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REVIEW

Disifin (Sodium tosylchloramide) and Toll-like receptors (TLRs): evolving importance in health and diseases

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Abstract Disifin has emerged as a unique and very effective agent used in disinfection of wounds, disinfection of surfaces, materials and water, and other substances contaminated with almost every type of pathogenic microorganism ranging from viruses, bacteria, fungi and yeast, and, very possibly, protozoan parasites, as well. The major active component of Disifin is tosylchloramide sodium (chloramine T). However, the mechanism by which Disifin suppresses the activities of pathogenic microbial agents remains enigmatic. The molecular mechanisms, and the receptors and the signal transducing pathways responsible for the biological effects of Disifin are largely unknown. Despite considerable advances, enormous investigative efforts and large resources invested in the research on infectious diseases, microbial infection still remains a public health problem in many parts of the world. The exact nature of the pathogenic agents responsible for many infectious diseases, and the nature of the receptors mediating the associated inflammatory events are incompletely understood. Recent advances in understanding the molecular basis for mammalian host immune responses to microbial invasion suggest that the first line of defense against microbes is the recognition of pathogen-associated molecular patterns (PAMPs) by a family of transmembrane pattern-recognizing and signal transducing receptor proteins called Toll-like receptors (TLRs). The TLR family plays an instructive role in innate immune responses against microbial pathogens, as well as the subsequent induction of adaptive immune responses. TLRs mediate recognition and inflammatory responses to a wide range of microbial products and are crucial for effective host defense by eradication of the invading pathogens. Now, recent updates demonstrated the ability of Disifin-derived products, Disifin-Animal and Disifin-Pressant to effectively suppress the progression and activities of Chikungunya fever and that of avian influenza A virus [*A/cardialis/Germany/72, H7N1: the agent of a highly pathogenic avian influenza (HPAI)*] infection, respectively. Overall, the above findings led me to suggest that Disifin and TLRs may mechanistically overlap in the processes of executing their functions against pathogenic microbial organisms. Thus, elucidating and better understanding of the molecular underpinnings responsible for the biochemical effects of Disifin-products, and the nature and mode of the interaction(s) of Disifin with TLRs in the process of exerting their biological effects may open a novel dimension in the research of infectious diseases, which may provide novel therapeutic targets for the prevention and treatment of a wide range of infectious diseases.

Keywords Disifin-(Sodium tosylchloramide) · Toll-like receptor (TLRs) · Pathogenic microbial organisms · Signal transducing pathway · Exploiting the roles of the Toll-like receptors

Introduction

Immune systems in vertebrates are divided into two basic categories: innate and adaptive arms of the immune system. Adaptive immunity, which is present in vertebrates, relies on antigen-specific receptors expressed on clonally expanded T and B lymphocytes that are generated by gene rearrangement and hyper-mutations. The innate immune

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system is evolutionarily conserved and is the first line of the defensive mechanisms for protecting the host from invading pathogens. [4]. It is now well established that both vertebrates and invertebrates' sense pathogens. This ultimately provokes the question: How does the host sense pathogens? Our present concepts emerged from the longstanding efforts to understand infectious disease. How pathogens harm the host, what molecules of these non-self objects (microbes) are sensed, and in commensurate to this, what is the type and nature of receptors that enable the host to sense the specific components of these pathogenic microbial organisms. Work in *Drosophila* opened the door to our understanding of aforementioned questioned and the roles of Toll in innate immunity. "Toll" is a German slang and it means, in this context, "fantastic". Toll mutant was discovered in the early 1980s by C. Nüsslein-Volhard and K. Anderson (two investigators working in Tuebingen, Germany) during a mutagenesis screen aimed at unravelling the genes involved in the establishment of dorso–ventral (DV) axis of *Drosiphila* embryo [7]. This work led to the discovery of 12 genes that are required for the determination of the DV axis of the embryo. Out of these 12 genes discovered, 11 of the genes were named: *pipe, windbeutel, nudel, gastrulation defective, snake, easther and spätzle* and the other four took the names: *tube, pelle, cactus and dorsal*. Hashimoto and colleagues cloned the Toll gene in 1988 and found it to encode a novel type of transmembrane receptor [39]. Its extracellular domain is composed of leucine-rich repeats flanked by characteristic cystein-rich motifs, while its intracytoplasmic domain did not resemble any structure known at that time. Subsequent investigative efforts in this direction revealed seven of the genes (*pipe, windbeutel, nudel, gastrulation defective, snake, easther and spätzle*) to code for factors required for activation of Toll [30], and the other four (*tube, pelle, cactus and dorsal)* to code for factors downscreen of the receptor. Further in-depth genetic studies involving loss-of-function mutations in these genes result in dorsalization of the embryo, with the exception of mutations in the *cactus* gene, which lead to ventralization of the embryos. Subsequent follow-up work, surprisingly and interestingly revealed, that both ventralizing and dorsalizing alleles of Toll were recovered in this screen (Hence, the name "Toll", which, as afore described, is a German slang term for "fantastic").

