Gene Expression **2024** vol. 000(000) | 000–000 Epub
DOI: 10.14218/GE.2023.00167

Review Article

Phagocyte-targeted Effects of *Yersinia pseudotuberculosis* Proteins and Their Role in Bacterial Colonization of the Liver

Lyudmila S. Dolmatova*

Laboratory of Biochemistry, V.I. Il'ichev Pacific Oceanological Institute, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia

Received: November 11, 2023 | **Revised:** June 06, 2024 | **Accepted:** June 18, 2024 | **Published online:** August 02, 2024

Abstract

Yersinia pseudotuberculosis (*Yptb*) causes intestinal infection and can spread to the liver, where the bacterium induces hemosiderosis, abscesses, and hepatitis. To evade the immune response of the host organism, *Yptb* expresses at least six plasmid-encoded *Yersinia* outer proteins belonging to the Type III secretion system, which suppress phagocytic activity. Recently, evidence has accumulated that chromosome-encoded protein toxins are also involved in the anti-phagocytic defense of *Yptb*. Most of these toxins have been found in isolates from patients with Far East scarlet-like fever, often accompanied by liver pathology. *Yersinia* proteins contribute to bacterial colonization of lymphoid organs through their effects on immune cells. A thorough understanding of the immunomodulatory effects of these toxic proteins on bacterial dissemination and colonization in the liver will contribute to the development of novel approaches to cure hepatic pathology during *Yptb* infection. The review aimed to summarize the current data on the mechanisms of effects of *Yptb* plasmid- and chromosome-encoded toxins on bacterial colonization in the liver. The review highlights the fine-tuning of immune system activity by toxins encoded by both a 70-kb plasmid and chromosomes, through various mechanisms of action of individual proteins and their interactions. The focus is on mechanisms that promote bacterial survival in macrophages, including those that facilitate bacterial-induced macrophage polarization towards the M2 phenotype. The role of a type of phagocyte death in bacterial dissemination and colonization in the organs is also discussed.

Introduction

The gram-negative bacterium *Yersinia pseudotuberculosis* (*Yptb*) is the causative agent of a severe infection known as pseudotuberculosis.[1,2] The gastrointestinal form of this infection is most common in cold temperate countries of Europe and North America.[3] Serious outbreaks of a variant of the disease, referred to as Far East scarlet-like fever (FESLF), have been reported from Russia and Japan, where the clinical symptoms of the *Yptb* infection are more diverse and severe than those observed in Europe.[3–5] In addition to damaging the gastrointestinal tract, the bacterium disseminates into internal organs rich in lymphoid elements and macrophages, such as the lungs, mesenteric lymph nodes, spleen, and liver.[6,7]

The bacterium has been reported to contribute to the progression of liver abscesses and sepsis in humans, especially in cases where liver disease or other immunosuppressive conditions are present, including during liver transplantation.[7,8] In cases of FESLF, liver damage is detected in 50% of infected individuals.[3] Liver disorders such as hemosiderosis, acute hepatitis, and chronic hepatitis have been found in infected humans as well as in many infected mammals (including primates, artiodactyls, lions, cows, and cats) and birds.[7,9–11] Once in the organs, *Yersinia* can persist there for a long time, causing multiple waves of the disease or chronic infection.[12] Particularly, FESLF is characterized as a disease that often progresses to a persistent form.[13]

Bacteria translocate from the intestine into the liver and other lymphoid element-rich organs via the bloodstream, after adhering to mucosal cells and subsequently penetrating adjacent Peyer's patches.[14] A subset of intestinal epithelial cells residing within the epithelium that covers gut-associated lymphoid tissue follicles (M cells) specializes in transferring bacteria from the gastrointestinal tract.[15] Bacteria usually enter the liver through the portal circulation or biliary tract.[16]

To colonize the organs, *Yptb* overcome the immune response of the host organism. For this purpose, the bacterium expresses a number of cytotoxic components of the Type III secretion system (T3SS, TTSS), which suppress host phagocyte function, as has long been believed.[17,18] *Yptb*, like *Yersinia pestis* (the causative agent of plague) and *Yersinia enterocolitica* (another pathogenic

Keywords: Phagocytes; *Yersinia pseudotuberculosis*; Bacterial toxins; Type III secretion systems; Liver abscess; Pyogenic.

***Correspondence to:** Lyudmila S. Dolmatova, Laboratory of Biochemistry, V.I. Il'ichev Pacific Oceanological Institute, Far Eastern Branch of the Russian Academy of Sciences, 43 Baltiyskaya, Vladivostok 690041, Russia. ORCID: https://orcid.org/0000-0002-5924-7687. Tel: +7-4232-2312580, E-mail: dolmatova@poi.dvo.ru

How to cite this article: Dolmatova LS. Phagocyte-targeted Effects of *Yersinia pseudotuberculosis* Proteins and Their Role in Bacterial Colonization of the Liver. *Gene Expr* 2024;000(000):000–000. doi: 10.14218/GE.2023.00167.

species), possesses a 70-kb plasmid, pYV (known as pCD1 in *Y. pestis*) (42–48 MDa).[5,19] This plasmid is the most common among those found in *Yersinia* species and ensures the pathogenicity of the strain that carries it. It encodes a unique TTSS system found only in pathogenic *Yersinia*, containing injectisomes that export pathogenic effector proteins with cytotoxic properties (known as *Yersinia* outer proteins, Yops) into the host organism.[20]

In addition to the Yops encoded by pYV, a number of toxins are encoded by chromosomal genes, such as *Yptb*-derived mitogen-superantigen (YPM), cytotoxic necrotizing factor (CNF_Y), and thermostable lethal toxin (TsTYp).[4,20] Recent research indicates that the activity of the *Yptb* anti-phagocytic system depends on the expression of both plasmid and chromosomal genes.[2]

The geographic variability in the severity of clinical forms of the disease caused by European and Far Eastern *Yptb* strains is also reflected in regional differences in the virulence factors synthesized by the bacteria.[4] In particular, YPM and the recently discovered Toll/interleukin-1 receptor (TIR)-domain-containing virulent protein (TcpYI) have been found almost exclusively in Far Eastern strains.[3,21] Furthermore, the extreme polymorphism of the clinical manifestations of FESLF is associated with the presence of several types of toxins.[22] Russian researchers have also identified that the causative agent of FESLF has a different sequence type (2ST) and a different allele of the *yadA* gene compared to European strains, leading to the hypothesis that Far Eastern strains form a clonal line of *Yptb*.[4]

Due to the severity of *Yptb*-induced liver lesions, research attention has focused on the role of *Yptb* protein toxins in bacterial dissemination and colonization in the liver over the last two decades. Understanding the mechanisms of action of these toxins and their contribution to fine-tuning the host immune response will aid in developing novel approaches to treating *Yptb* infection and related liver damage. This review aimed to summarize current data on the mechanisms and regulation of bacterial liver colonization by *Yptb* plasmid- and chromosome-encoded toxins. It specifically highlights the role of chromosome-encoded toxins produced by Far Eastern *Yptb* strains in exacerbating the infection. There is particular focus on how these toxins suppress inflammation, including through macrophage polarization via the M2 way, promoting bacterial dissemination to the liver.

Plasmid-encoded effector proteins/cytotoxins

At least six *Yersinia* effector proteins, including YopM, YopH, YpkA/YopO (in *Y. enterocolitica*), YopJ/YopP (in *Y. enterocolitica*), YopE, and YopT, have cytotoxic properties. With the help of some other Yops, they are translocated into host cells, primarily phagocytes.[20,23] They are all important for tissue colonization, but not all have been found to be involved in liver colonization by *Yersinia*. During *Yptb* infection, Yops target professional phagocytes, predominantly neutrophils in lymphoid organs, along with dendritic cells and macrophages that are selectively targeted in different tissues.[23]

In addition to effector proteins and proteins that promote their translocation into the cell, the 70-kb plasmid carries genes encoding only one T3SS regulating protein. LcrF, the *Yersinia* T3SS master regulator, controls T3SS expression during transitions into host cells. In particular, transcriptional activation or direct binding of LcrF to the promoter regions of *yopE* and *yopH* has been reported.[24] It has also been shown that antagonists of LcrF are required to control gene transcription. In turn, the transcription of *lcrF* is regulated by temperature through temperature-induced changes in chromosomal DNA topology.[25] When *Yersinia* enter cells of a warm-blooded host, the temperature shift affects the structure of RNA. This sensitivity of RNA structure to changes in temperature is used by the bacterium as an RNA thermometer (RNAT) that controls translation of LcrF to activate *yop* gene transcription.[26] LcrF is also post-transcriptionally regulated by the RNA-binding protein, carbon storage regulator system A. To increase *yop* gene expression, *Yptb* can also increase the number of pYV copies after contacting a host cell. Additionally, Yops expression in response to adverse external factors is regulated by the Rcs phosphorelay system, mediated by LcrF.[25]

YopM

YopM (42–54 kDa) is mostly composed of leucine rich repeats.[27] YopM does not exhibit enzymatic activity but has multidirectional effects on immune cells. It promotes the colonization of the liver and other organs by *Yptb*, while its action is aimed at reducing inflammation and stimulating the production of the anti-inflammatory cytokine interleukin (IL)-10.[28,29] Recently, it has been found that YopM stimulates IL-10 gene expression by promoting nuclear translocation of signal transducer and activator of transcription 3 (STAT3).[30] Release of IL-10 upon cell exposure to YopM is important for bacterial virulence and colonization of the liver, as evidenced by experiments on the YopM mutant strain and IL-$10^{-/-}$ animals.[29]

There are differences in the effect of YopM on bacterial colonization of the liver and spleen.[31] Experiments with the YopM mutant strain 32777 showed that colonization of the liver, to a greater extent than that of the spleen, depended on the presence of YopM.[29] Differences were also observed in the number of dendritic cells affected by YopM: in the spleen of mice infected with *Y. pestis* strain containing YopM, there was a decrease in only one subgroup of dendritic cells ($CD8^+$), associated with the production of pro-inflammatory cytokines, compared to those infected by the YopM mutant; in the liver, all three major subgroups of dendritic cells decreased in numbers. However, this difference was significant only at the late stage of infection, thereby raising the question of its role in the differential effects of YopM.[31] Moreover, Ye *et al.*[31] did not find any differences in the numbers of polymorphonuclear leukocytes (PMNs) in the livers and spleens of mice infected with the wild strain of *Y. pestis* or the YopM mutant, indicating that YopM does not affect the recruitment of PMNs to these organs. However, experiments on the mutant Δ*yopM* strain of *Y. pestis*, in which PMNs were suppressed, showed that PMNs were selectively involved in controlling the growth of the mutant bacteria in the liver, but not in the spleen.[32] These findings indicate that YopM targets PMNs in the liver to disrupt their function. Meanwhile, YopM has been shown to promote the apoptotic death of PMNs as well as inflammatory Kupffer cells (hepatic macrophages) and/or monocytes in the liver. It has also been shown that YopM-associated cell death is characteristic only of the liver, whereas cell death in the spleen is rather independent of YopM.[33] Apoptosis is a programmed cell death characterized by morphological features such as cell shrinkage, plasma membrane blebbing, apoptotic body formation, chromatin condensation, and fragmentation of nuclear DNA, and is considered a non-lytic homeostatic mode of cell death.[34] Caspase-3 has been identified as a cofactor or effector in YopM's proapoptotic action on PMNs.[28]

After being delivered into a cell, YopM is localized in the nucleus,[31] but significant amounts of the protein also reside in the cytoplasm.[35] To date, the mechanisms of its action have not been fully elucidated. Thus, YopM is reported to activate the protein

kinase C-related kinase 2 (PRK2) and 90-kDa ribosomal S6 kinase (RSK1).[33] PRK2 is one of the members of the PRK serine/threonine kinase family that are downstream effectors of the Rho family small G proteins.[36] The Rho guanosine triphosphate hydrolases (GTPases) play a role in switching important signaling pathways in the cell, followed by cytoskeleton rearrangement.[37] PRKs are regulated by multiple signaling pathways in different cellular locations, and accordingly, their switching effect depends on many factors.[38] One possible pathway for PRK2 activation is caspase 3-mediated proteolysis during apoptosis.[39] Hentschke *et al*.[40] found that RSK2 and PRK1 (PKN1) can also bind to YopM. However, PRK2 (PKN2) is not directly activated by YopM but indirectly by the overactivated RSK1.[41] Hentschke *et al.*[40] also suggested that activation of the extracellular signal-regulated kinase (ERK)-RSK pathway may be a common event during infection. It is worth noting that ERK has been shown to play a role in promoting cell migration and invasion.[42] Additionally, YopM increased the expression of IL-10 in *Y. enterocolitica*-infected human macrophages through interaction with RSK.[29] Supporting the hypothesis of Hentschke *et al*.,[40] it has been shown that the interaction of PRK2 with YopM is necessary for *Yptb* full virulence and may lead to the inactivation of PRK-dependent pyrin (marenostrin; TRIM200) inflammasome, which is responsible for IL-1 release from macrophages in response to bacterial infection.[29,43] At this level, YopM counteracts the effects of YopE and YopT, which trigger the assembly of the inflammasome.[44]

Inhibition of inflammasome formation by YopM is associated with downregulation of caspase-1.[45] Typically, caspase-1 activation occurs at the onset of infection and is accompanied by lysosome exocytosis and pyroptosis. The latter is a form of inflammatory cell death with cell membrane rupture and release of pro-inflammatory mediators, primarily IL-1β.[46] Thus, YopM may stimulate anti-inflammatory cytokine release while inhibiting pro-inflammatory cytokines, but the molecular basis for target selection has not yet been identified. Overall, the properties of YopM to induce apoptosis and abolish pyroptosis provide the toxin with the ability to kill cells while limiting inflammation.

