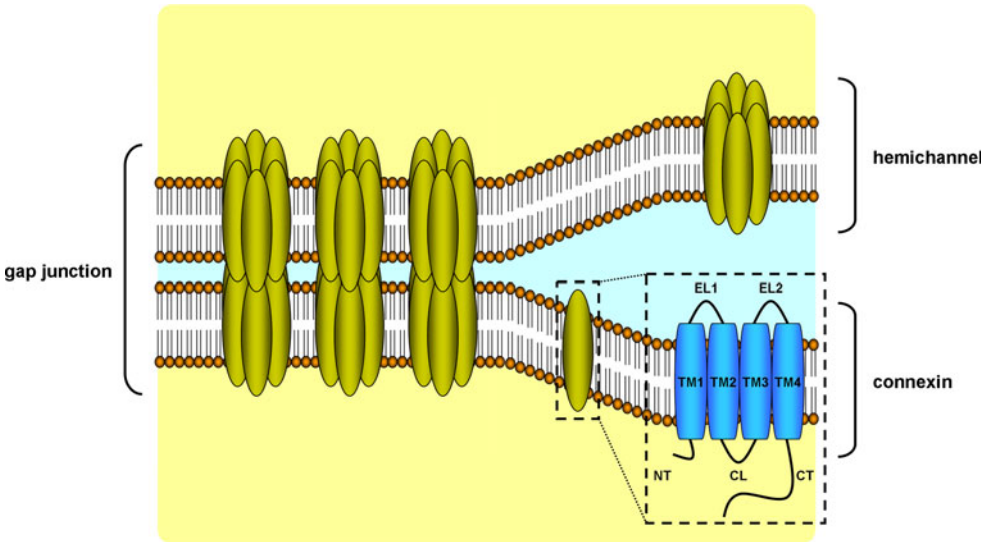

Fig. 1 Molecular architecture of gap junctions. Gap junctions are grouped in plaques at the cell plasma membrane surface and are composed of 12 connexin proteins, organized as two hexameric hemichannels or connexons of two apposed cells. The connexin protein as such is organized as four membrane-spanning domains (TM1-4), two extracellular loops (EL1-2), one cytoplasmic loop (CL), one cytoplasmic amino tail (NT), and one cytoplasmic carboxy tail (CT)

Function

Gap junctions provide an essential pathway for the intercellular exchange of small and hydrophilic molecules, including glucose, glutamate, glutathione, adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), inositol trisphosphate, and ions, like calcium, sodium, and potassium [3, 12]. The biophysical permeation characteristics of these substances rely on the nature of the connexin species that form the gap junction [12, 13]. For instance, ATP is more able to pass through Cx43-based gap junctions, compared to channels composed of Cx32 [14]. Obviously, numerous, if not all, physiological processes are driven by the substances that are conveyed via these channels, and hence gap junctional intercellular communication (GJIC) is considered as a key mechanism in the maintenance of tissue homeostasis [3, 7–9]. In the last decade, it has become clear that hemichannels in non-junctional areas at the cell plasma membrane surface can also function as transmembrane channels. In fact, connexin hemichannels foresee a pathway for communication between the intracellular compartment and the extracellular environment. The substances that travel through hemichannels are quite similar to those implied in GJIC, namely ATP, nicotinamide adenine dinucleotide, glutamate, glutathione, and prostaglandins [3, 6, 11, 15, 16]. Pannexin channels are also permeable for small signaling molecules, like ATP, thereby playing an important role in autocrine and paracrine purinergic signaling [11, 17]. As a result of their crucial role in maintaining tissue homeostasis, connexin and pannexin channels are also frequently involved in conditions of homeostatic imbalance, such as during inflammation. The latter has been most exemplified in the context of atherosclerosis, pulmonal inflammation, and ischemic brain damage [18]. In the current paper, the role of connexin-related signaling triggered by bacterial infection will be thoroughly discussed.

Regulation

A labyrinth of mechanisms underlies the regulation of the connexin life cycle and activity. Short-term control (i.e., second/minute range) of connexin channel functionality by the process of channel gating is governed by a number of factors, including transmembrane voltage, calcium ions, and hydrogen ions, though phosphorylation has gained most attention in this respect [7–9]. All connexins, with the exception of Cx26, are phosphoproteins. The outcome of the phosphorylation event, mainly occurring at the C-terminal connexin tail, depends on both the identity of the connexin species and the kinase type [19, 20]. Regulation of GJIC and hemichannel activity over the long-term (i.e., hour range) basically concerns peritranscriptional control of connexin expression. The structure of most connexin genes is rather simple and consists of a first exon, containing the 5'-untranslated region, which is separated by an intron from a second exon, bearing the complete coding sequence and the 3'-untranslated region [5, 21, 22]. Connexin gene transcription is ruled by conventional *cis*/*trans* actions, involving both ubiquitous transcription factors, like specificity protein 1 and activator protein 1, and tissue-specific transcription factors, such as hepatocyte nuclear factor 1 [22]. Epigenetic mechanisms, including histone acetylation and DNA methylation, predominate the pretranscriptional platform of connexin expression [8, 22]. Recently, microRNA species have been described as novel regulators of connexin expression at the posttranscriptional level [23–28].