Toll in *Drosophila* is thus involved in establishing the DV axis in embryogenesis, and flies with conditional mutations in Toll (expressed only in adult, allowing normal fly development) exhibit reduced survival against fungal infection. In other words, Toll is a *Drosophila* gene that is essential for ontogenesis and antimicrobial resistance in the insect. [53]. The finding that *Drosophila* Toll had an immune function prompted the search for Toll-like receptors in mammals [52]. In 1997, the successful cloning of a human homologue of *Drosophila* Toll was reported by Janeway and Medzhitov, and this Toll-like receptors (TLR) was shown to activate NF-kappaB. [61]. This was the first evidence that an immune response pathway was conserved between fruit flies and humans. The crucial link of this TLR to mammalian immunity was dramatically highlighted when Poltorak, Beutler and colleagues discovered that the *lps* mutation in mice, which abolishes the response to bacterial LPS, corresponded to a loss-of-function mutation in this receptor [73, 75]. These results, and the concomitant finding that mammalian genomes encoded a family of TLRs [78], opened a novel field in the study of recognition of infectious non-self, and more generally placed the TLR family of protein at the heart of mammalian innate immune reactions. The TLRs represent a major class of pattern recognition receptors (PRRs). Recognition of pathogen-associated molecular patterns (PAMPs) by TLRs, either alone or in heterodimerization with other TLR or non-TLR receptors, induces signal responsible for the activation of innate immune response. To date, ten members of TLRs have been identified in human, and 13 in mice, and a huge body of genetic studies has revealed their respective ligands [89]. TLRs are important transmembrane signal transducing receptor proteins that confer a significant degree of specificity to the cells of the innate arm of the immune system. TLRs have been implicated to distinctly recognize every known category of pathogens that cause human disease. They have the ability to recognize every trace of microbial components and subsequently mount an early defense, largely dependent on the activation of NF-kappaB, which in turn, after its translocation into the nucleus, activates multiple pro-inflammatory genes, including tumor necrosis factor, IL-1 and IL-6 [90].

A good number of Disifin-derived products are presently in use as very efficient disinfectants in many settings. These include Disifin Animal Tabs, for animal farming [Test results: Faerber WU, et al. 2000. Institut für Krankenhaushygiene und Infektionskrankheiten, GbR. ISO 9001 Reg. Nr: EQ-Zeit 97224-01, Siemens Str. 18, 35394 Giessen, Germany, unpublished]; Disifin Pond Top Tabs: for Fish farming, Disifin Med Tabs and Med dent Tabs for the clinics [Test results: Trenner P. 1998: Germany: unpublished, Heeg P. 1999. Krankenhaushygieniker, Universitätsklinikum, Calwer Str. 714, 72076 Tübingen, Germany, unpublished] Disifin Health Care Tabs for Drinking water purification [Test results: Trenner P. 1998. Aqua Kommunal- Service GmbH & Co. KG, Postfach 1327, 15208 Frankfurt (Oder), Germany, unpublished]; Disifin Food Tabs for the Food industry [Test results: Stolle A. 1998: The activity of chemical disinfectant "DISIFIN", Munich (Muenchen), Germany: unpublished]; and Disifin Pressant Tabs for poultry farming, and specially designed, for tackling and prevention of the spread of highly pathogenic avian

influenza (HPAI) virus infection. A German company, RMP GmbH & Co. KG, is presently the major and/or sole producer of Disifin-derived products worldwide. These products have the ability to effectively suppress the activities of almost all pathogenic microorganisms ranging from bacteria, viruses, fungi and yeast and, very probably, protozoan parasites. In line with the character of Disifin (Chloramine T)-derived products that belong to the family of oxidant disinfectants, exhaustive exploitation of the functions of NADPH oxidase is of crucial importance for these products in the process of exerting their function(s) of inactivating and killing of microbial pathogens [72, 85]. Disifin-derived products as produced by the German Company, RMP GmbH & Co. KG, are commercially available mainly in tablets. Disifin tablets are stable for, at least, 3 years at a storage temperature of 25°C. The active disinfectant compound of Disifin is sodium-N-chloro-paratoluenesulphonamide (Chloramine T; or Sodium tosylchloramide). Chloramine T, MG.227.67, has demonstrated wide antimicrobial activity, and it is thought to be the first successful organic chlorine derivative with documented bactericidal effects on the skin [34]. Chloramine T is stable in water–alcohol solution, and a mixture of methanol and chloramine T has been demonstrated to be highly sporicidal, thereby suggesting an important role for such mixture in disinfecting heat-sensitive instruments [21]. Application of chloramine T to contaminated wounds has been reported to be strongly bactericidal [25, 42]. Chloramine T, as a good oxidizing agent, is also used for other purposes including iodination of proteins [11], and in the chemical industry for bleaching of material, etc. [64]. Despite the broad application of Disifin products, the mechanisms by which Disifin-derived products suppress the activities of pathogens are incompletely defined. However, Chloramine T is a well-known efficient oxidant, and a wealth of scientific evidence has demonstrated the ability of ChloramineT to suppress the activities of bacteria, for instance, by inducing the generation of oxygen and chlorine-free radicals [33], and thereby initiating and mediating oxidative damage to the microbial pathogen [50, 72, 85]. Support for the efficiency of chloramine T (or Sodium tosylchloramide) to suppress the activities of microbial pathogen had been earlier robustly evidenced by Semmelweis [83]. This indicates that Sodium tosylchloramide destroys the activities of pathogen, among other things, through the process of oxidative stress cascade. Reactive oxygen species (ROS) are generated ubiquitously in aerobic organisms. However, when these cytotoxic agents overwhelm the endogenous antioxidant defense system, oxidative stress and oxidative damage occur as reflected by the oxidative modification of macromolecules such as lipid, protein and DNA [106]. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a multicomponent enzyme, is a major oxidant-generating enzyme, and when fully activated is known to play a crucial role in this cascade. Now, for NADPH oxidase to be activated, intracellular signaling should be of critical importance in facilitating the shift from resting state to an activated state upon encounter with the appropriate stimuli. Since disinfectants (the active substance of the Disifin-derived product) should directly or indirectly interact with pathogenic agents in the process of disactivating and/or killing them, coupled with the fact that oxidant disinfectants have been shown to exploit the functions of NADPH oxidase in the process of exerting their functions, it is thus reasonable to anticipate that innate immune system should be implicated with the processes of microbicidal activities of a variety of disinfectants.