It should also be noted that in response to various environmental signals, macrophages can transform into pro-inflammatory (M1 macrophages, "classically" activated) or anti-inflammatory (M2 macrophages, "alternatively" activated) phenotypes.[47] M1 macrophages play a key role in defense against bacterial infections, while M2 macrophages are involved in tissue repair. Induction of the M1 or M2 ways occurs upon exposure to Th1 or Th2 cytokines, respectively, and certain other stimuli, including IL-10.[48] In addition to the known mechanisms of cellular immunity suppression, bacteria have a relatively recently discovered and still poorly studied property of dysregulating the balance between host's M1 and M2 macrophages. In response to various groups of bacterial pathogens, a nonspecific stress reaction of macrophages occurs, accompanied by their functional and phenotypical transformation into the M1 type, providing protection against acute infection.[49] However, as shown for a number of bacteria, they cause a shift in macrophage polarization towards the M2 type, leading to decreased bactericidal activity of macrophages and contributing to chronic infection.[49,50] *Yptb* is also capable of causing the transformation of macrophages towards the M2 type. This process, according to Bi *et al*.,[1] likely involves certain bacterial factors and plays an important role in the immunosuppressive strategy of *Yptb*. Due to its ability to stimulate IL-10 production,[28,29] YopM is also assumed to cause a shift in macrophage polarization towards the M2 type. This assumption is supported by the anti-inflammatory effect of YopM mentioned above.[29,43]

YopJ

YopJ (31–32 kDa) possesses properties of a cysteine protease and deubiquitinase but is primarily known as an acetyltransferase.[51,52] The protein regulates the dissemination of *Yersinia* from mucosal tissues but not its replication in organs.[53]

YopJ induces apoptotic cell death in macrophages,[20] but not in neutrophils.[33] YopJ-induced apoptosis of macrophages in the mucosa reduces the immune response, thereby facilitating bacterial dissemination.[53,54] On the other hand, YopJ activates caspase-8, an upstream activator of caspase-3, and induces apoptosis of monocytes in lymphoid nodes,[55] which, according to Bliska *et al*.,[56] promotes the elimination of replicative cell niches, thereby limiting the rate of *Yptb* dissemination. To support this suggestion, Palace *et al*.[57] have demonstrated that YopJ suppresses immune cell recruitment in mouse liver *in vivo*.

YopJ acetylates serine and threonine residues on mitogen-activated protein (MAP) kinase 3, transforming growth factor beta-activated kinase 1 (TAK1), and some MAP2 kinases, resulting in their inactivation. Redundantly, YopJ inhibits the c-Jun N-terminal kinases (JNK), p38 mitogen-activated protein kinase, and nuclear factor kappa B (NF-κB) signaling pathways, thereby inhibiting IL-1β transcription. Moreover, YopJ's effects on TAK1 and other MAPKs are independent of each other, and p38 is involved only in the MAPK-dependent signaling pathway.[58] Thus, YopJ predominantly induces apoptotic signals inhibiting MAPKs and NF-κB signaling.[52,55]

In a study by Sheppe *et al*.,[52] prostaglandin (PG) E2, known as a lipid immunoregulator, stimulates the polarization of *Y. enterocolitica*-infected macrophages towards a proinflammatory M1 phenotype. Simultaneously, PGE2 reduces bacterial survival in macrophages. Both pathogenic bacteria, *Y. enterocolitica* and *Yptb*, inhibit PGE2 synthesis in macrophages, and YopJ contributes to this effect through MAPK inhibition. YopJ blocks PGE2-induced activation of IL-1β transcription by NF-κB, thereby enhancing bacterial survival in macrophages. It can be assumed that YopJ, by inhibiting PGE2 synthesis, also stimulates the polarization of macrophages towards the M2 phenotype.

To counteract this, and in accordance with the known data that different death scenarios can simultaneously develop in a cell,[59] phagocytes induce the assembly of a cytoplasmic death-inducing complex comprising receptor-interacting serine/threonine protein kinase 1 (RIPK1), Fas-associated protein with death domain (FADD), and caspase-8. This assembly is another way to limit bacterial dissemination, as RIPK1/caspase-8 induces proinflammatory cytokine release from uninfected cells.[55] Recently, the role of gasdermins, pore-forming proteins, in pyroptotic cell death has been demonstrated. YopJ, mediated by RIPK1 kinase activity, triggers gasdermin (GSDM) E-dependent, but not GSDMD-dependent, neutrophil pyroptosis, indicating an exclusive role of GSDME in neutrophil pyroptosis.[60] In macrophages, GSDMD has been shown to be involved in pore formation and pyroptosis.[60] Mitochondrial reactive oxygen species (ROS) have been found to augment GSDMD-dependent pore formation.[61] Stimulation of ROS production in mitochondria through caspase 8 and RIPK3 has been discovered as an alternative mechanism to NADPH oxidase-dependent ROS generation,[58] indicating that ROS are involved in both apoptotic and pyroptotic cell death.

The Toll-like receptor (TLR) 4-dependent activation of caspase-1 by YopP can also occur in macrophages. This activation

requires specific infection conditions, such as a high concentration of bacteria and prolonged contact with cells, ensuring that YopP enters macrophages at high concentrations.[62] However, there are allelic variations in genes encoding YopJ and YopP proteins. Data on the activating effect of YopJ on pyroptosis were also obtained from a model of *Yptb*-infected mouse myeloid cells, indicating that TLR4-dependent activation of macrophages shifts cell death from apoptosis to pyroptosis.[60] Thus, YopJ can be involved in regulating both apoptotic and pyroptotic cell death. Nevertheless, it remains unclear how signaling pathways switch under exposure to the effector, which determines the fate of the cell.

YpkA

YpkA (81.7 kDa) is a serine/threonine protein kinase homologous to that of eukaryotes.[51] YpkA plays a significant role in *Yptb* virulence, as shown in experiments with mutant strains expressing catalytically inactive YpkA.[63] YpkA also promotes the spread of *Yptb* to the brain and spleen,[64] but there is no available data regarding its spread to the liver.

YpkA directly inhibits Rho GTPases, including RhoA, Rac1, and Rac2.[57] To control actin remodeling necessary for macrophage phagocytosis, the protein contains a guanine nucleotide dissociation inhibitor-like domain, an N-terminal membrane localization domain, and a serine/threonine kinase domain.[65]

Additionally, YopO (homologous to YpkA) phosphorylates the actin-binding protein gelsolin, which is implicated in various cellular activities such as cell motility, signaling, apoptosis, and phagocytosis. Thus, YopO may attenuate host defense through its effects on gelsolin.[66]

YpkA phosphorylates the Gq protein alpha subunit (Gαq), a member of the family of heterotrimeric G protein alpha subunits. This leads to inhibition of the Gαq signaling cascade, which regulates a number of cell activities, including cell growth and proliferation.[67] This results in inactivation of the Rho GTPases and subsequent cytoskeleton disruption,[68] but there is still no full understanding of the signaling pathways that contribute to YpkA toxicity.

YpkA/YopO also induces phosphorylation of vasodilator-stimulated phosphoprotein (VASP), a regulator of actin dynamics,[69] and targets other actin-regulating proteins such as the actin filament elongator Ena/VASP-like protein and the nucleation-promoting factors: Wiskott-Aldrich syndrome protein and Wiskott-Aldrich syndrome protein-interacting protein.[67] Phosphorylation of these proteins presumably leads to the inhibition of actin rearrangement, as demonstrated for VASP, and further suppression of phagocytosis.[70] The importance of these multiple effectors is not clear, but a role in differential tissue tropism or effector kinetics has been suggested.[57]

Another way to suppress host immunity, as shown for YpkA in murine macrophages, is by inducing caspase-3-mediated apoptosis,[67] which may be independent of its effects on actin depolymerization.[43] Additionally, co-infection with YopT and YpkA inhibited YopT-enhanced neutrophil degranulation, indicating that YpkA inhibits neutrophil degranulation, which is part of host defense.[71]

YopH

The protein toxin YopH (51 kDa) belongs to the class of protein tyrosine phosphatases.[51] Experiments on wild-type and YopH-mutant strains have shown that YopH is crucial for the colonization of the liver and other lymphoid tissues, as well as for the virulence of pathogenic *Yersinia* species.[71,72] YopH targets neutrophils, and one of the presumed mechanisms is as follows: the toxin causes dephosphorylation of the adapter protein Src kinase-associated phosphoprotein-2 (SKAP2).[72] SKAP2 is involved in integrin-stimulated cytoskeletal changes in macrophages and neutrophils.[73] In addition, SKAP2 is required for ROS production. Furthermore, YopH inhibits both ROS production in neutrophils and phagocytosis. To inhibit ROS generation, YopH blocks both SKAP2-dependent (involving ERK1/2 phosphorylation) and independent (Fc gamma receptor-stimulated phosphorylation of proximal signaling proteins such as spleen tyrosine kinase, SH2 domain-containing leukocyte protein of 76 kDa, phospholipase Cγ 2, and PML-RARA-regulated adaptor molecule-1) mechanisms.[72]

YopH was also shown to inhibit neutrophil degranulation independently of SKAP2.[72] Another function of YopH is to prevent the uptake of *Yptb* by M cells and thereby reduce the dissemination of bacteria within lymph. Thus, YopH promotes bacterial colonization while reducing dissemination.[15]

YopE

The toxic properties of YopE (23 kDa) are associated with the modification of Rho GTPases.[44,51,74] According to recent data, YopE is a GTPase-activating protein that facilitates the hydrolysis of guanosine triphosphate (GTP) into guanosine diphosphate, thereby inactivating the Rho GTPases,[51] which leads to cytoskeleton disruption.[44]

An increase in the level of YopE is followed by restriction of bacterial dissemination to the liver.[75] However, YopE partly degrades within infected phagocytes.[51,76] Increased intracellular levels of YopE were shown in models of mutant strains of *Y. enterocolitica* serogroup O8, which avoided the degradation of YopE via the ubiquitin-proteasome pathway. The accumulation of degradation-resistant YopE was accompanied by a pronounced cytotoxic effect on infected cells and a decrease in the delivery of YopP/YopJ and YopH into cultured cells infected by *Yersinia*. A pathway influencing the translocation of other Yops by YopE has been suggested.[75] While activated Rho GTPases stabilize pores formed by T3SS in the host cell membrane via actin cytoskeleton manipulation, YopE-induced inhibition of Rho GTPases limits pore formation and further restricts the translocation of other Yops. Thus, variations in the level of YopE and its influence on Yops translocation may depend on the activity of the proteasome, which also optimizes the infection process.[75,77] This finding indicates that increased cytotoxicity limits bacterial dissemination. However, there is evidence that the direct anti-phagocytic effect of YopE released by *Yptb* is predominant compared to its regulatory role in Yop translocation.[75]

YopE was shown to inhibit caspase-1 and the release of IL-1β in macrophages.[78] This effect was observed with the inhibition of Rac2, but not RhoA, and was associated with the inhibition of ROS.[79] However, RhoA was the preferred target for YopE, the inhibition of which, in contrast, resulted in pyrin dephosphorylation and inflammasome activation.[44] As YopM usually inactivates pyrin, YopE initiates the assembly of inflammasomes only in the absence of YopM.[80] Output of pyrin dephosphorylation depends on the features of effectors, such as Rho GTPase specificity.[15] However, the mechanisms targeting specific Rho GTPases remain poorly understood. Hence, despite the fact that inhibition of RhoA led to activation of the inflammasome,[44] this did not restrict the survival of *Yersinia* in macrophages.[74] Therefore, the ability of YopE to kill phagocytes through the inflammasome pathway remains unclear.