Effects of bacterial pathogens and their toxins on connexin and pannexin channels

Gram-negative bacteria

Escherichia coli

Escherichia coli is a Gram-negative rod belonging to the family Enterobacteriaceae. In general, this bacterium is part of the normal microbiota of the lower bowel in humans and other mammals. Most strains of *E. coli* are non-pathogenic, but some, such as serotype O157:H7, can cause severe food-borne and life-threatening infections in man, while others can evoke meningitis and urinary tract infections [29]. Among the *E. coli* strains that can cause intestinal disease in healthy individuals, there are at least six well-characterized classes of pathotypes (Table 1). Some of them are also pathogenic for animals. However, dairy and beef cattle can also carry *E. coli* O157:H7 asymptomatically and shed it in their feces, hence acting as primary reservoirs of this *E. coli* serotype. Depending on the type of strain, *E. coli* infection causes a broad spectrum of intestinal and extra-intestinal syndromes by way of virulence factors for colonization and fitness, as well as toxic factors that distress a wide assortment of cellular processes. For a comprehensive review on *E. coli* virulence factors and their actions, the reader is referred to the paper of Kaper et al. [29]. *E. coli* infection has additionally been related to chronic disorders such as inflammatory bowel diseases, Crohn's disease and ulcerative colitis [30]. The various pathotypes of *E. coli* are likely clonal groups that are characterized by common O (lipopolysaccharide; LPS) and H (flagellar) antigens that characterize serogroups (O antigen only) or serotypes (O and H antigens). LPS is composed of three structural domains, more specifically lipid A, core oligosaccharide and O polysaccharides, and is

Table 1 Different pathotypes of *E. coli*, their hosts, and possible disease in humans

E. coli type	Host	Clinical picture in man	Reference
Enteropathogenic *E. coli* (EPEC)	Man[a], rabbit, dog, cat horse, pig, cattle, sheep	Profuse watery diarrhea, vomiting, low-grade fever, death	[183–187]
Enterohemorrhagic *E. coli* (EHEC)	Man, goat, sheep, cattle, pig, cat, chicken, gull	Bloody diarrhea[b], hemolytic-uremic syndrome, acute kidney failure	[183–185]
Enterotoxigenic *E. coli* (ETEC)	Man[c], pig, dog, goat, sheep, cattle, horse	(Traveler's) watery, secretory diarrhea, sometimes fever and vomiting, death	[183, 186, 188, 189]
Enteroaggregative *E. coli* (EAEC)	Man	Persistent watery, mucoid and secretory diarrhea	[183]
Enteroinvasive *E. coli* (EIEC)	Man	Abundant watery diarrhea, fever	[183]
Diffusely adherent *E. coli* (DAEC)	Man[d]	Watery diarrhea	[183]

[a] Often infants younger than 2 years

[b] Serotype O157:H7

[c] Healthy adults or children in developing world

[d] Typically in children and hospitalized patients

the major building stone of the outer membrane of the bacterial cell wall [31]. LPSs act as endotoxins and initiate strong immune responses in animals and man. As such, it was demonstrated that superantigens interact with LPS in an interferon (IFN)-γ-dependent way [32]. Lee et al. [31] showed that *E. coli*-derived LPS indeed significantly changed the level of IFN-γ amongst other cytokines in cystitis.

The effects of *E. coli*-derived LPS on connexin channels have been extensively documented and turn out to be complicated, whereby both, the identity of the connexin species and the cell type, are critical determinants. Thus, both downregulated [33–39] and upregulated [35, 37, 40–48] connexin protein quantities have been observed upon exposure of cells to LPS from *E. coli* in in vitro and in vivo settings, whether or not in combination with IFN-γ. In some cases [38, 41, 43, 44, 47, 48], but not all [34, 38], LPS also targeted connexin mRNA production. The deterioration of Cx32 protein in rat liver after LPS administration, for instance, was found to result from its corresponding mRNA degradation, which in turn was a consequence of shortening of the poly(A)tail [49, 50]. At an even more upstream level, *E. coli*-derived LPS can interfere with promoter activity of connexin genes, *in casu* Cx43, both in a negative [41] and in a positive [51] way. At the utter downstream platform of connexin expression, considerable attention has yet been paid to the impact of LPS on GJIC. As also noticed on the connexin protein level, both reduction [33, 34, 36–38, 43, 52–56] and enhancement [41, 42, 44, 47] of gap junction activity have been reported in several experimental models. Hemichannel activity, however, seems to be consistently upregulated by LPS [45, 57–60]. A number of mechanisms have been proposed to underlie LPS-induced modifications in GJIC, including nitric oxide signaling [43, 54, 56] and activation of kinase pathways [55, 61]. In fact, some of these pathways may be at the basis of the differential outcome observed for LPS on gap junctions and connexin hemichannels. Indeed, in Cx43-transfected human cervical carcinoma cells [58] and in co-cultures of primary mouse astrocytes and mouse microglial cells [59, 60], *E. coli*-derived LPS activated Cx43-hemichannels, but simultaneously inhibited their full channel counterparts. In the former case, this involved *c*-Src kinase, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2 and arachidonic acid signaling [58]. It has been suggested that such switching between GJIC and connexin hemichannel signaling through different regulation may serve to optimize cellular responses to newly occurring pathophysiological conditions [58], *in casu* LPS-induced inflammation. The importance of connexin signaling in the latter was recently confirmed by Okamoto and group, showing increased cytokine serum concentrations in Cx32 knock-out mice treated with LPS, thus suggesting that Cx32-based cellular communication protects against such insults [62]. In the last few years, it has become clear that pannexin hemichannels are also essentially involved in the biological effects that are triggered by LPS, particularly the inflammatory response. With respect to the latter, LPS from *E. coli* as well as from other Gram-negative bacteria, bind to a Toll-like receptor (TLR) to initiate the expression of inactive precursor interleukin (IL)-1β. Activation of precursor IL-1β occurs through cleavage by caspase 1, which itself becomes activated by processing within a cryopyrin-containing inflammasome. The subsequent extracellular release of active pro-inflammatory IL-1β requires the presence of ATP, which acts via the purinergic P_2X_7 receptor. Research from the group of Surprenant

showed that Panx1 hemichannels are crucial for the processing of caspase 1 and release of IL-1β. Panx1 hemichannel opening is hereby induced by ATP-stimulation of P_2X_7 receptors, as shown in mouse and human macrophages exposed to LPS [63–65]. It has been suggested that Panx1 hemichannels fulfill a critical role in the recognition and the intracellular delivery of bacterial molecules, including subsequent activation of the cryopyrin-mediated caspase 1 cleavage, independently of TLR signaling [66, 67]. The involvement of Panx1 hemichannels in inflammatory and immune reactions in response to bacterial molecules has also been demonstrated in other cell types, such as erythrocytes [68], dendritic cells [69] and neutrophils [70].