However, surprisingly, very little has been disclosed about the molecular mechanisms underlying the microbicidal actions of disinfection agents. The nature and form of the interactions between disinfectants, microbial pathogen complex on the one side, and the immune system, particularly the innate immune system, on the other side, are incompletely defined. The type of receptors and the nature of the signaling pathways, active in these processes, are equally poorly understood. Microbicidal effects are, however, thought to be mediated primarily by the generation of ROS [14], by NADPH oxidase system. Activation of NADPH oxidase, thus, represents an essential mechanism of defense against pathogens [26, 84], and a huge body of scientific evidence recently accumulating indicates that TLRs, and especially, TLR4 plays a crucially important role in mediating and/or regulating NADPH oxidase-dependent superoxide production following microbial infections [14, 27, 49, 87]. This points to a common line of function for TLRs and Disifin-derived products with regard to host defense against pathogens, and also to a possible role for TLRs in mediating largely and/or partially the biological effects of Disifin-derived products.

Together, the above notions are strongly suggestive of the existence of common and/or closely related signal transducing pathways, and close interactions between DISIFIN-derived products and the TLRs in the process of exerting their actions against microbial pathogens. Thus, untangling the signaling networks underlying the mechanisms of innate immune system-associated disinfectant–microbe interactions will be a challenge for the future and might provide key insights into the genesis of microbicidal processes, and thereby the circumstances linking disinfectants such as Disifin-derived products to microbial pathogens and the innate immunity. It is thus the subject of this review article to explore the molecular signaling of TLRs, and alongside discuss the possibilities, and ultimate importance of Disifin-derived products, working in concert with TLR proteins in host defense against microbial pathogens.

Toll-like receptors

The innate immune system

The innate immune response is the first line of defense against an invading pathogen.

The innate immunity is activated by a few highly conserved structures present in most pathogenic microorganisms. These structures are defined as PAMPs sensed by the PRRs. Among the well-defined PAMPs are bacterial lipopolysaccharide (LPS), peptidoglycan, mannans, bacterial DNA and double-stranded RNA. PAMPs are believed to be produced almost solely by microbial pathogens and are essential for the survival or pathogenicity of microorganisms [2, 47, 71]. PRRs are mainly expressed on effector cells, such as dendritic cells (DCs), macrophages and B cells. All PRRs are displayed by a given cell type and have identical specificities. When a PAMP is recognized, all cells are immediately triggered to perform their effector functions, leading to a rapid innate immune response [22, 90].

Toll-like receptors

Research over the past few years has greatly advanced our understanding of the mechanisms by which the immune system functions, and especially, the innate immune system [46, 60]. These advances suggest that the first line of defense against microbes is the recognition of PAMPs by the TLRs. The PAMPs such as lipoteichoic acids (LTA), lipopolysaccharide (LPS), peptidoglycan (PGN), other components of microbial cell walls [59, 89, 92], which enables the innate immune system to recognize invading micro-organisms and to induce a protective immune response. The mammalian TLRs are a family of highly conserved, germline-encoded transmembrane receptors that are critically involved in mammalian host defense. To date, 13 TLRs have been identified; 10 in humans and 12 in mice [16, 89]. Structurally, TLRs are characterized by the presence of a leucine-rich repeats domain in their extracellular regions and a Toll/IL-1R (TIR) domain in the intracellular regions. In respect of the amino acid sequence and genomic structure, TLRs can be divided into five subfamilies: TLR2, TLR3, TLR4, TLR5 and TLR9. The TLR2 subfamily is composed of TLR1, TLR2, TLR6 and TLR10, and TLR9 subfamily is composed of TLR7, TLR8 and TLR9. TLR1 and TLR6 form heterodimers with TLR2 [89]. Recent updates disclosed the identification of a human TLR11. This was originally isolated from murine. Murine TLR 11 appears to be closely related to TLR5, and it is expressed abundantly in kidney and bladder. TLR11-deficient mice are reported to be highly susceptible to infection of the kidney by uropathogenic bacteria [107], suggesting that TLR11 plays an important role in urinary tract infection. The function of the human TLR11 is not known because of the presence of a stop codon in the gene [107]. Important updates suggested that a microbial profilin-like molecule isolated from the protozoan parasite *Toxoplasma gondii* (*T. gondii*) functions as a ligand for TLR11 [104]. This profilin-like molecule (a protein) was shown to trigger IL-12 through TLR11.