YopE, like other Yops, is a multifunctional protein. The toxin can be recognized by phagocytes, with YopE being an antigen for T and B cell responses. Activation of phagocytes may play a role

in the progression of chronic lymph adenopathy.[79]

Similar to YopH, the toxin prevents the uptake of *Yptb* by M cells. This fact may explain the more efficient penetration of *Yptb* into M cells during early infection stages when YopE concentration is still low.[15]

YopT

The cytotoxin YopT (35.5 kDa), like YopE and YpkA, is a Rho-modifying toxin.[44,51,81] However, as a protease, it inactivates Rho GTPases, such as RhoA, Rac1, RhoG, and CDC42 *in vitro*, by cleaving the C-terminus, leading to their release from the membrane.[44,81] Meanwhile, *in vivo*, YopT induces the shift of RhoA, but not Rac1 or CDC42, from the cell membrane to the cytosol, indicating that RhoA is the primary target for the bacterially translocated YopT.[81] However, YopT has been shown to selectively target different Rho GTPases depending on the cell type.[67] YopT is highly cytotoxic, and it is the only *Yersinia* effector protein that can destroy actin filaments in HeLa cells.[82]

Similar to YopE, in relation to inhibiting RhoA GTPase, YopT also induces pyrin dephosphorylation in the absence of YopM, leading to the activation of inflammasome assembly, further activation of caspase-1, and eventually cell death by pyroptosis. As is known, pyroptosis limits bacterial growth in cells and their spread to deep tissues.[44] However, there is no experimental confirmation of the effect of YopT on bacterial dissemination.

YopT acts slower on pyrin dephosphorylation compared to YopE. For this reason, YopE is considered to play a dominant role in triggering this process.[44] However, YopT is able to decrease the translocation rate of YopE possibly by competing for the same Rho GTPases.[71] The exact mechanisms of this action remain poorly studied.

Chromosome-encoded toxins

Some chromosome-encoded genes in *Yptb* contribute to the coordinated activation of the expression of the 70-Kb plasmid (pCD1). For example, the *ctgA* (chromosomal T3SS secretion gene) mutant strain released fewer Yops than the parental strain.[83] Moreover, recently, some gene products (YlrA, YlrB, and YlrC) representing chromosome-encoded T3SS effector proteins of *Y.pestis* have been discovered for the first time. These proteins promote the optimal survival of *Y. pestis* in the presence of macrophages.[84] However, the putative role of these proteins in the virulence of *Yersinia* has only begun to be studied.

The chromosome-encoded toxins of *Yersinia,* whose action does not depend on the presence of the 70-Kb plasmid, have been previously described.[3,4,20] Over the past 20 years, evidence has emerged that toxins such as YPM, CNF_Y, TsTYp, and TcpYI may also contribute to the modulation of immune response and bacterial spread.

CNF_Y

CNF_Y catalyzes the deamidation of glutamine in molecules of small Rho GTPases, blocking their ability to hydrolyze GTP. In contrast to YopE, CNF_Y constitutively activates small Rho GTPases.[85,86] The molecular weight of CNF family proteins is usually estimated at approximately 114 kDa, but this applies to cytotoxic necrotizing factor 1 (CNF1) of *Escherichia coli*,[87] which shares only about 65% identity with CNF_Y.[86]

The CNF_Y toxin is an important virulence determinant expressed predominantly during the early stage of infection.[87] Temperature control is crucial for its activation upon entry into warm-blooded hosts; increased protein levels are recorded only after a shift to 37°C. However, the secondary structure of the protein in *Yptb* YPIII is impaired at temperatures up to 42°C, suggesting that *cnfY* RNAT confers temperature-dependent translational control to its downstream gene. The RNAT-controlled synthesis of CNF_Y is crucial for *Yptb* virulence and its spread to the liver, but the *cnfY* gene is not controlled by LcrF.[88]

RhoA GTPase plays a crucial role in CNF-promoted bacterial spread, as it is involved in cell membrane destabilization.[89] Specifically, CNF_Y activates Rho GTPases, enhancing the translocation of certain Yops.[85,86,90]

CNF-induced activation of Rho GTPases is associated with its ability to induce the formation of giant multinucleated cells. Studies using CNF1 have shown that toxin-induced stimulation of Rho protein activity leads to cellular DNA replication without concomitant cell division, resulting in multinucleation.[4] CNF_Y also induces dermonecrotic reactions *in vivo*.[85] Studies with CNF1 indicate that RhoA inactivation and Rac1 presence are critical for CNFs' ability to prevent cell apoptosis by stimulating the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/NF-κB signaling pathway, while CNF-mediated RhoA activation opposes pyrin inflammasome activation.[90]

Additionally, CNF_Y-induced activation of Rho GTPases leads to STAT3 activation in human embryonic kidney cells, suggesting this mechanism operates in various cell types.[90] STAT3 activation may lead to increased IL-10 release,[30] but its role in cell death during CNF_Y exposure remains unclear.

In the absence of CNF_Y, transcripts of enzymes involved in lymphatic tissue damage repair and regeneration (e.g., Adamts4, Adamtsl2, Lox, Wisp2/CCN5, Pappa2, and Retnlg) are found in lower numbers compared to wild-type-infected tissue,[91] suggesting CNF_Y's association with inflammatory response and tissue injury. The pro-inflammatory actions of CNFs are evidenced by the activation of cytokines IL-6 and IL-8, as well as mediators like monocyte chemotactic protein 1 and macrophage inflammatory protein-3 in CNF1-intoxicated endothelial cells.[92]

Heine *et al*.[91] demonstrated that CNF_Y played a role in the virulence of *Yptb*, promoting dissemination in lymphatic tissues and organs, including the liver, of infected mice, and inducing inflammatory reactions that lead to extensive tissue destruction. These authors suggested that CNF_Y accelerates bacterial dissemination through Rho GTPase-activated phagocyte motility and facilitates bacterial penetration into damaged tissues. However, this requires further study.

Meanwhile, CNF_Y-induced caspase-3/7-mediated apoptosis was observed in the human prostate cancer cell line LNCaP,[93] indicating that the cellular response to CNF_Y exposure depends on the cell type.

CNF_Y has been intensively studied worldwide since isolation from YPIII strain in 2002.[85] It's important to note that all the described experiments were conducted with CNF_Y isolated from YPIII bacteria.[94] The full-length *cnfY* gene encoding CNF_Y has only been identified in *Yptb* strains YPIII and IP2666 of serogroup III. Many other *Yptb* and all *Y. pestis* strains contain various deletions within *cnfY*.[89] The presence of *cnfY* increases the virulence of *Yptb*, presumably enhancing the translocation of certain Yops. However, considering that Yops such as YopH, Yop M, and YpkA reduce inflammatory responses,[91] while CNFs predominantly stimulate inflammation,[92] these proteins are unlikely to be associated with increased virulence of wild-type bacteria. It has been suggested that the increased virulence induced by CNF_Y in *Yptb* is rather a consequence of decreased translocation of YopJ, the most potent

inhibitor of the innate immune response,[53,54] as mRNA levels of YopJ were found to increase in Δ*cnfY* mutants.[91] Thus, the specific roles of certain Yops in CNF_Y effects remain to be elucidated.

Earlier, in 1996, a heat-labile protein toxin (HLT, 200 kDa) was isolated and purified in Russia from the YPIII strain 2517 obtained from France.[22,94] It is noteworthy that the first information about the *Yptb* heat-labile toxin (at that time the bacterium was considered to belong to the genus *Pasteurella*) appeared in 1969.[17] According to Somova *et al*.,[95] HLT causes a local dermonecrotic reaction and death in laboratory animals when administered parenterally.

Severe dystrophic and necrotic changes were found in organs, mainly in the liver and kidneys, already in the initial stages (3–6 h) after the administration of HLT. At 48 h post-administration, necrobiotic and necrotic changes in hepatocytes with a perifocal inflammatory reaction were observed in the liver. Subsequently, mass spectrometric analysis and a comparative study of the virulent and toxic properties (dermonecrotic reaction, heat lability, and similar immune response) of CNF_Y and HLT on macrophages identified HLT as CNF_Y.[94] However, these findings need further confirmation. In particular, the molecular targets of HLT remain unexplored.

It has also been reported that in the Far East of Russia, there are strains having the *cnfY* gene, which differs from that of the YPIII strain by the presence of three nonsynonymous mutations, resulting in amino acid substitutions Asn192Ser, Glu304Gly, and Ala394Thr (registered as "allele 2 of the *cnfY* gene (KR028010)"). Nevertheless, a comparison of the activity of the Russian and American variants of the toxin has revealed their complete similarity.[4] However, most isolates in Russia and all isolates obtained from patients with FESLF (0:1b serotype) contain an inactivated variant of CNF_Y. This gene (registered in the GenBank database as "*Yptb cnf* gene allele 1 (KR028011)") contains two deletions and a number of nucleotide substitutions, one of which, T355G, causes the formation of a stop codon.[96]

Previously, a study of HLT activity showed that the toxin stimulated lactate and succinate dehydrogenase activities in resident macrophages after the first 2–6 h of incubation, while simultaneously decreasing the activity of the inducible nitric oxide synthase system.[97] Nitric oxide (NO) is a marker of M1 macrophages, whereas M2 macrophages demonstrate an oxidative metabolic profile.[48] These findings are in favor of the hypothesis that this toxin can cause macrophage polarization through the M2 pathway in the initial period of incubation. Apparently, the reparative changes in the transcriptome in wild-type-infected tissues compared to those of Δ*cnfY* mutant infected cells may also be associated with the shift from M1 to M2 macrophage phenotypes in response to CNF_Y action,[91] which can reduce inflammation induced by Yops translocation.

A significant accumulation of NO by macrophages in response to HLT was recorded at 24 h.[97] Thus, the toxin exhibits biphasic action, inducing a presumed pro-inflammatory effect at a later stage of infection, likely related to polarization towards the M1 macrophage type.

However, there is a question regarding the role of CNF_Y inactivation in worsening infection during FESLF. It is likely that the characteristics of the bacterial strain serotype 0:1b, prevalent in patients with FESLF, including the presence of an additional plasmid pYV82 (82 MDa),[94] may influence the ratio and activity of effector proteins translocated under CNF_Y exposure. The presence of this plasmid, which has only been recorded in Russian strains in addition to pYV, leads to a more severe clinical course of the disease compared to strains that have only pYV.[4]

Obviously, a balance between the effects of CNF_Y and other Yops is crucial for the infection outcome. However, the mechanisms regulating this balance in FESLF remain unclear.

YPM

YPM (14 kDa) is encoded by the chromosomal gene *ypm*.[3] This superantigen binds directly to MHC II molecules, rapidly activating both innate immune cells and up to 20% of T cells.[2] YPM also activates hepatotoxic $CD4(^+)$ T cells.[5] In contrast to Gram-positive bacterial superantigens, which increase inflammatory cytokine production by phagocytes, YPM stimulates IL-4 release and strengthens the Th2 immune profile.[3,5,98]

YPM enhances the toxicity of *Yptb* in systemic infections but not in gastroenteric infections in mice. Injection of YPM into mice induces massive hepatocyte necrosis. Further, transaminase activity in plasma of YPM-containing *Yptb*-infected mice increases.[5,98] It has been suggested that granzymes (cytotoxic serine proteases) released from YPM-stimulated $CD4^+$ T cells damage the liver via anoikis, a variant of apoptosis.[5] Data from a YPM mutant indicates that the superantigen promotes bacterial colonization in the mouse liver.[99]

YPM is produced by all clinical strains from Far Eastern regions causing FESLF but is absent in almost all European non-FESLF-causing strains. Superantigen-producing strains can be categorized into three clusters containing YPMa, YPMb, or YPMc encoded by the *ypmA*, *ypmB*, and *ypmC* genes.[3] Each of these variants activates a specific clone of T-lymphocytes.[100] YPMa is detected in 98% of Far Eastern clinical strains, YPMc in five strains of serotype O:3 from Western countries and Japan, while YPMb has not been found in clinical strains at all.[101] This suggests that YPMa is implicated in the mechanisms underlying the severe form of the disease.