Shigella flexneri

Shigella flexneri, also called Group B *Shigella*, is one of the four *Shigella* species belonging to the family *Enterobacteriaceae* next to *E. coli*, though could equally be envisaged as an *E. coli* species as such based upon genome comparison. Infection with this Gram-negative microorganism may be water- or food-borne and is transmitted from an infected individual to another, mostly by the fecal–oral route. This often happens when basic hygiene conditions and habits are scarce, such as upon inadequate hand washing. *S. flexneri* infection results in shigellosis characterized by a wide range of symptoms, including bloody diarrhea originating from the colon, stomach cramps and fever. This type of dysentery may also be attended with a late-onset complication, called Reiter's syndrome, in approximately 3% of those infected [71, 72]. Bacterial invasion of the colonic mucosa represents a fundamental step in the pathogenesis of shigellosis. Invasion and replication within the colonic epithelium subsequently result in an intense inflammatory reaction by the host and eventually epithelial destruction [72]. *S. flexneri* pathogenesis is a complex process that involves several types of host immune cells. Assisted by polymorphonuclear leukocytes, *S. flexneri* reaches the basolateral epithelial cell pole, which is then invaded by this pathogen. Further invasion and lateral spreading of the bacteria within the epithelium causes tissue destruction that eventually burgeons into the typical *S. flexneri*-related clinical symptoms. Using an antibody approach, Clair and group found that Cx26, Cx32 and Cx43 hemichannel activity is crucial during *S. flexneri* invasion in a human Caco-2/TC7 intestinal epithelial cell line [73]. Further work from the same group also showed that infection of Cx26-transfected HeLa cells with *S. flexneri* induces Cx26 hemichannel opening in an actin- and phospholipase C-dependent way. As a result, ATP becomes released in the extracellular environment, which in turn further favors bacterial invasion and dissemination [74]. These findings were confirmed by Stella Man et al. [75], whereby it was also demonstrated that such bacterial spreading scenario is not present in HeLa cells transfected with deafness-associated mutated Cx26, Cx30 or Cx31. Recently, gap junctions were found to mediate the generation of an inflammatory response (i.e., IL-8 production) in uninfected epithelial cells from neighboring cells infected with *S. flexneri*, a process called bystander activation [76].

Yersinia enterocolitica

As a human pathogen, *Y. enterocolitica,* also belonging to the family *Enterobacteriaceae*, is most frequently associated with a broad spectrum of clinical symptoms such as acute diarrhea, terminal ileitis, mesenteric lymphadenitis, and pseudoappendicitis. This zoonotic pathogen has even approached the level of *Salmonella* and *Campylobacter* being a major cause of acute bacterial gastroenteritis in various countries. Human yersiniosis is mostly attributed to contaminated pork meat, milk and water, as well as blood transfusion [77, 78]. Once adhered to the intestinal epithelium overlying the Peyer's patches, *Y. enterocolitica* invades the epithelium and proliferates in the underlying lymphoid tissue [79]. In fact, this microorganism has developed several strategies at the molecular level allowing the accomplishment of a persistent infection and the encouragement of invasion of the host cells. Both the role of the 70–75 kb virulence plasmid, encoding for a type III secretion apparatus, in the evasion of phagocytosis leukocytes [80–82] and the role of integrin receptors on the mammalian cell surface in bacterial internalization [83–85] have been described on several occasions. Recently, Velasquez-Almonacid and colleagues [86] also demonstrated that connexin hemichannels contribute to *Y. enterocolitica* pathogenesis. In particular, infection of Cx43-tranfected HeLa cells with *Y. enterocolitica* triggers tyrosine phosphorylation of Cx43. This favors Cx43 hemichannel opening, thereby facilitating intracellular uptake of *Y. enterocolitica*.

Helicobacter pylori

The genus *Helicobacter* nowadays includes at least 32 species with validly published names [87]. The genus can roughly be divided into two groups, namely the enterohepatic and gastric *Helicobacter* species of which *H. pylori* is the type strain. This Gram-negative bacterium colonizes the stomach of about 40% of the human population in developed countries and even 80% of all humans in developing countries. *H. pylori* infection has been related to chronic active gastritis without clinical symptoms, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma [88–91].

Its role in the development of gastric cancer can be explained by the bacterium's ability to influence inflammatory cytokine secretion, apoptosis, cell proliferation, and cell differentiation through the activation of a number of oncogenic pathways [88, 92]. Among the various virulence factors that control this process, cytotoxin-associated antigen A (CagA) is the most prominent one, particularly in the context of *H. pylori*-induced gastric cancer [89, 93–96]. CagA affects cellular signaling as a result of altered epithelial cell permeability, cell contacts and cell polarity [88]. Indeed, *H. pylori* $CagA^+$ and $CagA^-$ strains both abolish GJIC in a human gastric epithelial cell line, associated with inhibition of cell proliferation [97]. Upon administration of water extracts of $CagA^+$ *H. pylori* to rats in which gastric ulcers were induced by acetic acid, healing and reappearance of Cx32 protein expression in gastric mucosa are significantly delayed [98]. $CagA^+$ *H. pylori* also downregulates Cx43 production in cultured human gastric carcinoma cells [99]. Likewise, in precancerous gastric lesions of patients with *H. pylori* infection, especially the $CagA^+$ variant, Cx32 and Cx43 levels are more reduced compared to non-infected patients [100, 101]. Eradication of *H. pylori* usually results in restoration of connexin expression in human gastric cells, both in vitro [99] and in vivo [100].