Recent studies disclosed that TLRs do not only mediate recognition and inflammatory responses to a wide range of microbial products but also to non-microbial endogenous proteinaceous molecules, heparan sulfate and RNA, DNA and small molecular synthetic products [13, 48, 68, 94]. These, thus, present TLRs as appealing target for pharmacological intervention in pathological conditions in which inflammatory consequences and the threat of infection pose great problems.

TLR signaling pathways

TLRs activate distinct signaling cascades via four different TIR domain-containing adapter proteins. The four adapter proteins are MyD88 (myeloid differentiation factor 88), MAL/TIRAP (MyD88-adapter-like/TIR-associated protein), TRIF or TICAM-1/Toll-receptor-associated activator of interferon) and TRAM (Toll-receptor-associated molecule). The aforementioned four adapter proteins transduce signals from all of the TIR domains, activating protein kinases and then the transcription factors that lead to inflammatory effects. Recent discoveries disclosed the identification of a possibly fifth TIR adapter protein, designated, Sarm [17]. However, the function of Sarm is presently unknown.

Despite divergent PAMP ligands, all TLRs with the exception of TLR3 activate MyD88-dependent pathways to induce a core of stereotyped responses such as inflammatory events. The pathways that transduce TLR signals in mammals appear to have both similar and dissimilar characteristics from those in *Drosophila* [62]. In *Drosophila* the Toll-IMD-pathways are crucial for antifungal and anti-Gram negative bacterial responses, respectively. In mammals the host defense against microorganisms mainly relies on pathways that originate from the common TIR domain of TLRs. The TLR family signaling pathway is highly homologous to that of IL-1R family. Both TLR and IL-1R interact with an adapter protein MyD88, which has a TIR domain in its C-terminal segment but a death domain (DD) in its N-terminal segment instead of the transmembrane domain found in TLRs. MyD88 associates with both the TLRs and IL-1R via interaction between the respective TIR domains. Upon stimulation by a ligand, MyD88 recruits a death domain-containing serine kinase, the IL-1R-associated kinase [IRAK (IRAK 1 and IRAK4)]. IRAK is

activated by phosphorylation through its N-terminal death domain and then activates TNFR associated factor 6 (TRAF6) to stimulate IKappaB Kinase (IKK) complex and MAP kinase. Phosphorylation of IkappaB by IKK complex induces the degradation of Ikappa B through the ubiquitin-proteasome pathway, and subsequent nuclear translocation of liberated NF-kappaB mediates transcription of pro-inflammatory cytokine gene [3, 32].

The TLR3 ligand, double-stranded RNA has been reported to induce the activation of NF-kappaB in MyD88 knockout (KO) mice, thereby buttressing the notion that TLR3 signaling is independent of MyD88 [6] Furthermore, signaling can occur independently of MyD88 for TLR4, which also activates NF-kappaB through the adapter protein TIRAP and for TLR3, as indicated above, which induces an antiviral interferon (IFN)-β response through TICAM-1 [10, 89]. Recently, a germline-induced mutation in TRIF led to identification of another adapter molecule with a TIR domain; TRIF-related adopter molecule (TRAM), shown to be required by TLR4, but not TLR3-mediated IFN response. In Tram-deficient mice, LPS induced persistent NF-kappaB activation, whereas the expression of IFN-inducible genes was defective, thereby strongly pointing to a crucially pivotal role for TRAM in the TLR 4-mediated MyD88-independent signaling pathway [28, 103]. These compellingly indicate that studies designed to determine the activities of TLRs in any specific pathological condition, whereby strictly MyD88-deficient model was tested (solely putting MyD88-dependent pathway into consideration), could almost impossibly lead to findings, which could be enough to categorically rule out the involvement of TLRs in the development and resolution of the pathology in the concerned model. Thus, individual TLR signaling pathways are divergent, although MyD88 is common to almost all TLRs. Support for this notion is evidenced by a plethora of data [23, 69, 80, 88]. Judging from the enormous interest in research on these transmembrane receptor proteins, the TLRs, it is very likely that more TLR ligands and signaling pathways will be identified in the future. It is also of note that signaling pathways mediated by TLRs have also been revealed to be cell type-specific [81, 99]. This indicates that, under certain conditions, some cells and tissues might be prone to favor this pathway to the other. The molecular mechanisms underlying this still have to be fully resolved.