Furthermore, higher titers of anti-YPM were observed in patients presenting systemic symptoms such as lymphadenopathy, transient renal dysfunction, and arthritis, but not in patients with liver diseases.[102] This raises questions about the role of the toxin in *Yptb* colonization in the liver.

TsTYp

TsTYp (45 kDa) is produced by bacteria of I-VI serovariants identified in various regions of Russia and other countries.[22,103,104] It is produced by more than 80% of *Yptb* strains isolated from patients with FESLF and from the environment. TsTYp causes a general vascular reaction in the liver and other organs that results in dystrophic and necrotic changes. Diffuse and focal hyperplasia of Kupffer cells has been recorded, indicating their involvement in the inflammatory response.[22] TsTYp has been used as an antigen in a test system for the diagnosis of pseudotuberculosis.[105,106] This toxin causes death in mice when administered parenterally. TsTYp toxicity depends on the temperature during its isolation and is lower at 6–8°C compared to that at 37°C. Such a wide temperature spectrum of TsTYp activity suggests toxin involvement in *Yptb* pathogenicity in various organisms, including invertebrates.[22]

In the last two decades, evidence has emerged that TsTYp is able to affect the mechanisms of phagocytic activity in neutrophils and monocytes.[22,106] *In vitro* studies have shown that TsTYp has an apoptosis-modulating effect on rat neutrophils, dependent on its concentration and incubation time.[107]

Apoptosis is closely associated with ROS levels, and antioxidant enzymes limit the damaging effect of ROS on cells.[108,109] TsTYp (in the concentration range of 0.2–2.5 μg/mL) had a significant impact on mononuclear leukocytes after 30 min of incuba-

tion *in vitro*, causing suppression of ROS generation at a low but not higher concentration. In neutrophils, the toxin had the opposite effect, corresponding to changes in phagocytic activity.[22] The response of mononuclear cells even to a lower concentration of TsTYp indicates that these cells are more sensitive to the action of TsTYp than neutrophils, despite neutrophils being the first line of defense against bacteria.[50,110] Such early suppression of the functional activity of mononuclear leukocytes at low concentrations of TsTYp can be considered one of the strategic mechanisms of bacterial suppression of host immunity.

TsTYp also modulated the level of cAMP in phagocytes in a concentration-dependent manner, and the changes were biphasic in the studied concentration range of 1–5 μg/mL. A similar biphasic effect was shown for the adenylate cyclase toxin of *Bordetella pertussis* when incubated for 2 h with rat fat cells. The cholera toxin stimulated a linear accumulation of GTP, closely associated with that of cAMP, in a concentration range of 1–3 μg/mL, and at 3–5 μg/mL, the toxin began to decrease the GTP level.[111] Moreover, the biphasic concentration mechanism of effect (also called hormetic) is basic and common to biological systems, representing stimulatory or overcompensation responses to relatively low damaging doses of agents of various origins.[112] A biphasic dose-dependent response to TsTYp has also been demonstrated in mouse leukocytes *in vivo*. At a dose of 0.01 mg/mouse, TsTYp promoted leukopenia; at a dose of 0.1 mg/mouse, TsTYp stimulated leukocytosis, in addition to the release of pro-inflammatory cytokines and the synthesis of anti-inflammatory cytokine IL-4.[18]

However, the concentration-dependent impact of TsTYp on cAMP accumulation in human peripheral blood monocytes was opposite to that in neutrophils, which was consistent with the opposite effects of the toxin on ROS levels in these two types of leukocytes.[22] These results are consistent with reports indicating an inverse relationship between increased levels of cAMP and stimulation of the immune response.[113] The involvement of cAMP in the mechanisms of the apoptosis-modulating effect of TsTYp on rat neutrophils was demonstrated using caffeine, an inhibitor of cAMP phosphodiesterase activity. The combined addition of caffeine and the toxin reduced the level of apoptosis compared to the effect of TsTYp alone, indicating that an increase in toxin-induced apoptosis occurs against the background of a reduced cAMP level.[107] This agrees with available data on the cAMP-dependent suppression of apoptosis in neutrophils.[114]

For *Yptb*, it has been shown that the inhibition of ROS production in blood phagocytes promotes bacterial colonization in the host's organs.[58,115] However, the effect of TsTYp on bacterial colonization in the liver has not been studied yet.

The toxin does not have ADP-ribosyltransferase activity, which is a feature of some other enterotoxins that enhance the accumulation of cAMP.[22] The mechanisms of TsTYp's effect on cAMP accumulation remain unclear. As known, the signaling cascades following the increase in cAMP levels generally include the activation of protein kinase A (PKA), which catalyzes the phosphorylation of a number of proteins, including the cyclic AMP response element-binding protein transcription factor (CREB), and subsequent expression of target genes.[116,117] Activation of the cAMP/PKA signaling pathway may lead to a decrease in ROS generation,[118] as observed in mononuclear cells exposed to low concentrations of the toxin.[22]

Cyclic AMP can also induce activation of a PKA-independent signaling pathway, which includes the binding of cAMP by proteins of the exchange proteins activated by the cAMP family, involved in the regulation of cell migration.[116] Apparently, through the modulation of cAMP accumulation, TsTYp can counteract or, *vice versa,* promote the effects of Yops and CNF_Y depending on the concentration and stage of infection. Further study on TsTYp-induced cAMP activation downstream effects will help to understand their interaction.

Of particular interest is the role of cAMP in the activation of ERK 1/2 through ROS-dependent activation of Ras GTPases.[119] The ERK 1/2 - MAPK signaling pathway, in turn, can contribute to the polarization of macrophages towards the M2 phenotype.[120] In addition, Ras stimulates the activity of PI3K.[116] The PI3K/Akt signaling pathway may contribute to an increase in the number of M2 macrophages.[121] Apparently, these mechanisms induce a cAMP-dependent shift towards a decrease in the content of Th1 lymphocytes and, respectively, M1 macrophages when exposed to bacterial toxins that increase the level of cAMP in host cells.[122] Based on these data, it can be assumed that the cAMP-elevating effect of TsTYp in macrophages may also induce a shift in macrophage polarization towards the M2 phenotype, facilitating *Yptb* survival in cells. This assumption was confirmed through model experiments on marine invertebrates. *Yptb*, like other *Yersinia* species, successfully adapts to the external environment.[123] *Yptb* is able to survive for a long time in seawater and in the bodies of some marine invertebrates, including sea cucumbers.[22] The two types of phagocytes in these animals differ phenotypically and functionally and display markers (NO level or arginase activity), suggesting that these two types of phagocytes are analogs of M1 and M2 macrophages.[124] TsTYp dysregulates the functional activity of the two types of phagocytes and causes a shift towards a dominance of the activity of cells with a phenotype similar to that of M2 macrophages.[125]

TcpYI

The recently discovered TcpYI (24.6 kDa) was isolated from FES-LF serotype I strains. TcpYI contains a C-terminal hydrophobic 115 amino acid domain similar to that of the TIRI domain family. The TcpYI gene is located on a genomic island known as the *Yersinia* adhesion pathogenicity island. TcpYI protein causes virulence in the absence of the pYV virulence plasmid. The toxin promotes intracellular survival of *Yptb* within macrophages and in mice spleens *in vivo*.[21] As for the effects of TcpYI in the liver, there is no available data to date.

As shown previously by Rana *et al*.,[126] a common strategy for Tcp-containing bacteria to suppress host defenses involves targeting pyroptosis-inducing TLR4 signaling via specific protein-protein interactions. Another mechanism through which some Tcp-containing bacteria demonstrate virulence is their nicotinamide adenine dinucleotide (NAD^+)-hydrolase activity, leading to NAD^+ depletion and cell death.[127] TcpYI blocks TIR signaling by binding to TIR,[21] which may protect host cells from NAD^+ depletion.[128] However, the downstream effects of TcpYI remain unstudied.

Data on the activity of *Yptb* plasmid-encoded effector proteins and chromosome-encoded toxins are summarized in Table 1.[2,3,5,15,18,21,22,28–30,36,39,41,43–45,51–58,60,62,64,66–73,75,78,81,82,85,86,89–92,98,99,107,125]

Multidirectional regulations of survival and dissemination of *Yptb* by protein toxins

Both Yops and chromosome-encoded toxins provide bacteria with protection from the host's immune system.[3,4] All of them have a toxic effect on the cells of the phagocytic system, causing their death or decrease in activity (Fig. 1). Simultaneously, most pro-

Table 1. Anti-phagocytic and dissemination-regulating effects of protein cytotoxins/toxins of *Yersinia pseudotuberculosis*

Protein	Enzyme activity	Cellular effect	Target molecule	Dissemination/liver colonization effect
YopM	No activity[28]	Stimulation of IL-10 release[28] Inhibition of IL-1 release[29] Caspase-3-mediated apoptosis[39] Suppression of caspase-1[45]	PRK2[36] RSK1[41] STAT3[30]	Promotes liver colonization[28]
YopJ	Acetyltransferase, cysteine protease and deubiquitinase[51,52]	Apoptosis of macrophages in the mucosa[53,54] Apoptosis of monocytes in lymphoid nodes[55] Prevention of inflammasome assembly[52,55,58] Blocking IL-1β secretion[55] Increase in release of proinflammatory cytokines[55,68] Pyroptosis in neutrophils[60] IL-1β release[60] Pyroptosis in macrophages[60,62]	Caspase-8[55] TAK1[58] NF-κB[52,55,58] MAPKs[58] JNK[58] p38[58] PGE2[52] Cytoplasmic death–inducing complex (RIPK1, FADD and caspase-8)[55] GSDME[60] GSDMD[60] Caspase-1[62]	Promotes bacterial dissemination to lymphoid tissues[53,54] Not obligatory for colonization[53] Limits rates of dissemination[55–57] Suppresses immune cell recruitment into the liver[57]
YpkA	Serine threonine kinase.[51]	Disruption of the actin cytoskeleton[70] Suppression of phagocytosis[70] and neutrophil degranulation[71] Caspase-3-mediated apoptosis[43,67]	RhoA, Rac1, and Rac2[57] Gαq[67] VASP[69] EVL[67] WASP[67] WIP[67] Gelsolin[66] NFκB[68] ROS[68]	Promotes bacterial dissemination to brain and spleen[64] No data for the liver
YopH	Tyrosine phosphatase[71]	Inhibition of ROS production and phagocytosis in neutrophils[72] Inhibition of neutrophil degranulation, decrease in immune response[72] Preventing the uptake of *Yptb* by M cells[15]	SKAP2 Syk, SLP-76, PLCγ2, and PRAM-1[72,73]	Promotes colonization of lymphoid tissues/liver[71,72] Reduces bacterial dissemination[15]
YopE	GTPase-activating protein[51,75]	Promotion of cytoskeleton disruption[44] Inhibition/activation of caspase-1 activity and release of IL-1β in macrophages[78] Prevents the uptake of *Yptb* by M cells[15] Limits the translocation of other Yops[75]	Rho GTPases[44] Rac 2/ Rho A[78]	Restricting of bacterial dissemination to the liver[75] Inhibits bacterial dissemination[71]

(continued)

Table 1. *(continued)*

Protein	Enzyme activity	Cellular effect	Target molecule	Dissemination/liver colonization effect
YopT	Protease[51]	Disruption of cytoskeleton[82] Activation of inflammasome assembly[44]	Rho GTPases[44,51,81]	No data available
CNF_Y	Catalyzes the deamidation of glutamine in Rho GTPases, constitutively activating them[85,86]	Cell membrane destabilization[89] Reduction of phagocytosis[89] Pyrin inflammasome assembly inhibition[89] Preventing apoptosis[89] IL-6, IL-8, MCP1, and MIP-3α release[91]	Rho GTPases[85,86] Rho A[89] Rac, PI3K/ Akt/NF-κB[89] STAT3[89]	Promotes bacterial dissemination and liver colonization[90,92] Promotes bacterial dissemination[89]
YPM	No activity[3]	IL-4 release, strengthening the Th2 immune profile[3,5,98]	MHC II molecules[2]	Promotion of bacterial growth rate[99]
TsTYp	No activity[22]	A biphasic changes in pro-and anti-inflammatory cytokine release in mice[18] Inhibition of the phagocytic activity of neutrophils and macrophages[22] Apoptosis in neutrophils[107] Modulation of ROS generation in monocytes and neutrophils[22] Dysregulation of the functional activity of the two types of phagocytes of the sea cucumber[125]	cAMP[22]	No data available
TcpYI	No activity[21]	Decrease in macrophage activity[21]	TLR signaling[21]	Promotes bacterial dissemination with lymph[21]