Bordetella pertussis

Currently, the genus *Bordetella* is comprised of nine recognized species of which *B. pertussis* and *B. parapertussis*$_{hu}$ in humans, and *B. bronchiseptic* and *B. parapertussis*$_{ov}$ in animals are the most common ones. These Gram-negative bacteria produce a toxin, called lethal toxin or dermonecrotic toxin (DNT), which elicits a variety of precarious disorders amongst several organs in experimental animals. As such, DNT of *B. bronchiseptica* is considered to play a role in the pathogenesis of porcine atrophic rhinitis resulting from turbinate atrophy due to a deficient osteoblastic differentiation [102]. In humans, infection of the airways with *B. pertussis* has been recognized as a significant hazard to newborns and infants and is increasingly acknowledged as a cause of pertussin, or whooping cough, in adolescents and adults. Common complications of the disease, sometimes fatal, include bronchopneumonia and encephalopathy [103–105]. Although the pathogenesis of *B. pertussis* infection and disease largely remains to be established, a number of virulence-associated factors are known to drive these processes. These include calmodulin-activated adenylate cyclase toxin, tracheal cytotoxin and pertussin toxin (PTX) [106, 107]. The latter is envisaged as the main etiological agent of whooping cough that plays a crucial role in the initial colonization stage of the infection. PTX is able to bind to the surface of phagocytes, which then take up the bacteria. The lipid moiety of PTX is thought to be a major modulator of the host immune defenses. PTX has been found to inhibit GJIC in Novikoff hepatoma cells. This was not a result of changes in Cx43 mRNA or protein levels, nor was it associated with modifications of its phosphorylation pattern, but was due to inhibition of gap junction assembly at the cell plasma membrane surface, which in turn stemmed from aberrant Cx43 trafficking [108].

Aggregatibacter (Actinobacillus) actinomycetemcomitans

Aggregatibacter, formerly *Actinobacillus*, *actinomycetemcomitans* [109] is a capnoic Gram-negative coccobacillus known to produce localized juvenile periodontitis (LJP), osteomyelitis, and infections of the heart, brain and urinary tract [110, 111]. It has been suggested that *A. actinomycetemcomitans* induced-apoptosis of alveolar bone cells plays an important role in periodontal diseases [112]. This bacterial agent forms several putative virulence factors, such as a secreted chaperonin 60 and repeat toxin leukotoxin, that both affect several white blood cell types, proteins possessing the ability to block eukaryotic cell cycle progression, including cytolethal distending toxin, and proteins that can provoke diverse types of pro-inflammatory cytokine networks [113–115]. Other proteins secreted by *A. actinomycetemcomitans* play a central role in binding to endothelium or epithelium and/or for possible invasion of host cells. This specifically holds true for sarcosyl-insoluble outer membrane protein (OMP) members, which may underlie *A. actinomycetemcomitans*-induced LJP [116, 117]. As such, both *A. actinomycetemcomitans* and its OMP29 product reduce GJIC in cultures of human gingival epithelial cells. This is associated with downregulated levels of phosphorylated and non-phosphorylated Cx43 protein and concomitant lowered cAMP amounts. In the same study, it was also demonstrated that the anti-ulcer agent irsogladine maleate is able to counteract the deleterious actions of OMP29 on Cx43-based gap junctions [118].

Pseudomonas aeruginosa

Pseudomonas aeruginosa is one of the 13 members of the genus *Pseudomonas*, which are Gram-negative, aerobic rods. It is ubiquitous in soil and water as well as on surfaces in contact with soil or water. In addition, it can be present in a biofilm attached to some surface or substrate. This bacterial agent is considered occasionally as a pathogen of plants, but it also has become more and more accepted as an emerging opportunistic pathogen of clinical relevance in animals and humans. The main requisite for the development of an infection with this microbe is a

compromised immune system [119, 120]. Populations of patients prone to acquire *P. aeruginosa* infection are those suffering from cancer, cystic fibrosis, and burns. *P. aeruginosa* can cause multiple health-care associated nosocomial infections such as septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis [121, 122]. The secretion of effector proteins is a crucial step during *P. aeruginosa* infection. These effector proteins disrupt the epithelial barrier and hamper wound repair by limiting cell migration and cell proliferation, causing apoptosis or necrosis and abolishment of tight junctions. They also impede macrophage and neutrophil function and migration [123]. Furthermore, one of the broad arsenals of virulence determinants produced by this bug that plays an important role in the development of these infections is LPS [124]. Intratracheal instillation of *P. aeruginosa*-derived LPS in mice results in upregulated alveolar Cx43 production. This is thought to be crucial for neutrophil recruitment to the lung [125]. By contrast, intranasal instillation of LPS in mice downregulated the Cx40 expression in lung [126]. Similarly, in cultured nasal epithelial cells, *P. aeruginosa* LPS negatively affected Cx43 expression [127].

Citrobacter rodentium

Citrobacter rodentium is a natural pathogen of mice using attaching and effacing (A/E) lesion formation as an apparatus of tissue targeting and infection, similar to enterohemorrhagic *E. coli* (EHEC) and Enteropathogenic *E. coli* (EPEC) in humans and domestic animals. A diversity of clinical manifestations and differing lethality degrees are noted, albeit with one vast similitude, namely hyperplasia of the colon. Important to mention is that *C. rodentium*-infected laboratory mice are in particular a powerful in vivo model for studying the pathogenesis of infectious gastroenteritis [128, 129]. Using this model, Guttman and colleagues recently reported increasing levels of Cx43 in mouse colon, whereby unpaired hemichannels were formed at both the apical and the lateral membrane surface of the colonocytes. By applying animals genetically deficient in Cx43, it was subsequently demonstrated that Cx43 hemichannel opening triggers water release during *C. rodentium*-induced diarrhea [130].