Disifin (Chloramine T)-derived products and Toll-like receptors: evolving importance in human health

A hallmark and one of the fascinating episodes during the development of hygiene was the beating of the puerperal fever by Semmelweis [83], who attained this goal by introducing chloride of lime (bleach) as a rapid disinfecting agent. After this, chlorine and other active chlorine compounds, bearing O–CI or N–CI functions emerged as indispensable substances in the practice of disinfection [24]. In line with the findings of Semmelweis [83] and others [24], and extending on these findings, a German company, RMP GmbH & Co. KG, concentrated on, and specialized in the developing of Disifin-derived products as effective disinfectants for a wide range of settings. Chloramine T-derived disinfectants have not only been associated with wide antimicrobial activity [25] but also been shown to control the spread of viral infection very efficiently [18]. Support for this notion could be evidenced by important updates reporting the capability of Disifin-Animal Tabs and Disifin-Pressant Tabs, both at relatively low concentrations, to effectively suppress the lethal activities of both Chikungunya virus and that of avian influenza A virus (*A/cardialis/Germany/72, H7N1*) infection: the agent of a highly pathogenic avian influenza (HPAI), respectively [Kaleta EF., Yilmaz A. Test Report: prEN 14675 Virucidal Activity of Disifin Animal against Chikugunya virus and Virucidal activity of Disifin Pressant against Influenza A/Carduelis/Germany/72 (H7N):agent of highly pathogenic avian influenza (HPAI): virucidal conditions for disinfection of influenza viruses determined according to the prEN 14675 standard (obligatory conditions), University of Giessen, Giessen, Germany, March 2006. unpublished]. Chikungunya virus (CHIK V) is a member of the Alphavirus genus of the family of Togaviridae, is a single-stranded positive RNA enveloped virus, firstly isolated from a human serum in 1953 in Tanzania [77]. CHIKV is widely spread throughout Africa, South East Asia, Western Pacific and India [51]. CHIK V is transmitted from primates to humans generally by *Aedes aegpti* and also by various aidine mosquitoes species [102] Recent resurgence of chikungunya disease is believed to represent a worldwide public health problem. [36].

Avian influenza (A1) virus engages animals and humans

It is now well established that avian influenza (A1) virus poses significant threats to both animal and human health. Since 2000 a huge number of birds have died or have been culled worldwide as a result of avian influenza (A1) virus infection. Highly pathogenic avian influenza (HPAI) is a disease of poultry caused by H5 or H7IA A strains, with mortality that ranges up to 100%. A disturbing number of outbreaks has been documented in the past few years: Hong Kong (1997), Italy(1999), Chile (2002), the Netherlands's (2003), Canada (2004), outbreaks in Southeast Asia (2003–2005) [65, 97], and in Nigeria in February 2006. H5 and H7 subtypes have been reported to cross species barrier and caused fatal disease in humans in several Asian countries and in the Netherlands. This represents a particularly

serious threat in terms of loss of human lives and the opportunity to generation of new human pandemic virus [19, 43]. The source of this virus remains enigmatic. However, it is now widely believed that circulating virus in domestic poultry represents the main source of infectious virus for humans. This, thus, underscores the urgency and importance to pro-actively address this problem. To reduce the primary risk of human HPAI infection, it is crucial to prevent infection of poultry. Among the common methods used to control the outbreaks of HPAI viruses, such as killing and destruction of the infected poultry, preemptive culling, bio-security measures and vaccinations, the most desirable measures should have the potential to prevent viral transmission from animal to animal, and/or viral spread between animals in a flock and, subsequently, the spread of virus between flocks to such an extent that a major outbreak will not occur. Importantly and unfortunately, very little is presently known about the ability of AI vaccines, working alone, to reduce substantially both the transmission of HPAI viruses in chickens and the spread of the virus within a flock, suggesting that consequent application of disinfectant is capable to suppress significantly the progression, and the spread of HPAI viruses should be highly promising for the management of HPAI infection.

Toll-like receptors and viruses

Viruses contain genetic material, which consists of either DNA or RNA (but not both) that encodes viral structural components and synthetic and replication enzymes. Various structural components, including double-stranded RNA (dsRNA) viral DNA, single -stranded RNA (ss-RNA) and surface glycoproteins, are recognized as PAMPs by TLRs and other PRRs. Among the TLRs: TLR3, TLR7, TLR8 and TLR9 are the major PRRs that are recognized by distinct types of virally determined PAMPS. The recognition of viral components by PRRS commonly induces type I interferons (IFN-alpha/beta) production that can activate target cells in different ways, in both autocrine and paracrine manners. Now, CHIK disease and HPAI microbial pathogens are notably viruses, and as viruses the recognition of their viral nucleic acids released from infected apoptotic cells should or may occur in the intracellular compartments, such as phagolysosomes, for the TLR proteins, which are in a position to recognize viral components (TLR3, TLR7/8 and TLR 9) are exclusively localized to endosomal compartments, but not on cell surfaces [3, 55, 89] This implies that, before the activation of the immune system, these viruses must have been sensed and localized by specific members of TLR family (such as TLR3, TLR7/8 and TLR 9), which have the capacity to sense and locate specific pathogen-associated patterns of viruses in the intracellular compartments, such as phagolysosomes. Disifin-derived products: Disifin-Animal Tabs, and Disifin-Pressant Tabs, as aforementioned [Kaleta EF., Yilmaz A. Test Report: prEN 14675 Virucidal Activity of Disifin Animal against Chikugunya virus and Virucidal activity of Disifin Pressant against Influenza A/Carduelis/Germany/72 (H7NI): agent of highly pathogenic avian influenza (HPAI): virucidal conditions for disinfection of influenza viruses determined according to the prEN 14675 standard (obligatory conditions), University of Giessen, Giessen, Germany, March 2006. unpublished], have been recently demonstrated to have the ability to suppress significantly the activities or actions of CHIK and HPAI viruses in well planned in vitro set of experiments. This, thus, strongly suggests that Disifin-derived products may interact very closely with TLRs in the process of exerting their functions, and/or strongly exploit the roles of TLR proteins in the process of killing microbial pathogens.