Akt, protein kinase B; cAMP, cyclic adenosine monophosphate; CNF_Y, cytotoxic necrotizing factor of *Yersinia pseudotuberculosis*; EVL, Ena/VASP-like protein; FADD, Fas-associated protein with death domain; Gαq, Gq protein alpha subunit; GSDMD, gasdermin D; GSDME, gasdermin E; GTPase, guanosine triphosphate hydrolase; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MAPKs, mitogen-activated protein kinases; MHC, major histocompatibility complex; MIP-3α, macrophage inflammatory protein-3α; MCP1, monocyte chemotactic protein 1; NF-κB, nuclear factor kappa B; p38, p38 mitogen-activated protein kinase; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; PLCγ2, phospholipase Cγ 2; PRAM-1, PML-RARA regulated adaptor molecule 1; PRK2, protein kinase C-related kinase 2; RIPK1, receptor-interacting serine/threonine protein kinase 1; ROS, reactive oxygen species; RSK1, 90-kDa ribosomal S6 kinase; SLP-76, SH2 domain-containing leukocyte protein of 76 kDa; SKAP2, Src kinase-associated phosphoprotein-2; STAT3, signal transducer and activator of transcription 3; Syk, spleen tyrosine kinase; TAK1, transforming growth factor beta-activated kinase 1; TcpYI, Toll/interleukin-1 receptor domain-containing virulence protein of *Y. pseudotuberculosis*; TLR, Toll-like receptor; TsTYp, thermostable toxin of *Y. pseudotuberculosis*; VASP, vasodilator-stimulated phosphoprotein; WASP, Wiskott-Aldrich syndrome protein; WIP, WASP-interacting protein; YPM, *Y. pseudotuberculosis*-derived mitogen.

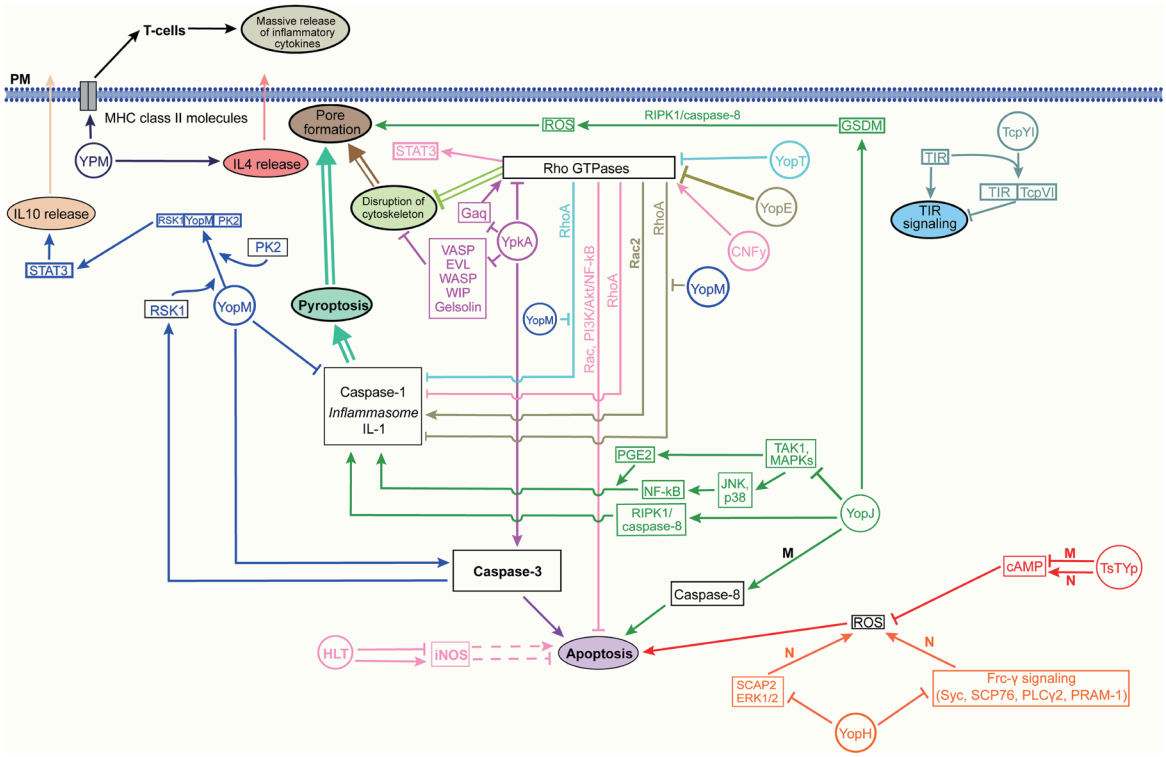

Fig. 1. Scheme of modulation of signaling pathways in phagocytes by protein effectors/toxins of *Yersinia pseudotuberculosis*. To maintain a balance between the death of the host cell and saving a niche for survival and spread, *Yptb* uses a set of toxic proteins. The proteins exert their action through different signaling pathways. The activity of the crucial signaling pathways that determine the type of cell death is controlled simultaneously by several proteins, which can both mutually enhance each other's effects or compete with each other. YopM activates PRK2 by preliminary binding and activating RSK1; caspase-3 is a cofactor in the activation of the latter. The downstream effector STAT3 stimulates the expression of the anti-inflammatory cytokine IL-10. Additionally, YopM has an anti-inflammatory effect through inhibition of the activity of caspase-1, which is part of the pyrin-inflammasome complex, and the synthesis of the pro-inflammatory cytokine IL-1, which induces a pro-inflammatory type of cell death, pyroptosis. YopM also exerts an anti-inflammatory effect through inhibition of the pro-inflammatory activity (activation of inflammasome assembly) of YopE and YopT. YopJ induces apoptotic cell death in macrophages but not in neutrophils through activation of caspase-8. In addition, YopJ prevents activation of the MAPKs (including TAK1 and MAP2 kinase) and downstream activation of JNK and/or p38 and NF-κB-dependent pathways. Redundantly, YopJ inhibits biosynthesis of PGE2, which is involved in the activation of NF-κB-dependent transcription of IL-1β. Thus, inhibiting the MAPK cascade leads to blocking IL-1β secretion and preventing pyroptosis. However, YopJ can also have a pro-inflammatory effect, activating inflammasome assembly via the RIPK1/caspase-8-dependent pathway, as well as stimulating GSDME- and GSDMD-dependent and controlling by RIPK1 and caspase-8 ROS activation and pore formation in neutrophils and macrophages, respectively. YpkA inhibits Rho GTPases and the rearrangement of the actin cytoskeleton directly or through phosphorylation of Gαq. In addition, YpkA can promote pore formation through phosphorylation of actin-binding proteins, such as VASP, EVL, WASP, WIP, and gelsolin. These pathways lead to pyroptosis. Meanwhile, the anti-inflammatory effect of YpkA also occurs through stimulation of caspase-3-mediated apoptosis. YopH inhibits ROS generation in neutrophils and apparently limits inflammation through inhibiting SKAP2-dependent ERK1/2 signaling pathway and FcγR-dependent signaling pathway, including phosphorylation of the proximal signaling proteins such as Syk, SLP-76, PLCγ2, and PRAM-1. YopE suppresses Rho GTPases and selectively inhibits caspase-1 and the release of IL-1β to protect host cells from pyroptosis via inactivating Rac2. Inhibition of RhoA, on the contrary, results in inflammasome activation. YopE manipulates the assembly of inflammasomes only in the absence of YopM. YopT cleaves RhoA GTPase, leading to the disruption of actin structures and contributing to pore formation and the anti-phagocytic effect of *Yersinia*. YopT also activates inflammasome assembly and cell death by pyroptosis. In this case, YopT acts slower than YopE and activates inflammasome assembly only in the absence of YopM. CNF_Y constitutively activates Rho GTPases. This leads to STAT3 activation, which may be involved in the anti-inflammatory response, including iNOS inhibition as described for HLT (identified as CNF_Y) in the early stage of incubation. In addition, RhoA stimulation by CNF_Y leads to suppression of inflammasome assembly. Conversely, Rac1 activation by the toxin through the PI3K/Akt/NF-κB signaling pathway prevents apoptosis. YPM binds directly to MHC II molecules and activates both innate immune cells and T cells, followed by a massive release of pro-inflammatory cytokines. Simultaneously, YPM stimulates IL-4 release, strengthening the Th2 immune profile apparently aimed to restrict inflammatory death of phagocytes. TsTYp modulates the accumulation of cAMP and ROS-mediated apoptosis, negatively associated with cAMP level. The toxin affects the level of cAMP in neutrophils and mononuclear cells in the opposite way. TcpYI blocks TIR signaling via binding to TIR. Akt, protein kinase B; cAMP, cyclic adenosine monophosphate; CNF_Y, cytotoxic necrotizing factor of *Yersinia pseudotuberculosis*; EVL, Ena/VASP-like protein; FADD, Fas-associated protein with death domain; Gαq, Gq protein alpha subunit; GSDM, gasdermin; JNK, c-Jun N-terminal kinase; ERK1/2, extracellular signal-regulated kinase 1/2; FcγR, Fc gamma receptor; GTPase, guanosine triphosphate hydrolase; HLT, heat-labile toxin; IL, interleukin; iNOS, inducible nitric oxide synthase; M, macrophage; MAPKs, mitogen-activated protein kinases; MHC, major histocompatibility complex; N, neutrophil; NF-κB, nuclear factor kappa B; p38, p38 mitogen-activated protein kinase; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; PLCγ2, phospholipase Cγ 2; PM, plasma membrane of the phagocyte; PRAM-1, PML-RARA regulated adaptor molecule 1; PRK2, protein kinase C-related kinase 2; RIPK1, receptor-interacting serine/threonine protein kinase 1; ROS, reactive oxygen species; RSK1, 90-kDa ribosomal S6 kinase; SLP-76, SH2 domain-containing leukocyte protein of 76 kDa; SKAP2, Src kinase-associated phosphoprotein-2; STAT3, signal transducer and activator of transcription 3; Syk, spleen tyrosine kinase; TAK1, transforming growth factor beta-activated kinase 1; TcpYI, Toll/interleukin-1 receptor domain-containing virulence protein of *Y. pseudotuberculosis*; TIR, Toll/interleukin-1 receptor; TLR, Toll-like receptor; TsTYp, thermostable toxin of *Y. pseudotuberculosis;* VASP, vasodilator-stimulated phosphoprotein; WASP, Wiskott-Aldrich syndrome protein; WIP, WASP-interacting protein; YPM, *Y. pseudotuberculosis*-derived mitogen.

teins exert multidirectional effects on the dissemination of *Yptb* and its further colonization in the liver (Table 1).

Yersinia usually replicates as an extracellular pathogen that evades phagocytosis by neutrophils and monocytic cells in lymphoid tissues. Facultatively, bacteria are able to survive and replicate in phagocytes, leading to increased pathogenicity.[129] Once inside a cell, bacteria avoid the host's immune defenses and can also enter lymphoid organs, including the liver. In this case, the immune cell dies, which may be accompanied by the release of antibacterial substances. Moreover, apoptotic dendritic cells release antimicrobial agents that can prime $CD8^+$ T cells.[130] Hence, the death of immune cells has been considered a strategy for *Yersinia* to eliminate host phagocytes. On the other hand, macrophage death, in the context of *Yersinia* infection, is protective for the host animal.[50,131] Thus, at least two scenarios of phagocyte death may unfold simultaneously.[57,61,69] The choice of the most beneficial variant for the bacterium is ensured by cytotoxic Yops and other toxins.

Typically, the activation of caspase-1 occurs at the beginning of infection, immediately after entry into the cell, and is accompanied by lysosome exocytosis and pyroptosis.[41,132] The latter is a form of inflammatory cell death, followed by the release of proinflammatory mediators that have antimicrobial effects.[76] Activation of caspase-1 was shown only for YopT and YopE,[44,77] and under certain conditions, for YopJ.[59] YopE could also inhibit the enzyme, but only in the absence of YopM,[77] while YopM, vice versa, inhibited the activation of caspase-1 by YopT,[44] indicating that YopM plays an important role in maintaining the balance of cytotoxicity between these two Yop proteins.