Gram-positive bacteria

Clostridium species

Clostridium botulinum *Clostridium* bacteria are Gram-positive anaerobic organisms. The genus is comprised of approximately 100 species that contain ubiquitous bacteria as well as important pathogens including *C. botulinum* [131]. The obligate anaerobe *C. botulinum* is worldwide present in aquatic sediments and soils and may hence contaminate vegetables. The bacterium is, however, also able to colonize the gastrointestinal tract of mammals, birds and fish. *C. botulinum*, and rarely *C. butyricum* and *C. baratii*, produce highly potent botulinum neurotoxins that are responsible for botulism, a severe neuroparalytic disease. Seven serologically distinct neurotoxins (A–G) that differ in structure, toxicity, and host species specificity have been described. Most strains produce one neurotoxin, but a few produce two [132]. Neurotoxins A, B, E, F and rarely G give rise to flaccid paralysis in humans. Yet, human botulism is divided into five clinical classes, namely food-borne, wound, infant, adult infectious and inadvertent botulism [133]. Two other toxins are produced by some strains of *C. botulinum* types C and D, namely binary actin-modifying C2 toxin and C3-like exoenzyme [132]. The latter displays adenosine diphosphate (ADP)-ribosyltransferase activity and negatively affects Rho family guanosine triphosphate hydrolyzing enzymes (GTPases). It has been reported that inhibition of Rho GTPases by the C3 toxin in cultured cancerous human astrocytes results in repression of extracellular ATP release, probably through connexin or pannexin hemichannels [134]. Reduction of ATP-related communication through hemichannels has also been observed in cultured primary rat astrocytes exposed to *C. botulinum* toxin A [135]. Furthermore, *C. botulinum* toxin C3 also influences gap junction activity, as it represses GJIC in cultures of primary rat cardiomyocytes. This is not associated with modifications in Cx43 protein levels or with changes in the Cx43 phosphorylation pattern. The interaction between Cx43 and *zonula occludens* 1 (ZO-1), increases by the C3 toxin. Cx43-ZO-1 interaction typically occurs at the periphery of gap junction plaques and is thought to control gap junction turnover. Thus, Cx43-ZO-1 interaction is inversely related to gap junctional plaque size and therefore negatively affects GJIC [136]. In the eye lens of transgenic mouse expressing *C. botulinum*-derived C3 ADP-ribosyltransferase and in cultures of primary rabbit corneal epithelial cells of, *C. botulinum* toxin C3 reduced Cx50 immunostaining [137] and Cx43 immunoreactivity [138], respectively.

Clostridium perfringens *Clostridium perfringens*, is, similar to *C. botulinum*, found in soil and marine samples as well as in the intestinal environment of domestic animals and human beings as a benign component. Nonetheless, when this environment is distorted by sudden changes in diet or other factors, it also can act as an opportunistic veterinarian and human pathogen, resulting in a plethora of syndromes such as food poisoning, necrotic enteritis and gas gangrene in man, and enterotoxaemia in cattle, sheep, horses and pigs, necrotic enteritis

in poultry and typhlocolitis in equines. Development of these disorders can mainly be attributed to potent toxins that act locally or, when absorbed, systemically with usually destructive consequences for the host [139–142]. Next, *C. perfringens* infection also may result in a decreased cardiac contractility, mainly due to phospholipase C and may therefore be lethal [143]. *C. perfringens* strains have the ability to produce a large number of toxins, including enterotoxin, beta2 toxin, perfringolysin O, alpha toxin, beta toxin, epsilon toxin and iota toxin (ITX) [142]. ITX appears to influence several actin isoforms including alpha-actin of the heart muscle as shown in an in vitro study conducted by the group of Gabbiani [144]. Derangeon et al. [136] additionally tested the outcome of a chimeric toxin constructed of *C. perfringens*-derived iota toxin component Ib and *C. botulinum*-derived C3 ADP-ribosyltransferase on gap junctions in cultures of ventricular cardiomyocytes from neonatal rats and found that it decreases GJIC and Cx43 gap junctional plaque size. Furthermore, exposure of isolated gap junction-rich cell membrane fractions from mouse liver to phospholipase C from *C. perfringens* resulted in disappearance of gap junctions [145].

Clostridium difficile *Clostridium difficile* is a major causative agent of antibiotic-associated diarrhea that can result in serious complications, including *Pseudomembranous colitis*. This is often a consequence from eradication of the residual gut microbiota by antimicrobials. In most cases however, *C. difficile*-infection passes by without any symptoms [141]. *C. difficile* associated disease may also be represented as an extracolonic manifestation causing infection of the small intestine, reactive arthritis, osteomyelitis, various skin diseases and bacteraemia [146]. In addition, brain astrocytes, and more specifically the cytoskeleton, may be affected as shown in an in vitro study of Ciesielski-Treska and co-researchers [147]. The pathogenicity of *C. difficile* is interceded by two large exotoxins, namely Toxin A and Toxin B. Once transported into the cytoplasm of the host cell, both toxins act as negative effectors of Rho family GTPases. Most of *C. difficile* isolates with mutations in the *Toxin A* and *Toxin B* genes produce another toxin, *C. difficile* toxin. This iota-like toxin has ADP-ribosyltransferase activities, but is thus far assumed to be non-essential in the development of colitis caused by *C. difficile* [146, 148]. It also inhibits Rho family GTPases and Blum and colleagues reported that this action results in a drop of extracellular ATP release in cultured human cancerous astrocytes. Although unequivocal scientific evidence is lacking, this study suggests that the ATP release system affected by Toxin B might be a hemichannel composed of either connexins or pannexins. However, the relevance of this finding is yet unclear [133].