Innate immune system: evolving in disinfectant–micobial pathogen interactions

Innate immune system provides the first line of defense against invading pathogens. Innate immunity is mediated by cells such as macrophages, neutrophils and DCs. Macrophages and neutrophils seem to play a pivotal role in mediating the activities in the innate immune system. These phagocytic cells have the capability to detect infectious organisms through a plethora of receptors, engulf and kill the pathogens, and orchestrate an appropriate host response by secretion of cytokines and chemokines and activate the adaptive immune system to provide protective immunity [1]. Now, compelling scientific evidence, derived from recent discoveries, strongly suggests that the mechanisms by which the innate immune system recognizes “nonself” are largely based on the actions of a family of type I transmembrane (TM) proteins designated TLRs, which play a front-line role in the defense of the host against infection by microbial pathogens. TLRs constitute a family of transmembrane protein that differentially recognize pathogen-associated molecular patterns through an extracellular domain and initiate inflammatory signaling pathway through an intracellular domain [3, 15, 47]. The TLRs are thus thought to have diverse functions, including ligand recognition, signal transduction and subcellular distribution [66].

NADPH oxidase and host defense against pathogens

As aforementioned, microbicidal effects are mediated primarily by the NADPH oxidase-dependent generation of ROS [14] and reactive nitrogen species.

NADPH oxidase, a specialized multienzyme system, consists of five essential protein complex, two in the

membrane and three in the cytosol. It plays a pivotally crucial role in this process of host defense function against pathogens [9, 58]. NADPH oxidase is an enzyme that is located in neutrophils, eosinophils, and mononuclear phagocytes and catalyzes the generation of superoxide from oxygen and NADPH. Concurrent with the activation of the NADPH oxidase, intracellular granules fuse with phagosomal membrane or plasma membrane to release an array of biologically active molecules that are critical for host defense. These include proteases, antimicrobial proteins and peroxidases. The H_20_2 generated by the oxidase serves as a cosubstrate for peroxidases, either myeloperoxidase (MPO) in neutrophils and monocytes or eosinophil peroxidase in eosinophils, in the oxidation of halides to generate hypohalous acids. In the case of neutrophils, the HOCL produced represents a potent antimicrobial agent that in turn spawns long-lived chloramines that are themselves cytotoxic [5, 38]. Activation of NADPH oxidase thus represents an essential mechanism of host defense against pathogens, and it is widely believed that a variety of disinfectants exploit these mechanisms of action by their actions against microbial pathogens with the aim of killing them [33, 72, 85] To this end, recent studies have demonstrated that the activation of NADPH following exposure to microbial component largely implicates the actions of TLRs (functions of TLRs and TLR signaling). These include direct interaction of TLR 4 with NAHPH oxidase [70], activation of NFkappaB via TLRs [14, 96], and TLR 4-mediated activation of the DCs [98]. In addition to this, in contrast to the previous concept, important updates suggested that the microbicidal effects observed after the activation of NADPH oxidase could be accomplished through many ways, including the robust generation of free radicals [9, 38, 58] modulation of redox-sensitive signal transduction pathway in immune cells [82] and through NADPH-dependent TLR2 interaction with TRAF4 leading to blockage of TLR4 signaling, which then results in protection of host cells from adverse over-responses to pathogens [91]. Together, the above findings buttress the notion arguing in favor of the enormous importance, and broad implications of TLRs in host defense against pathogens

Molecular underpinnings responsible for microbicidal effects of Disifin (ChloramineT)-derived products and other related disinfectants