The action of most cytotoxic effector proteins is generally aimed at preventing pyroptosis-associated inflammasome assembly and caspase-1 activation (YopM, YpkA, and YopE),[45,62] and causing cell death through apoptotic mechanisms (YopJ, YopH, and TsTYp),[53,54,107,123] while keeping the phagocyte cell membrane intact. One might expect that TcpYI and CNF_Y are also capable of inducing apoptosis since they block TIR signaling associated with Toll receptor activation,[21] and NO production,[95] respecrively, involved in apoptosis regulation mechanisms.[61]

It should also be noted that the early response of macrophages to different infections is associated with polarization towards the M1 phenotype, while in chronic infection, polarization occurs via the M2 way.[133,134] As a result, several toxins and effector proteins of *Yptb* display activities that may be associated with their ability to induce anti-inflammatory M2-type polarization of macrophages. Thus, YopM and YPM induce the release of IL-10 and IL-4, respectively.[5,28,98] These cytokines belong to the Th2 immune profile.[5,98] The ability of TsTYp to dysregulate the activities of two types of phagocytes in sea cucumbers has been shown in model-based experiments followed by the activation shift in one of them, considered analogous to M2 macrophages.[126] One result of such polarization, as demonstrated in tuberculosis infection, is that M2 macrophages contain more bacteria compared to M1 cells.[134] This suggests that polarization towards the M2-type macrophages promotes the survival and growth of bacteria in macrophages.

Protection from pro-inflammatory substances may be expected to promote the replication of bacteria within a cell and their dissemination inside phagocytes to the liver and other organs. Indeed, as shown in Table 1, YopM and YpkA promoted bacterial dissemination and colonization in the liver.[28,62] Speculatively, CNF_Y could be considered in this series.[92] Regarding TcpYI, it promotes *Yptb* dissemination to the spleen,[21] but its effect on the liver is unknown.

YopE has been shown to promote *Yptb* colonization in lymphoid tissues, but different thresholds of the toxin are observed between different tissues. The effect also depends on the level of its antiphagocytic activity and ROS inhibition.[78] These findings support the idea that inhibition of the inflammatory profile of cells facilitates *Yptb* colonization in organs. However, the effect of YopE on bacterial colonization in the liver has not been studied to date.

Evidence that YopJ-induced apoptosis facilitates *Yptb* dissemination has supported the assumption about the relationship between the type of host cell death and *Yptb* dissemination and colonization in the liver, where apoptosis is the preferred option for bacterial dissemination.[53,54] However, Viboud and *Bliska* obtained opposite results,[20] showing that YopJ-induced apoptosis was followed by slowing down *Yersinia* dissemination. These inconsistencies may be explained by differences in the models used, highlighting the need to consider various factors when assessing the effects of toxins, including their interplay.

Many facts indicate that *Yptb* limits toxicity during long-term infection.[4,120] To maintain a balance between the death of the host cell and saving a niche for survival and spread, *Yptb* uses a panel of toxic proteins, whose complex action provides fine-tuning of the bacterial survival mechanism. The proteins exert their action through different signaling pathways (Fig. 1). This provides reliability of regulation, where damage to one mechanism can be compensated by another. Moreover, the activity of the crucial pathways that determine the type of cell death is controlled simultaneously by several proteins, which can both mutually enhance each other's effects and compete with each other. Thus, the activity of the caspase-1–inflammasome complex is regulated by YopM, YopJ, YopE, and YopT, with only YopM displaying exclusively inhibitory activity.[44,45,60] Thus, considering that YopE can suppress caspase-1 activity, YopM appears to shift a balance between YopE-induced pyroptosis and apoptosis towards the latter. However, YopM also activates caspase-3,[28] and YopJ supports this pro-apoptotic effect through the activation of caspase-8.[1,20]

In turn, YopE, YopT, YpkA, and also CNF_Y are involved in the manipulation of the cytoskeleton, disturbances of which are closely associated with cell death.[66,75,82,85,86] Yops modulate the activity of Rho GTPases in different ways, including by competing between certain proteins (YopE, YopT), and presumably by binding to different Rho GTPases, mainly RhoA, or Rac1 and Rac2. These proteins have a wide range of activities, particularly in regulating the actin cytoskeleton and coordinating cell migration.[74] Additionally, these GTPases stabilize the formation of pores through which Yops are translocated. Therefore, YopE, by inhibiting Rho GTPases, can reduce the entry of other Yops into the cell and thus decrease the possibility of extrusion and replication of *Yptb* in the cell.[75] It may be assumed that YopT and YpkA are also involved in the regulation of pore formation. CNF_Y, which has been shown to increase the translocation of Yops into a cell, can be expected to act in the same mode by regulating pore formation.[94]

YopH, like YopJ, inhibits ROS-dependent signaling by two independent mechanisms, indicating the importance of this pathway and its redundant regulation to increase signaling reliability.[72,120,132] TcpYI is assumed to be involved in the suppression of inflammasome assembly by inhibiting the TIR signaling pathway, which leads to pyroptosis.[135]

TsTYp appears to induce apoptosis via a cAMP-dependent pathway.[22] However, there is a lack of experimental data to elucidate details.

Experiments with staphylococcal enterotoxin superantigen have shown that the activation of T cells is accompanied by apoptosis of antigen-presenting macrophages.[136] Based on these findings, YPM

may be expected to induce apoptosis through T-cell activation mediated by MHC class II molecules. However, in view of some differences between YPM and superantigens of Gram-positive bacteria,[98] this idea also needs to be tested.

The multiplicity of bacterial effector proteins and toxins is apparently associated with their different roles and importance for *Yptb* at different stages of infection and under different environmental conditions. In particular, temperature is a significant factor influencing the expression of plasmid- and chromosome-encoded genes.[83] In addition, there are differences in the mechanisms of the effects of certain toxins on macrophages and neutrophils. Thus, YopJ is known to cause apoptosis in macrophages, whereas neutrophils do not undergo apoptosis in response to YopJ exposure. The apoptosis-inducing effect of YopM may be most strongly manifested on neutrophils in the liver.[31,50,137] Monocytes are also more sensitive to the apoptotic effects of TsTYp than neutrophils.[22] This difference determines the preferential target cells for toxins with different levels of activity at certain stages of infection. It also determines the selectivity of colonization of one or another organ, since they differ in cell composition.[138]

The increase in bacterial resistance to antibiotics observed over recent decades requires novel drugs with new mechanisms of action. A promising approach is the development of drugs targeting the regulation of bacterial survival and dissemination. One such target is the T3SS of *Yptb*, and the search for its inhibitors is already underway.[139,140] However, it should be borne in mind that the pathogenicity of *Yptb* is not limited to the T3SS, and Yops proteins interact both with each other and with proteins encoded by chromosomes, and the effects they cause are specific to different cells of the immune system.

Conclusion

Recent studies have shown that the anti-phagocytic defense and bacterial dissemination in host organs are "fine-tuned" by both plasmid- and chromosome-encoded proteins. Toxins such as YPM, TcpYI, and CNF_Y have been shown to stimulate bacterial dissemination even in the absence of Yops. For efficient bacterial replication and colonization of host organs, phagocyte death by a non-inflammatory type, such as apoptosis, is favored by *Yersinia*. However, apoptosis may be accompanied by the activation of pro-inflammatory mechanisms of antibacterial defense, leading to pro-inflammatory cell death, namely pyroptosis. As a result, bacteria need to maintain a balance between killing cells via pyroptosis or apoptosis. Yops proteins regulate this balance using different host cell signaling pathways, often duplicating the effects of several proteins on crucial molecular components triggering cell death, and also by modulating the activity and/or rate of translocation of some proteins by others. The chromosome-encoded proteins can directly affect the mechanisms of cell death activation, and some of them even appear to influence the outcome of Yops activity, changing the rate of release or translocation of toxins into the cell or interfering with them. The chromosome-encoded proteins released by Far-Eastern strains play an important role in aggravating the course of *Yptb* infection. Some Yops and chromosome-encoded proteins, such as YopM, YPM, and TsTYp, induce metabolic changes in macrophages, indicating a shift towards an anti-inflammatory M2 phenotype. This provides new insights into the mechanisms of anti-phagocytic effects of *Yptb* toxins.

Meanwhile, a number of studies have shown that the effect of Yops on the colonization of different organs is specific, and the colonization of the liver differs from that of the spleen. Moreover, individual Yops may have different effects on bacterial colonization of the liver. However, the liver has often been overlooked in studies of lymphoid organ colonization. Further studies are required to elucidate the mechanisms of the effect of certain Yop proteins and chromosome-encoded toxins on *Yptb* colonization in the liver. This may provide novel approaches for the development of new types of drugs to target blocking the replication and dissemination of bacteria for the treatment of *Yersinia*-induced liver injury and FESLF.

Acknowledgments

The author thanks Dr. N.F. Timchenko and Dr. I.Yu. Dolmatov for valuable advice in preparing the manuscript.

Funding

This work was implemented under the state assignment of the Ministry of Science and Higher Education of the Russian Federation (No. 124022100077-0).

Conflict of interest

The author declares no conflict of interests related to this publication.

Author contributions

LSD is the sole author of the manuscript.

References

[1] Bi Y, Wang X, Han Y, Guo Z, Yang R. *Yersinia pestis* versus *Yersinia pseudotuberculosis*: effects on host macrophages. Scand J Immunol 2012;76(6):541–551. doi:10.1111/j.1365-3083.2012.02767.x, PMID: 22882408.

[2] Somova LM, Shubin FN, Plekhova NG, Drobot EI, Lyapun IN. Inflammation induced by different plasmid types of russian *Yersinia pseudotuberculosis* strains. Russ J Infect Immun 2019;9:369–374. doi:10.15789/2220-7619-2019-2-369-374.

[3] Amphlett A. Far East scarlet-like fever: a review of the epidemiology, symptomatology, and role of superantigenic toxin: *Yersinia pseudotuberculosis*-derived mitogen A scarlet-li. Open Forum Infect Dis 2016;3(1):ofv202. doi:10.1093/ofid/ofv202, PMID:26819960.

[4] Somova LM, Antonenko FF, Timchenko NF, Lyapun IN. Far Eastern scarlet-like fever is a special clinical and epidemic manifestation of Yersinia pseudotuberculosis infection in Russia. Pathogens 2020;9(6):436. doi:10.3390/pathogens9060436, PMID:32498317.

[5] Goubard A, Loïez C, Abe J, Fichel C, Herwegh S, Faveeuw C, *et al*. Superantigenic *Yersinia pseudotuberculosis* induces the expression of granzymes and perforin by CD4+ T cells. Infect Immun 2015;83(5):2053–2064. doi:10.1128/IAI.02339-14, PMID:25754199.

[6] He YX, Ye CL, Zhang P, Li Q, Park CG, Yang K, *et al*. Yersinia pseudotuberculosis exploits CD209 receptors for promoting host dissemination and infection exploits CD209 receptors for promoting host dissemination and infection. Infect Immun 2019;87(1):e00654–18. doi:10.1128/IAI.00654-18, PMID:30348825.

[7] Lee YJ, Kim J, Jeon JH, Seok H, Choi WS, Chang EA, *et al*. Extraintestinal manifestation of Extraintestinal manifestation of *Yersinia pseudotuberculosis* bacteremia as acute hepatitis: case report and review of the literature bacteremia as acute hepatitis: case report and review of the literature. Pathogens 2021;10(10):1255. doi:10.3390/pathogens10101255, PMID:34684204.

[8] Dudina M, Søgaard KK, Deleuran T, Joensen KG, Frøkjær JB, Nielsen HL. A rare Danish case of Extraintestinal manifestation of *Yersinia*

pseudotuberculosis bacteremia as acute hepatitis: case report and review of the literature Extraintestinal manifestation of *Yersinia pseudotuberculosis* bacteremia as acute hepatitis: case report and review of the literature. Clin Case Rep 2022;10(10):e6464. doi:10.1002/ccr3.6464, PMID:36276910.

[9] Thompson D. Successful treatment of *Yersinia pseudotuberculosis* hepatitis in a cat presenting with neurological abnormalities. JFMS Open Rep 2019;5(1):2055116919853644. doi:10.1177/2055116919853644, PMID:31210959.