Streptococcus pneumoniae

Streptococcus pneumoniae, also referred to as pneumococcus, a member of the genus *Streptococcus*, is a normal inhabitant of the human upper respiratory tract, but may also act as an important human pathogen which is the causative agent of numerous disorders ranging from pneumonia, usually of the lobar type, acute paranasal sinusitis and otitis media to meningitis, bacteremia, sepsis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, necrotizing fasciitis, and brain abscess in humans and/or animals. Moreover, this Gram-positive bacterium is at this moment the prime cause of invasive bacterial disease in infants and the elderly [149–154]. With regard to acute otitis media, *S. pneumoniae* is one of the top-three isolates found in this pervasive illness in infants and children, next to *Haemophilus influenzae* and *Moraxella catarrhalis* [155] with the highest rates in developed and emerging countries [156]. An important consequence of middle ear infection is sensorineural hearing loss due to cochlear damage. This is associated with loss of Cx26 expression in the spiral ligament [157]. Cx26-based gap junctions are known to play a role in the cycling of potassium ions and intercellular metabolite sharing, which is essential for the maintenance of the ionic composition of the endolymph and the endocochlear potential in the cochlea. Deterioration of these critical functions may results in cochlear dysfunction and deafness [158].

Staphylococcus aureus

Staphylococcus aureus, a Gram-positive spherical bacterial microorganism, can be cultured from nasal passages of clinically healthy humans, but most other anatomical locales, such as the skin, oral cavity and gastrointestinal tract may also frequently yield this bacterial species. Methicillin-resistant *S. aureus* (MRSA) has been well-established in hospitals for several decades, though MRSA strains have additionally emerged more and more outside the hospital becoming identified as community associated-MRSA (CA-MRSA) strains of the organism, which now predominate staphylococcal infections observed in clinic settings [159, 160]. Since 2005, a MRSA clone CC398 has been reported to colonize pigs, veal calves, and broiler chickens and to infect dairy cows. It also has the capacity to spread to humans [161]. *S. aureus* is the leading bacterial cause of gastroenteritis as a result of the consumption of contaminated food. More specifically, staphylococcal food poisoning is attributable to the uptake of enterotoxins belonging to the family of pyrogenic toxins produced by *S. aureus*. Types of diseases caused by *S. aureus* and its virulence determinants include, next to food-borne

originated-gastroenteritis, skin infections possibly evolving into impetigo or cellulitis or scalded skin syndrome, lactational mastitis, bacteremia or sepsis, pneumonia, endocarditis which may lead to heart failure, osteomyelitis, chorioamnionitis and neonatal sepsis in pregnancy, brain abscess, and toxic shock syndrome and circulatory collapse eventually leading to death mainly in people with severe burns over large areas of the body [160, 162–166]. Peptidoglycan present in the cell wall of *S. aureus* appears to play an important role in the toxic shock syndromes' pathogenesis. It has indeed been acknowledged that this macromolecule, and more specifically its embedded TLR2 ligand, operates as a pathogen-associated molecular trigger that activates the pro-inflammatory innate immune reaction. However, it also has the ability to modulate the anti-inflammatory response related to its pathogenicity [167]. Such contradictory actions are also found at the gap junctional platform. Thus, *S. aureus*-derived peptidoglycan was reported to induce GJIC in cultures of primary mouse microglia, which was linked to enhanced Cx43 gene transcription and translation [168]. In cultures of primary mouse astrocytes, though, both *S. aureus* and its peptidoglycan silence gap junction activity, a process that involves the p38 MAPK signaling cascade. At the connexin level, a decrease in mRNA and protein amounts of both Cx30 and Cx43 was observed, whereas Cx26 production became induced [169]. In a recent study, Karpuk and colleagues reported downregulated astrocyte GJIC in brain slices of mice infected with *S. aureus*. Interestingly, this coincided with activation of hemichannel activity, which in turn was paralleled by increased expression of Cx30 and Cx43 [170]. Such opposite outcome on both channel types is reminiscent of the differential actions of LPS on hemichannels and gap junctions [58–60], and thus suggests specific functions for these channels in these conditions. On the other hand, peptidoglycan, albeit derived from *S. epidermidis*, was recently found to increase Cx43 mRNA and protein abundance in cultured murine endothelial cells and by doing so, both GJIC and Cx43-based hemichannel activity became elevated. The latter was probably triggered through Cx43 phosphorylation and in turn caused induction of IL-6 and TLR2 expression. Thus, Cx43-based hemichannels are likely to play an important role in the initiation of early innate immune responses in the endothelium [171].