The chlorination (the formation of "chlorine cover") of microbes prior to their destruction by active chlorine compounds has been shown in a variety of disinfection processes [35, 50]. To this end, it is important to note that all organic chlorine derivatives, including Chloramine T, and N-chlorotaurine, develop "chlorine covers" [35, 50]. However, the consequence of this chlorination event is incompletely understood. Now, recent studies by Gottardi and Nagl [35] suggested that this may represent the first sign of interaction between disinfection agent and microorganisms, and concomitantly, represent the first step of action of the disinfectant against the microbial pathogen. These findings, interestingly, strongly suggest an important role for "Chlorine cover" in the induction of innate immune response. The enormous importance of oxidants as disinfectants in medicine is today well established [101]. These include Disifin (Chloramine T)-derived products and related disinfectants. In spite of the importance and wide use of these disinfectants, and in particular, Chloramine T-derived products, which have been in use for decades, relatively little is known about the molecular mechanisms underlying the actions of these products in inhibiting the actions of pathogens and killing the pathogenic agents. However, it is largely believed that the molecular mechanisms by which Disifin-derived products and related disinfectants kill pathogens might be largely consistent with the mechanisms underlying the bactericidal effects of NADPH oxidase system-dependent methods [14, 70, 82, 96, 98]. The respiratory burst generated by the NADPH oxidase [9, 58] is critical for defending the host against invading pathogens. As aforedescribed, host defense functions are attributed largely to direct microbicidal action of toxic intermediates such as H_2O_2 and OH ions. The interaction between the disinfectant (Disifin-derived product, for instance) and the microbial pathogen leading to the formation of the complex designated "chlorine cover" has been reported to represent the first step of disinfectant action against the microbial pathogen [35], and this can probably and/or plausibly serve as an adequate stimulus for the activation of NADPH oxidase via the TLR proteins. If this happens, it might equally lead to the aforementioned reactions implicated in the host defense against pathogens following activation of NADPH oxidase [14, 70, 82, 96, 98]. Second, the nuclear factor kappa B (NF-kappaB) is an important intracellular signaling pathway for both innate and acquired immunity. The NF-kappaB family of transcription factors have been reported to exert host defense against infectious agents by inducing the expression of inflammatory genes, whose target genes are transcriptionally regulated by NF-kappaB and they play an important role in host defense. These include pro-inflammatory cytokines, chemokines, enzymes such as inducible NO synthase and beta-defensins [12, 14, 20, 31, 96]. ROS, a major product of activated NADPH oxidase has been shown to be implicated in signaling by activation of transcription factors, such as NF-kappaB, as evidenced by a plethora of in vitro studies [29, 44, 54]. It is important to note at this point that activation of NF-kappaB following exposure to microbial agents in macrophages and other cell types is largely mediated via TLR signaling actions [14, 70, 96]. Now, earlier discoveries

disclosed that direct treatment of some cells with oxidants activated NF-kappaB [45, 86]. This concept further buttresses the finding describing oxidants as important tools to attack and kill pathogens [85, 95]. This, therefore, widely implies that direct contact of Disifin-derived products (a well-known oxidant disinfectant) with some cells could lead to activation of NF-kappaB, among other things. Therefore, it could also be anticipated that Disifin-derived products may equally carry out their host defense function against pathogens through modulation of redox-sensitive signal transducing pathways.

Receptors, signaling pathways and proteins in a position to transduce the necessary signals are crucial for these processes. Since the immune system, and especially important cells of the innate immune system are intimately implicated in these processes, it is thus reasonable to hypothesize that TLR proteins may have an important role to play in the molecular underpinnings responsible for the biological and biochemical effects of Disifin-derived products in exerting their actions against microbial pathogens. Additionally, in this regard, earlier studies carried out by Nagl et al. [63] described chloramines as powerful tools for granulocytes to impact host defense against pathogen, by inducing immune modulatory effects. Extending these findings to the nucleus of this manuscript, and coupled with what we today understand of the functions of TLRs, and TLR signaling, the aforementioned discoveries of Gottardi and associates [63] strongly points to a crucial role for TLRs in driving the host defense function against pathogens executed by Disifin-derived products, and closely related disinfectants. In addition to this, the findings of Marcinkiewicz [56], associating the activities of chloramines with the both arms of the immune systems, further buttress the notion of immunomodulatory ability of chloramines. Therefore, the aforementioned observations strongly, and consistently, strengthen the concept that Disifin-derived products may share biological effects and mechanisms of actions with the TLR proteins. Thus, improved understanding of the interplay between the innate immune response and the Disifin-derived products may be fundamental to probing into the molecular basis underlying the microbicidal actions of oxidant disinfectants, generally, and, perhaps, other related disinfectants, as well. Furthermore, it may be reasonable to anticipate that Disifin-derived products may contribute to their microbicidal functions through many other ways other than direct damage on microbes by free radicals generated following the activation of NADPH oxidase, but, in addition to this, also through modulation of redox-sensitive signal transducing pathways [37, 82], and through blocking the TLR signaling to evade adverse cellular activation brought about by pathogens [91]. These findings thus indicate that the simple scheme, which for many years has served largely as a satisfactory working theory with regards to mechanisms of microbicidal effects by various disinfectants, including Disifin-derived products (oxidant disinfectants) is inadequate. The immense importance of the TLR proteins in the immune system and especially in the innate arms of the immune systems, coupled with the broad implications of TLRs in host defense against pathogens and in the inflammation events, and the notions that Disifin-derived products and, perhaps, other closely related disinfectants, and the TLR proteins overlap both functionally and in their mechanisms of actions, present Disifin (Chloramine T)-derived products as exceptionally efficient disinfectants. Together, this broadly implies that Disifin-derived products and, perhaps, other closely related disinfectants might be exerting their microbicidal functions largely through exploitation of mechanisms of innate immunity in pathogen–host interaction. Despite the fact that the notions in this review are largely based on the light of known and published information; this review is primarily meant to provide exciting and intellectual stimulations to scientists and clinicians for future studies on this subject. Increased studies and better understanding of the molecular underpinnings responsible for the innate immune responses in the processes of disinfectant, (Disifin-derived products) interactions with pathogens with the aim of killing the pathogens could lead to facilitate our understanding of the mechanisms underlying the capability of disinfectants to mount an attack that either protects against lethal effects of pathogens or, possibly, contribute to cellular damage. Such advances could lead to changes in outcome of combating pathogenic microorganisms with a wide variety of disinfectants.

