Old War, New Battle, New Fighters!

Sandra Sousa, Francisco Sarmento Mesquita, and Didier Cabanes
Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal

(See the major article by Wang et al on pages 1376–87.)

**Keywords.** fisetin; *Listeria monocytogenes*; listeriolysin O; natural flavonoid; antivirulence strategies.

Since the discovery of penicillin, antibiotics have been of critical importance in the control of infectious diseases. However, the extensive use and misuse of antibiotics during recent decades led to the widespread development and distribution of resistance to multiple drugs among bacteria. With the alarming levels of antibiotic resistance and the difficulties associated with discovering novel antibiotics, researchers are now investigating alternatives to treat infectious diseases. In contrast to traditional strategies, which aim to kill bacteria or prevent their growth, these new approaches intend to block the ability of bacteria to harm the host by directly inhibiting bacterial virulence factors and are thought to use less selective pressures that limit the development of bacterial resistance. These emerging strategies benefit from the detailed knowledge of the functional and molecular mechanisms underlying key pathogenic determinants acquired during the last decades of research in host-pathogen interactions [1]. Bacterial toxins in particular are primary targets for these novel antivirulence strategies. In this issue of *The Journal of Infectious Diseases*, Wang et al show that fisetin, a natural flavonoid with negligible antimicrobial activity, has effective antivirulence activity against *Listeria monocytogenes* by directly interfering with a secreted bacterial toxin.

*Listeria monocytogenes* is a facultative intracellular human foodborne pathogen that causes gastroenteritis, meningitis, encephalitis, and maternofetal infections. Listeriosis is the most frequent cause of death from consumption of contaminated food in Europe and has the third highest cost of illness and loss of quality of life among foodborne infections [2, 3]. *Listeria monocytogenes* enters the host via the ingestion of contaminated foods, invades the intestine, translocates to mesenteric lymph nodes, and spreads to the liver, spleen, brain, and placenta. During infection, *L. monocytogenes* has the ability to cross the intestinal, blood-brain, and placental barriers, entering, surviving, and multiplying inside phagocytic and nonphagocytic cells [4]. To establish and sustain infection, *L. monocytogenes* uses an arsenal of virulence factors to hijack host-signaling pathways [5, 6]. While remaining a real public health concern, *L. monocytogenes* has emerged as an exceptional model to address the different facets of host-pathogen interactions and the design of new therapeutic strategies.

Listeriolysin O (LLO) is a crucial virulence factor produced by *L. monocytogenes* [7]. It is a pore-forming toxin member of the cholesterol-dependent cytolysin family [8]. LLO monomers are secreted by the bacteria and oligomerize into a ring at the surface of target cell [9]. Membrane insertion of LLO results in ion fluxes across damaged membranes and ultimately leads to cell lysis in conditions of extensive damage and/or inefficient membrane repair mechanisms [10]. Inactivation of LLO results in the inability of *L. monocytogenes* to escape from the internalization vacuole, thereby decreasing the virulence potential of *L. monocytogenes* [11, 12]. Besides membrane lysis, it has become apparent that LLO acting from the intracellular or extracellular milieu exerts additional effects on the host cell [7]. Intracellular LLO affects host cell signaling [13, 14], induces autophagy [15], and suppresses reactive oxygen species [16]. LLO was also shown to deregulate host SUMOylation [17], to induce endoplasmic reticulum stress [18] and mitochondrial fragmentation [19], and to promote regulatory epigenetic changes [20].

A previous study showed that subinhibitory concentrations of plant essential oils could inhibit LLO activity and decrease *L. monocytogenes* virulence, but the specific compound responsible for this effect remained unknown [21]. Wang et al now report the discovery of the natural flavonoid fisetin as an effective antivirulence agent against LLO activity. They showed that fisetin inhibits...
the hemolysis capacity of LLO and is able to protect mice from lethal infection by *L. monocytogenes*. Molecular modeling studies demonstrated that fisetin directly engages LLO, causing a conformational shift of the LLO domains critical for its binding to cholesterol and oligomerization. Coupling mutational and biochemical approaches, they identified LLO amino acid residues involved in sensitivity to fisetin. This work establishes fisetin as a novel antivirulence compound that targets LLO by a unique mechanism, counteracting toxin binding to host cells and oligomerization. Interestingly, fisetin is able to decrease *L. monocytogenes* virulence in tissue-cultured cells and animal infection models, without affecting the *L. monocytogenes* growth or the phagocytic capacity of macrophages. Notably, while new roles have been frequently assigned to LLO, this study supports the concept that, during infection, pore formation remains the toxin’s principal function.

Antivirulence strategies targeting bacterial toxins to prevent their deleterious effects were previously developed. Such approaches were based on the use of soluble analogs or specific antibodies against toxins, thereby preventing their interaction with their receptors at the host cell membrane. Alternatively, they aim at blocking toxin pores, using synthetic compounds [1]. In the case of LLO, neutralizing monoclonal antibodies were previously described to control *L. monocytogenes* intracellular growth and virulence [22]. One important caveat, however, is that these strategies are often associated with high costs of production and maintenance. In contrast, fisetin is present in many fruits and vegetables associated with low costs of production and was shown to have broad biological properties, ranging from antioxidative to cancer therapeutic effects. The bioavailability and toxicity of fisetin are also well established [23]. In addition, an important advantage associated with dietary plant flavonoids is that they are perceived as nontoxic and have wide human acceptance [24]. This study thus paves the way for the development of broad antivirulence approaches based on natural products. Importantly, as cholesterol-dependent cytolysins generally have structural similarities, it would be interesting to test the effect of fisetin on cholesterol-dependent cytolysins produced by other bacterial pathogens, such as *Streptococcus pyogenes, Streptococcus pneumoniae, Arcanobacterium pyogenes,* and *Clostridium perfringens* [25]. Indeed, an optimal therapeutic agent would target virulence factors present in several pathogens. Results presented by Wang et al regarding the recent determination of the LLO crystal structure [26] could also allow the generation of fisetin derivatives with improved activity against cholesterol-dependent cytolysins.

Because of their rapid evolution rate, bacteria are experts at finding alternative routes to achieve growth and infection. Because most virulence traits are nonessential for bacterial survival, therapeutic strategies based on the inhibition of virulence should apply mild evolutionary pressure and limit the development of resistance. These promising approaches are sought to dampen pathogen progression, allowing the control of infection through an effective host immune response or increasing the efficacy of classic therapies targeting bacterial growth. Indeed, in the presence of antivirulence compounds, bacteria can still grow and produce the targeted virulence determinants and are thus able to reinjure the host in the absence of suitable inhibitors. Coupled with traditional antibiotics, antivirulence therapies may provide an important advantage in the fight against infectious diseases.

Although resistance development and side effects, including disruption of microbiota, are always possible, the report by Wang et al opens new possibilities for natural compounds as effective antivirulence strategies against human bacterial pathogens.

**Notes**

**Financial support.** This work was supported by the Fundo Europeu de Desenvolvimento Regional – Programa Operacional Factores de Competitividade (FEDER-COMPETE) and Fundação para a Ciência e a Tecnologia (FCT) (PTDC/BIA-BCM/111215/2009/FCOMP-01-0124-FEDER-014178, PTDC/SAU-MIC/111581/2009 FCOMP-01-0124-FEDER-0158449, and InfectERA PROANTILIS/0001/2013 to D. C.), the European Molecular Biology Organization (EMBO) (to F. S. M.), and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (to S. S.).

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