Conclusions and perspectives

Infectious diseases are caused by a plethora of pathogenic microorganisms, among which bacteria are prominent ones. Upon bacterial infection, epithelial barriers become compromised, which is unavoidably accompanied by disruption of cell junctions. *Rickettsia* infection, for instance, is associated with abrogation of adherens junction formation in host cells [172], whereas desmosomes are targets for exfoliative toxin released by *S. aureus* [173]. Likewise, several bacterial pathogens modulate tight junctional structure and function, including *E. coli*, *S. flexneri*, *H. pylori*, and *C. perfringens* [174–176]. Given their key roles in the maintenance tissue homeostasis, it is not surprising that connexins and pannexins, as well as their channels, are also affected during infectious diseases [3, 4, 9] from bacterial, viral or parasitic origin. As such, the classical swine fever virus [177], the Borna disease virus [178] and the human cytomegalovirus [179] have been found to downregulate connexin expression. Similar findings were reported for the protozoan parasites *Trypanosoma cruzi* [180, 181] and *Toxoplasma gondii* [181]. As specifically addressed in the current paper, bacterial pathogens and their toxins also typically modify connexin production in host cells. A variety of mechanisms hereby seems to be involved, implying both the most upper regulatory levels of connexin expression and the downstream platform of posttranslational control of connexin channel functionality (Table 2). The overall impact of these events on GJIC and connexin hemichannel functionality is complex and depends on a number of parameters, including the cellular context and the connexin species. Consequently, the biological relevance of these communicative changes yet remains largely unclear. Although exceptions exist, most reports support a scenario whereby pathogens modify connexin-related signaling pathways in such a way that endogenous communication in host cells, especially GJIC, becomes lost at the expense of the host-pathogen interaction. The latter is likely to contribute to the pathogenesis of the invading organism. This particularly holds true for connexin hemichannels. In fact, opening of connexons following bacterial infection not only foresees a direct route for the cellular uptake of the pathogen [86], but also provides a pathway for extracellular release of ATP [74] and water [130], which may favor bacterial invasion and spreading [73, 74, 86]. For pannexin hemichannels, a more specific role has been reported, namely their involvement in the processing of caspase 1 and release of IL-1β during LPS-evoked inflammation [63–65]. It should be noted, however, that the research field of hemichannels composed of connexins or pannexins, is still in its infancy, mainly because of the ubiquitous lack of exploratory agents that allow unequivocal discrimination from either their full channel counterparts, as in the case of connexins, or from other channel types, especially applying to pannexins. It can be anticipated that more light will be shed on the involvement of each of these connexin-related signaling pathways in bacterial pathogenesis upon introduction of appropriate

Table 2 Effects of bacterial pathogens and their toxins and connexin-related signaling

Agent	Cell type	Effect	Mechanism	Reference
CNF1 (*E. coli*)	Rat primary ventricular cardiomyocytes	↑GJIC	Activation of RhoA GTPase ↑Cx43 junctional plaque size ↓Cx43/ZO-1 interaction	[134]
LPS (*E. coli*)	Co-culture of rat primary astroglial cells and rat primary microglial cells	↓GJIC	↓Cx43 protein expression	[33, 34]
LPS (*E. coli*)	Co-culture of mouse primary astrocytes and mouse primary microglial cells	↓GJIC		[57, 58]
LPS (*E. coli*)	Co-culture of mouse primary astrocytes and mouse primary microglial cells	↑HC		[59, 60]
LPS (*E. coli*)	Rat primary neonatal astrocytes	↓GJIC	↓Cx43 mRNA and protein expression Activation of inducible NO synthase ↑TLR4 expression ↑ERK1/2 phosphorylation ↓Caveolin-3 expression	[43]
LPS (*E. coli*)	Human Cx43-transfected cervical carcinoma HeLa cells	↑HC	Activation of arachidonic acid signaling	[58]
LPS (*E. coli*)	Human Cx26-transfected cervical carcinoma HeLa cells	↑HC		[58]
LPS (*E. coli*)	Human Cx43-transfected cervical carcinoma HeLa cells	↓GJIC		[58]
LPS (*E. coli*)	Human primary microglial cells	~GJIC		[190]
LPS (*E. coli*)	Rat microvascular endothelial cells from skeletal muscle	↓GJIC	Activation of tyrosine kinases	[55]
LPS (*E. coli*)	Mouse aortic endothelial cells[b]	↓GJIC	↓Cx40 protein expression	[36]
LPS (*E. coli*)	Rat primary astrocytes	↓GJIC	Activation of NO synthase	[56]
LPS (*E. coli*)	Rat primary hepatocytes	~GJIC		[37]
LPS (*E. coli*)	Co-cultures of rat primary hepatocytes with Kupffer cells	↓GJIC		[37]
LPS (*E. coli*)	Isolated rat livers from rats[2]	↓GJIC	↓Cx32 mRNA and protein expression ↓Cx26 protein expression	[38]
LPS[a]	Human mesenchymal stem cells	↑HC		[57]
LPS[a]	Mouse microglial cells	↑HC	↑Cx32 cell plasma membrane expression	[45]
LPS[a]	Rat hepatic stellate cells	~GJIC		[48]
LPS[a]	Co-culture of human umbilical vein endothelial cells and human umbilical vein smooth muscle cells	↓GJIC		[191]
LPS[a]	Co-culture of mouse $CD4^+$ T lymphocytes and mouse macrophages	↓GJIC		[53]
LPS[a]	Co-culture of rat IEC-6 enterocytes and mouse J774 macrophages	↓GJIC	↓Cx43 phosphorylation Cx43 redistribution to cytosol ↑NO production	[54]
LPS (*E. coli*) + IFN-γ	Rat and mouse primary microglial cells	↑GJIC	↑Cx43 protein expression	[42]
LPS (*E. coli*) + IFN-γ	Mouse epidermis-derived XS52 dendritic cells	↑GJIC		[44]