[10] Ceccolini ME, Macgregor SK, Spiro S, Irving J, Hedley J, Williams J, *et al*. *Yersinia pseudotuberculosis* infections in primates, artiodactyls, and birds within a zoological facility in the United Kingdom. J Zoo Wildl Med 2020;51(3):527–538. doi:10.1638/2019-0205, PMID:33480528.

[11] Womble M, Cabot ML, Harrison T, Watanabe TTN. Outbreak in African lions of Yersinia pseudotuberculosis infection, with aberrant bacterial morphology. J Vet Diagn Invest 2022;34(2):334–338. doi:10.1177/10406387211072822, PMID:35037547.

[12] Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol 2016;13(3):267–276. doi:10.1038/cmi.2016.3, PMID:27063467.

[13] Somova LM, Andryukov BG, Lyapun IN, Drobot EI, Ryazanova OS, Matosova EV, *et al*. Pathomorphology of experimental infection caused by dormant *Yersinia pseudotuberculosis* strains. Russian Journal of Infection and Immunity 2022;12(1):69–77. doi:10.15789/2220-7619-POE-1674.

[14] Böhme K, Heroven AK, Lobedann S, Guo Y, Stolle AS, Dersch P. The small protein YmoA controls the Csr system and adjusts expression of virulence-relevant traits of *Yersinia pseudotuberculosis*. Front Microbiol 2021;12:706934. doi:10.3389/fmicb.2021.706934, PMID:34413840.

[15] Fasciano AC, Dasanayake GS, Estes MK, Zachos NC, Breault DT, Isberg RR, *et al*. *Yersinia pseudotuberculosis* YopE prevents uptake by M cells and instigates M cell extrusion in human ileal enteroid-derived monolayers. Gut Microbes 2021;13(1):1988390. doi:10.1080/19490976.2021.1988390, PMID:34793276.

[16] Zhang X, Liu H, Hashimoto K, Yuan S, Zhang J. The gut-liver axis in sepsis: interaction mechanisms and therapeutic potential. Crit Care 2022;26(1):213. doi:10.1186/s13054-022-04090-1, PMID:35831877.

[17] Brown JA Jr, West WL, Banks WM, Marshall JD Jr. Some characteristics of a heat-labile toxin from *Pasteurella pseudotuberculosis*. J Infect Dis 1969;119(3):229–236. doi:10.1093/infdis/119.3.229, PMID:4976343.

[18] Tsybulsky AV, Timchenko NF, Kostetsky EY, Vorobieva NS. A study of immunogenic and protective properties of the heat-stable lethal toxin of *Yersinia pseudotuberculosis* and its effects upon hematological and blood cytokine parameters of laboratory mice. Medical Immunology (Russia) 2014;16(3):227–236. doi:10.15789/1563-0625-2014-3-227-236.

[19] Zhou Y, Zhou J, Ji Y, Li L, Tan Y, Tian G, *et al*. Bioluminescent tracing of a *Yersinia pestis* pCD1(+)-mutant and *Yersinia pseudotuberculosis* in subcutaneously infected mice. Microbes Infect 2018;20(3):166–175. doi:10.1016/j.micinf.2017.11.005, PMID:29180033.

[20] Viboud GI, Bliska JB. *Yersinia* outer proteins: role in modulation of host cell signaling responses and pathogenesis. Annu Rev Microbiol 2005;59:69–89. doi:10.1146/annurev.micro.59.030804.121320, PMID:15847602.

[21] Nörenberg D, Wieser A, Magistro G, Hoffmann C, Meyer C, Messerer M, *et al*. Molecular analysis of a novel Toll/interleukin-1 receptor (TIR)-domain containing virulence protein of *Y. pseudotuberculosis* among Far East scarlet-like fever serotype I strains. Int J Med Microbiol 2013;303(8):583–594. doi:10.1016/j.ijmm.2013.08.002, PMID:24018301.

[22] Timchenko NF, Nedashkovskaya EP, Dolmatova LS, Somova-Isachkova LM. *Yersinia pseudotuberculosis* perifocal perifocal toxins. Primpoligrafcombinat Publ 2004.

[23] Durand EA, Maldonado-Arocho FJ, Castillo C, Walsh RL, Mecsas J. The presence of professional phagocytes dictates the number of host cells targeted for Yop translocation during infection. Cell Microbiol 2010;12(8):1064–1082. doi:10.1111/j.1462-5822.2010.01451.x, PMID:20148898.

[24] Liu L, Huang S, Fei K, Zhou W, Chen S, Hu Y. Characterization of the binding motif for the T3SS master regulator LcrF in *Yersinia pseudotuberculosis*. FEMS Microbiol Lett 2021;368(6):fnab031. doi:10.1093/femsle/fnab031, PMID:33705525.

[25] Schwiesow L, Lam H, Dersch P, Auerbuch V. *Yersinia* Type III Secretion System Master Regulator LcrF. J Bacteriol 2015;198(4):604–614. doi:10.1128/JB.00686-15, PMID:26644429.

[26] Pienkoß S, Javadi S, Chaoprasid P, Holler M, Roßmanith J, Dersch P, *et al*. RNA thermometer-coordinated assembly of the *Yersinia* injectisome. J Mol Biol 2022;434(18):167667. doi:10.1016/j.jmb.2022.167667, PMID:35667470.

[27] Evdokimov AG, Anderson DE, Routzahn KM, Waugh DS. Unusual molecular architecture of the *Yersinia pestis* cytotoxin YopM: a leucine-rich repeat protein with the shortest repeating unit. J Mol Biol 2001;312(4):807–821. doi:10.1006/jmbi.2001.4973, PMID:11575934.

[28] Höfling S, Grabowski B, Norkowski S, Schmidt MA, Rüter C. Current activities of the *Yersinia* effector protein YopM. Int J Med Microbiol 2015;305(3):424–432. doi:10.1016/j.ijmm.2015.03.009, PMID:25865799.

[29] McPhee JB, Mena P, Zhang Y, Bliska JB. Interleukin-10 induction is an important virulence function of the *Yersinia pseudotuberculosis* type III effector YopM. Infect Immun 2012;80(7):2519–2527. doi:10.1128/IAI.06364-11, PMID:22547545.

[30] Berneking L, Bekere I, Rob S, Schnapp M, Huang J, Ruckdeschel K, *et al*. A bacterial effector protein promotes nuclear translocation of Stat3 to induce IL-10. Eur J Cell Biol 2023;102(4):151364. doi:10.1016/j.ejcb.2023.151364, PMID:37806297.

[31] Ye Z, Kerschen EJ, Cohen DA, Kaplan AM, van Rooijen N, Straley SC. Gr1+ cells control growth of YopM-negative *Yersinia pestis* during systemic plague. Infect Immun 2009;77(9):3791–3806. doi:10.1128/IAI.00284-09, PMID:19581396.

[32] Ye Z, Uittenbogaard AM, Cohen DA, Kaplan AM, Ambati J, Straley SC. Distinct CCR2(+) Gr1(+) cells control growth of the *Yersinia pestis* ΔyopM mutant in liver and spleen during systemic plague. Infect Immun 2011;79(2):674–687. doi:10.1128/IAI.00808-10, PMID:21149593.

[33] Ye Z, Gorman AA, Uittenbogaard AM, Myers-Morales T, Kaplan AM, Cohen DA, *et al*. Caspase-3 mediates the pathogenic effect of *Yersinia pestis* YopM in liver of C57BL/6 mice and contributes to YopM's function in spleen. PLoS One 2014;9(11):e110956. doi:10.1371/journal.pone.0110956, PMID:25372388.

[34] Obeng E. Apoptosis (programmed cell death) and its signals - A review. Braz J Biol 2021;81(4):1133–1143. doi:10.1590/1519-6984.228437, PMID:33111928.

[35] Berneking L, Schnapp M, Rumm A, Trasak C, Ruckdeschel K, Alawi M, *et al*. Immunosuppressive *Yersinia* effector YopM binds DEAD box helicase DDX3 to control ribosomal S6 kinase in the nucleus of host cells. PLoS Pathog 2016;12(6):e1005660. doi:10.1371/journal.ppat.1005660, PMID:27300509.

[36] Patel H, Li J, Herrero A, Kroboth J, Byron A, Kriegsheim AV, *et al*. Novel roles of PRK1 and PRK2 in cilia and cancer biology. Sci Rep 2020;10(1):3902. doi:10.1038/s41598-020-60604-3, PMID:32127582.

[37] Bourguignon LY, Gilad E, Peyrollier K, Brightman A, Swanson RA. Hyaluronan-CD44 interaction stimulates Rac1 signaling and PKN gamma kinase activation leading to cytoskeleton function and cell migration in astrocytes. J Neurochem 2007;101(4):1002–1017. doi:10.1111/j.1471-4159.2007.04485.x, PMID:17403031.

[38] Sophocleous G, Owen D, Mott HR. The structure and function of protein kinase C-related kinases (PRKs). Biochem Soc Trans 2021;49(1):217–235. doi:10.1042/BST20200466, PMID:33522581.

[39] Takahashi M, Mukai H, Toshimori M, Miyamoto M, Ono Y. Proteolytic activation of PKN by caspase-3 or related protease during apoptosis. Proc Natl Acad Sci U S A 1998;95(20):11566–11571. doi:10.1073/pnas.95.20.11566, PMID:9751706.

[40] Hentschke M, Berneking L, Belmar Campos C, Buck F, Ruckdeschel K, Aepfelbacher M. *Yersinia* virulence factor YopM induces sustained RSK activation by interfering with dephosphorylation. PLoS One 2010;5(10):e13165. doi:10.1371/journal.pone.0013165, PMID:20957203.