Conclusions and perspectives

Recent studies have disclosed the mechanisms of innate immune recognition via the TLR family of proteins. TLRs recognize general molecular patterns often associated with invading pathogens or foreign genetic material, and trigger a specific network of signaling pathways that lead to production of cytokines and interferons [41]. Binding of these patterns to TLR leads to a cascade of signaling pathways that is evolutionarily conserved from plants to insects to mammals and humans and ultimately activates NF-kappaB and interferon production. Dendritic cells show up-regulation of MHC antigens and costimulatory molecules such as CD80 and CD 86. The latter are crucial for proper antigen presentation by antigen presenting cells (APC). Thus, thereby, the TLR creates links between innate and adaptive immunity [57]. The complement system is notably an integral component of the innate immune system, and it is widely thought to play an important role in host defense. The complement, a double-edged sword, which largely

contributes to recognition and destruction of pathogens and other invaders and to assist in the phagocytosis of waste materials. The major components of the complement system execute four major functions: (1) recognition, (2) opsonization, (3) inflammatory stimulation through anaphylotoxins, and (4) mediate killing through membrane attack complex (MAC) [74, 100]. To date, very little attention has been invested in the role the complement plays in the orchestrated sequence of events involved in the microbicidal actions of disinfectants; activation of NADPH oxidase and exploitation of the functions of NADPH oxidase in the host defense against pathogens. To this end, it has been demonstrated, McGeer and associates [93], that free radicals generated by a free radical-generating system unregulated significantly the transcription of important complement elements subsequently leading to membrane attack complex- associated injury. This finding widely implies that TLR-dependent activation of NADPH oxidase following microbial stimulus could plausibly lead to activation and/or overactivation of the complement system. This strongly suggests that the TLRs, may be, under certain conditions, working in concert with the complement system in driving both the pathophysiology and pathogenesis of a wide range of diseases, and they may also be working in concert with disinfectants in the disinfection processes with the aim of killing the pathogens. The notion that TLRs and complement system interact greatly in executing their actions has been recently evidenced in two presentations [40, 67]. Based on the strength of these notions, and coupled with the thoroughness and elegancy of certain sets of experiments arguing in favor for these notions, it is thus reasonable to anticipate that Disifin-derived products may equally be exploiting the roles of the complement system in executing their functions. Studies to elucidate the cellular and molecular mechanisms underlying the nature, mode and the consequences of the interactions between the complement system and the disinfectant–microbe complex, and increased studies on the cross-talk between the TLRs and the complement system may be fruitful for the development of new generation of more effective agents for both prevention of infection and treatment of diseases. Recent discoveries have identified some TLR-independent sensors important in driving innate immunity by their ability to recognize some microbial components. These include nucleotide-binding oligomerisation domain (NOD) receptor proteins [8] and retinoic acid-inducible gene I (RIG-I) [79, 105], thereby suggesting that host cells posses multiple mechanisms against microbial infection. NODs are a family of intracellular PRRs that include apoptotic protease activating factor 1 and mammalian NOD-LRR proteins [76]. Nevertheless, a wealth of overwhelming scientific evidence has demonstrated a crucial involvement of TLRs in the recognition of all the major classes of microbial pathogens, and thereby indicating an exceptionally crucial role of TLRs in driving the immune responses and in host defense against pathogenic microorganisms. Now, in addition to earlier notions: (Official Test Results; Stolle A, 1998: Disifin Food, Faerber WU. et al, 2000, Disifin Animal, Trenner P., 1998: Disifin Med., Heeg P., 1999: Disifin Med.: unpublished], recent work demonstrating the ability of Disifin-Animal Tabs and Disifin-Pressant Tabs to effectively suppress the activities of CHIKV and H7N1 virus at a relatively low concentration strongly buttresses the concept that Disifin-derived products represent effective disinfecting agents. Based on the scope of what we understand today about the nature, functions and biochemical actions of TLRs, I am tempted to suggest that Disifin-derived products (in virtue of their active component: sodium tosylchloramide) and TLR proteins share largely common functions and mechanisms of actions. In addition, the mere fact that we could to date plausibly and, to a considerable extent, correctly, associate the actions of TLRs with the notions treated in the following works [34, 35, 37, 50, 63, 72, 85] strongly indicates significant advances made in the past 6 years in understanding the components of the innate immune system, including the pathogen sensing mechanisms, receptor and intracellular signaling pathways, linkage to adaptive immune system, and the effectors of the innate immune response. Despite enormous advances in this area of research in the past decade, infectious disease caused by microbial pathogens remains one of the major causes of death. This notion is evidenced by the presence of new diseases such as acquired immune deficiency syndrome, avian influenza, severe acute respiratory syndrome and the resurgence and expansion of diseases including chikugunya fever, West Nile and Ebola hemorrhagic fevers. This fact underscores the importance and urgency for increased studies in this area of research. Therefore, elucidation of the nature, the mode and the consequence of the interactions between the active component of Disifin-derived products and the TLRs and their signaling pathways will increase our understanding of the mechanisms responsible for triggering the activation of innate immunity, the connection between innate immunity and adaptive immunity, and may help to identify potential targets for future therapeutic interventions to regulate host defense. Information gained from these studies may directly contribute to the development of effective agents, presently not available, for both prevention and management of wide range of infections and infectious diseases.

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