Table 2 continued

Agent	Cell type	Effect	Mechanism	Reference
LPS (*E. coli*) + IFN-γ	Mouse bone marrow-derived dendritic cells	↑GJIC	↑Cx43 mRNA and protein expression	[44]
LPS[a] + IFN-γ	Human primary monocytes	↑GJIC	↑Cx43 mRNA and protein expression	[41]
LPS[a] + IFN-γ	Mouse peritoneal macrophages	~GJIC		[192]
LPS[a] + IFN-γ	Mouse J774 macrophages	~GJIC		[192]
LPS[a] + hypoxia + reoxygenation	Mouse microvascular endothelial cells from skeletal muscle	↓GJIC	↓Cx40 PKA-specific serine phosphorylation	[61]
S. flexneri	Human Cx26-transfected cervical carcinoma HeLa cells	↑HC	Actin polymerization Activation of PLC	[74]
S. flexneri	Human Cx26-transfected cervical carcinoma HeLa cells	↑HC		[75]
S. flexneri	Human mutated Cx26-transfected cervical carcinoma HeLa cells	~HC		[75]
S. flexneri	Human Cx30-transfected cervical carcinoma HeLa cells	~HC		[75]
S. flexneri	Human Cx31-transfected cervical carcinoma HeLa cells	~HC		[75]
S. flexneri	Human Caco-2/TC7 intestinal epithelial cells	↑HC		[73]
Y. enterocolitica	Cx43-transfected human cervical carcinoma HeLa cells	↑HC	↑Cx43 phosphorylation	[85]
H. pylori $CagA^+/CagA^-$	Human gastric SGC-7901 epithelial cells	↓GJIC		[96]
B. pertussis toxin	Rat Novikoff hepatoma cells	↓GJIC	↓Gap junction assembly ↓Cx43 trafficking	[107]
A. actinomycetemcomitans	Human primary gingival epithelial cells	↓GJIC		[116]
OMP29 (*A. actinomycetemcomitans*)	Human primary gingival epithelial cells	↓GJIC	↓Cx43 protein expression ↓cAMP levels	[116]
C. rodentium	Mouse colonocytes[2]	↑HC	↑Cx43 protein expression	[129]
Toxin A (*C. botulinum*)	Rat primary astrocytes	↓HC		[135]
Toxin B (*C. botulinum*)	Human Cx43-transfected cervical carcinoma HeLa cells	↑HC		[58]
Toxin C3 (*C. botulinum*)	Rat primary ventricular cardiomyocytes	↓GJIC	Inhibition of RhoA GTPase ↓Cx43 junctional plaque size	[134]
Toxin C3 (*C. botulinum*)	Human 1321N1 astrocytes	↓HC Inhibition of RhoA GTPase ↑Cx43/ZO-1 interaction		[133]
Chimera of toxin C3 (*C. botulinum*) and Iota toxin (*C. perfringens*)	Rat primary ventricular cardiomyocytes	↓GJIC Inhibition of RhoA GTPase		[134]
Iota toxin (*C. perfringens*)	Rat primary ventricular cardiomyocytes	~GJIC		[134]
Toxin B (*C. difficile*)	Human 1321N1 astrocytes	↓HC Inhibition of RhoA GTPase		133
S. typhimurium	Mouse B16F10 melanoma cells	↑GJIC	↑Cx43 protein expression	[182]
S. typhimurium	Co-culture of mouse B16F10 melanoma cells and mouse DC1 dendritic cells	↑GJIC	↑Cx43 protein expression	[182]

Table 2 continued

Agent	Cell type	Effect	Mechanism	Reference
S. aureus	Mouse brain slices[b]	↑HC ↓GJIC	↑Cx43/Cx30 protein expression	[170]
S. aureus	Mouse primary astrocytes	↓GJIC	↓Cx43/Cx30 mRNA and protein expression ↑Cx26 mRNA and protein expression Activation of p38 MAPK pathway	[168]
Peptidoglycan (*S. epidermidis*)	Mouse b.End5 endothelial cells	↑GJIC ↑HC	↑Cx43 mRNA and protein expression	[171]
Peptidoglycan (*S. epidermidis)*	Cx43-transfected human cervical carcinoma HeLa cells	↑HC		[171]
Peptidoglycan (*S. aureus*)	Mouse primary microglia	↑GJIC	↑Cx43 mRNA and protein expression	[167]
Peptidoglycan (*S. aureus*)	Mouse primary astrocytes	↓GJIC	↓Cx43/Cx30 mRNA and protein expression ↑Cx26 mRNA and protein expression Activation of p38 MAPK pathway	[168]

[a] Source not specified

[b] In vivo/ex vivo study

cAMP cyclic adenosine monophosphate, *CNF1* cytotoxic necrotizing factor 1, *Cx* connexin, *ERK1/2* extracellular signal-regulated kinase 1/2, *GJIC* gap junctional intercellular communication, *GTPase* guanosine triphosphate hydrolyzing enzyme, *HC* connexin hemichannel communication, *IFN* interferon, *LPS* lipopolysaccharide, *MAPK* mitogen-activated protein kinase, *NO* nitric oxide, *OMP29* outer membrane protein 29, *PKA* protein kinase A, *PLC* phospholipase C, *TLR* Toll-like receptor, *ZO-1* zonula occludens 1

experimental tools. The fundamental knowledge that will be gained by doing so is not only of major importance for molecular biologists, but is also of great interest to clinical scientists. Indeed, connexins and their channels potentially represent new targets for the treatment of infectious diseases. The anticonvulsant levetiracetam [33] and the glucocorticoid dexamethasone [34], for example, were both reported to alleviate LPS-induced reduction of GJIC and Cx43 expression levels in co-cultures of primary rat astroglial cells and primary rat microglial cells. Similarly, abrogation of GJIC and Cx43 production in human gingival epithelial cells exposed to *A. actinomycetemcomitans*-derived OMP29 could be counteracted by the anti-ulcer agent irsogladine maleate [118]. On the other hand, the effects of bacteria on connexin signaling might be exploited in cancer therapy. In this context, Saccheri and group recently reported increased Cx43 levels in *S. typhimurium*-infected human and mouse melanoma cells, resulting in the establishment of a gap junction network with adjacent dendritic cells. As a result, antigenic peptides are transferred from the melanoma cells to the dendritic cells, which then present these peptides on their surface. The latter then trigger activation of cytotoxic T cells against the tumor antigen and thus an anti-tumor response [182]. Collectively, these data show that further exploitation of this emerging research field may open new perspectives for the development of new site-directed strategies for the clinical treatment of various diseases.

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