[41] McDonald C, Vacratsis PO, Bliska JB, Dixon JE. The *Yersinia* viru-

lence factor YopM forms a novel protein complex with two cellular kinases. J Biol Chem 2003;278(20):18514–18523. doi:10.1074/jbc.M301226200, PMID:12626518.
[42] Samson SC, Khan AM, Mendoza MC. ERK signaling for cell migration and invasion. Front Mol Biosci 2022;9:998475. doi:10.3389/fmolb.2022.998475, PMID:36262472.
[43] Park YH, Wood G, Kastner DL, Chae JJ. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. Nat Immunol 2016;17(8):914–921. doi:10.1038/ni.3457, PMID:27270401.
[44] Medici NP, Rashid M, Bliska JB. Characterization of pyrin dephosphorylation and inflammasome activation in macrophages as triggered by the *Yersinia* effectors YopE and YopT. Infect Immun 2019;87(3):e00822–18. doi:10.1128/IAI.00822-18, PMID:30602502.
[45] LaRock CN, Cookson BT. The *Yersinia* virulence effector YopM binds caspase-1 to arrest inflammasome assembly and processing. Cell Host Microbe 2012;12(6):799–805. doi:10.1016/j.chom.2012.10.020, PMID:23245324.
[46] Sollberger G, Strittmatter GE, Garstkiewicz M, Sand J, Beer HD. Caspase-1: the inflammasome and beyond. Innate Immun 2014;20(2):115–125. doi:10.1177/1753425913484374, PMID:23676582.
[47] Wang C, Ma C, Gong L, Guo Y, Fu K, Zhang Y, *et al*. Macrophage polarization and its role in liver disease. Front Immunol 2021;12:803037. doi:10.3389/fimmu.2021.803037, PMID:34970275.
[48] Dolmatova LS, Dolmatov IY. Tumor-associated macrophages as potential targets for anti-cancer activity of marine invertebrate-derived compounds. Curr Pharm Des 2021;27(28):3139–3160. doi:10.2174/1381612827666210319125652, PMID:33745429.
[49] Thiriot JD, Martinez-Martinez YB, Endsley JJ, Torres AG. Hacking the host: exploitation of macrophage polarization by intracellular bacterial pathogens. Pathog Dis 2020;78(1):ftaa009. doi:10.1093/femspd/ftaa009, PMID:32068828.
[50] Dolmatova LS, Dolmatov IY. Different macrophage type triggering as target of the action of biologically active substances from marine invertebrates. Mar Drugs 2020;18(1):37. doi:10.3390/md18010037, PMID:31906518.
[51] Tseneva GY, Solodovnikova NY, Voskresenskaya EA. Molecular aspects of *Yersinia* virulence. Chemical microbiology and anti-microbe therapy 2002;3:248–266.
[52] Sheppe AEF, Santelices J, Czyz DM, Edelmann MJ. *Yersinia pseudotuberculosis* YopJ limits macrophage response by downregulating COX-2-mediated biosynthesis of PGE2 in a MAPK/ERK-dependent manner. Microbiol Spectr 2021;9(1):e0049621. doi:10.1128/Spectrum.00496-21, PMID:34319170.
[53] Monack DM, Mecsas J, Bouley D, Falkow S. Yersinia-induced apoptosis in vivo aids in the establishment of a systemic infection of mice. J Exp Med 1998;188(11):2127–2137. doi:10.1084/jem.188.11.2127, PMID:9841926.
[54] Mills SD, Boland A, Sory MP, van der Smissen P, Kerbourch C, Finlay BB, *et al*. Yersinia enterocolitica induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. Proc Natl Acad Sci U S A 1997;94(23):12638–12643. doi:10.1073/pnas.94.23.12638, PMID:9356502.
[55] Weng D, Marty-Roix R, Ganesan S, Proulx MK, Vladimer GI, Kaiser WJ, *et al*. Caspase-8 and RIP kinases regulate bacteria-induced innate immune responses and cell death. Proc Natl Acad Sci U S A 2014;111(20):7391–7396. doi:10.1073/pnas.1403477111, PMID:24799678.
[56] Bliska JB, Brodsky IE, Mecsas J. Role of the *Yersinia pseudotuberculosis* virulence plasmid in pathogen-phagocyte interactions in mesenteric lymph nodes. EcoSal Plus 2021;9(2):eESP00142021. doi:10.1128/ecosalplus.ESP-0014-2021, PMID:34910573.
[57] Palace SG, Proulx MK, Szabady RL, Goguen JD. Gain-of-function analysis reveals important virulence roles for the *Yersinia pestis* type III secretion system effectors YopJ, YopT, and YpkA. Infect Immun 2018;86(9):e00318–18. doi:10.1128/IAI.00318-18, PMID:29891548.
[58] López-Pérez W, Sai K, Sakamachi Y, Parsons C, Kathariou S, Ninomiya-Tsuji J. TAK1 inhibition elicits mitochondrial ROS to block intracellular bacterial colonization. Proc Natl Acad Sci U S A 2021;118(25):e2023647118. doi:10.1073/pnas.2023647118, PMID:34161265.
[59] Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 2014;15(2):81–94. doi:10.1038/nrm3735, PMID:24401948.
[60] Chen KW, Demarco B, Ramos S, Heilig R, Goris M, Grayczyk JP, *et al*. RIPK1 activates distinct gasdermins in macrophages and neutrophils upon pathogen blockade of innate immune signaling. Proc Natl Acad Sci U S A 2021;118(28):e2101189118. doi:10.1073/pnas.2101189118, PMID:34260403.
[61] Orning P, Weng D, Starheim K, Ratner D, Best Z, Lee B, *et al*. Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. Science 2018;362(6418):1064–1069. doi:10.1126/science.aau2818, PMID:30361383.
[62] Bergsbaken T, Cookson BT. Macrophage activation redirects *Yersinia*-infected host cell death from apoptosis to caspase-1-dependent pyroptosis. PLoS Pathog 2007;3(11):e161. doi:10.1371/journal.ppat.0030161, PMID:17983266.
[63] Navarro L, Koller A, Nordfelth R, Wolf-Watz H, Taylor S, Dixon JE. Identification of a molecular target for the *Yersinia* protein kinase A. Mol Cell 2007;26(4):465–477. doi:10.1016/j.molcel.2007.04.025, PMID:17531806.
[64] Najdenski H, Vesselinova A, Golkocheva E, Garbom S, Wolf-Watz H. Experimental infections with wild and mutant *Yersinia pseudotuberculosis* strains in rabbits. J Vet Med B Infect Dis Vet Public Health 2003;50(6):280–288. doi:10.1046/j.1439-0450.2003.00679.x, PMID:14628999.
[65] Lee WL, Singaravelu P, Wee S, Xue B, Ang KC, Gunaratne J, *et al*. Mechanisms of *Yersinia* YopO kinase substrate specificity. Sci Rep 2017;7:39998. doi:10.1038/srep39998, PMID:28051168.
[66] Singaravelu P, Lee WL, Wee S, Ghoshdastider U, Ding K, Gunaratne J, *et al*. *Yersinia* effector protein (YopO)-mediated phosphorylation of host gelsolin causes calcium-independent activation leading to disruption of actin dynamics. J Biol Chem 2017;292(19):8092–8100. doi:10.1074/jbc.M116.757971, PMID:28280241.
[67] Pha K, Navarro L. Yersinia type III effectors perturb host innate immune responses. World J Biol Chem 2016;7(1):1–13. doi:10.4331/wjbc.v7.i1.1, PMID:26981193.
[68] Blonska M, Lin X. NF-κB signaling pathways regulated by CARMA family of scaffold proteins. Cell Res 2011;21(1):55–70. doi:10.1038/cr.2010.182, PMID:21187856.
[69] Chung LK, Bliska JB. *Yersinia* versus host immunity: how a pathogen evades or triggers a protective response. Curr Opin Microbiol 2016;29:56–62. doi:10.1016/j.mib.2015.11.001, PMID:26638030.
[70] McCormick LE, Suarez C, Herring LE, Cannon KS, Kovar DR, Brown NG, *et al*. Multi-monoubiquitylation controls VASP-mediated actin dynamics. J Cell Sci 2024;137(2):jcs261527. doi:10.1242/jcs.261527, PMID:38277158.
[71] Logsdon LK, Mecsas J. Requirement of the *Yersinia pseudotuberculosis* effectors YopH and YopE in colonization and persistence in intestinal and lymph tissues. Infect Immun 2003;71(8):4595–4607. doi:10.1128/IAI.71.8.4595-4607.2003, PMID:12874339.
[72] Shaban L, Nguyen GT, Mecsas-Faxon BD, Swanson KD, Tan S, Mecsas J. *Yersinia pseudotuberculosis* YopH targets SKAP2-dependent and independent signaling pathways to block neutrophil antimicrobial mechanisms during infection. PLoS Pathog 2020;16(5):e1008576. doi:10.1371/journal.ppat.1008576, PMID:32392230.
[73] Djavaheri-Mergny M, Javelaud D, Wietzerbin J, Besançon F. NF-kappaB activation prevents apoptotic oxidative stress via an increase of both thioredoxin and MnSOD levels in TNFalpha-treated Ewing sarcoma cells. FEBS Lett 2004;578(1-2):111–115. doi:10.1016/j.febslet.2004.10.082, PMID:15581626.
[74] Wang X, Parashar K, Sitaram A, Bliska JB. The GAP activity of type III effector YopE triggers killing of *Yersinia* in macrophages. PLoS Pathog 2014;10(8):e1004346. doi:10.1371/journal.ppat.1004346, PMID:25165815.
[75] Gaus K, Hentschke M, Czymmeck N, Novikova L, Trülzsch K, Valentin-Weigand P, *et al*. Destabilization of YopE by the ubiquitin-proteasome pathway fine-tunes Yop delivery into host cells and facilitates systemic spread of *Yersinia enterocolitica* in host lymphoid tissue. Infect Immun 2011;79(3):1166–1175. doi:10.1128/IAI.00694-10, PMID:21149597.
[76] Forsberg A, Rosqvist R. In vivo expression of virulence genes of *Yersinia pseudotuberculosis*. Infect Agents Dis 1993;2(4):275–278.

PMID:8173809.
[77] Schoberle TJ, Chung LK, McPhee JB, Bogin B, Bliska JB. Uncovering an important role for YopJ in the inhibition of caspase-1 in activated macrophages and promoting *Yersinia pseudotuberculosis* virulence. Infect Immun 2016;84(4):1062–1072. doi:10.1128/IAI.00843-15, PMID:26810037.
[78] Schotte P, Denecker G, Van Den Broeke A, Vandenabeele P, Cornelis GR, Beyaert R. Targeting Rac1 by the *Yersinia* effector protein YopE inhibits caspase-1-mediated maturation and release of interleukin-1beta. J Biol Chem 2004;279(24):25134–25142. doi:10.1074/jbc.M401245200, PMID:15060067.
[79] Songsungthong W, Higgins MC, Rolán HG, Murphy JL, Mecsas J. ROS-inhibitory activity of YopE is required for full virulence of *Yersinia* in mice. Cell Microbiol 2010;12(7):988–1001. doi:10.1111/j.1462-5822.2010.01448.x, PMID:20148901.
[80] Ramachandran RA, Lupfer C, Zaki H. The inflammasome: regulation of nitric oxide and antimicrobial host defence. Adv Microb Physiol 2018;72:65–115. doi:10.1016/bs.ampbs.2018.01.004, PMID:29778217.
[81] Aepfelbacher M, Trasak C, Wilharm G, Wiedemann A, Trulzsch K, Krauss K, *et al*. Characterization of YopT effects on Rho GTPases in *Yersinia enterocolitica*-infected cells. J Biol Chem 2003;278(35):33217–33223. doi:10.1074/jbc.M303349200, PMID:12791693.
[82] Zumbihl R, Aepfelbacher M, Andor A, Jacobi CA, Ruckdeschel K, Rouot B, *et al*. The cytotoxin YopT of *Yersinia enterocolitica* induces modification and cellular redistribution of the small GTP-binding protein RhoA. J Biol Chem 1999;274(41):29289–29293. doi:10.1074/jbc.274.41.29289, PMID:10506187.
[83] Houppert AS, Kwiatkowski E, Glass EM, DeBord KL, Merritt PM, Schneewind O, *et al*. Identification of chromosomal genes in *Yersinia pestis* that influence type III secretion and delivery of Yops into target cells. PLoS One 2012;7(3):e34039. doi:10.1371/journal.pone.0034039, PMID:22479512.
[84] Schesser Bartra S, Lorica C, Qian L, Gong X, Bahnan W, Barreras H Jr, *et al*. Chromosomally-encoded *Yersinia pestis* type III secretion effector proteins promote infection in cells and in mice. Front Cell Infect Microbiol 2019;9:23. doi:10.3389/fcimb.2019.00023, PMID:30854334.
[85] Lockman HA, Gillespie RA, Baker BD, Shakhnovich E. *Yersinia pseudotuberculosis* produces a cytotoxic necrotizing factor. Infect Immun 2002;70(5):2708–2714. doi:10.1128/IAI.70.5.2708-2714.2002, PMID:11953417.
[86] Schweer J, Kulkarni D, Kochut A, Pezoldt J, Pisano F, Pils MC, *et al*. The cytotoxic necrotizing factor of *Yersinia pseudotuberculosis* (CNFY) enhances inflammation and Yop delivery during infection by activation of Rho GTPases. PLoS Pathog 2013;9(11):e1003746. doi:10.1371/journal.ppat.1003746, PMID:24244167.
[87] Falbo V, Pace T, Picci L, Pizzi E, Caprioli A. Isolation and nucleotide sequence of the gene encoding cytotoxic necrotizing factor 1 of *Escherichia coli*. Infect Immun 1993;61(11):4909–4914. doi:10.1128/iai.61.11.4909-4914.1993, PMID:8406895.
[88] Twittenhoff C, Heroven AK, Mühlen S, Dersch P, Narberhaus F. An RNA thermometer dictates production of a secreted bacterial toxin. PLoS Pathog 2020;16(1):e1008184. doi:10.1371/journal.ppat.1008184, PMID:31951643.
[89] Hofman P, Le Negrate G, Mograbi B, Hofman V, Brest P, Alliana-Schmid A, *et al*. *Escherichia coli* cytotoxic necrotizing factor-1 (CNF-1) increases the adherence to epithelia and the oxidative burst of human polymorphonuclear leukocytes but decreases bacteria phagocytosis. J Leukoc Biol 2000;68(4):522–528. PMID:11037974.
[90] Chaoprasid P, Dersch P. The cytotoxic necrotizing factors (CNFs)-a family of Rho GTPase-activating bacterial exotoxins. Toxins (Basel) 2021;13(12):901. doi:10.3390/toxins13120901, PMID:34941738.
[91] Heine W, Beckstette M, Heroven AK, Thiemann S, Heise U, Nuss AM, *et al*. Loss of CNFY toxin-induced inflammation drives *Yersinia pseudotuberculosis* into persistency. PloS Pathog 2018;14(2):e1006858. doi:10.1371/journal.ppat.1006858, PMID:29390040.
[92] Munro P, Flatau G, Doye A, Boyer L, Oregioni O, Mege JL, *et al*. Activation and proteasomal degradation of rho GTPases by cytotoxic necrotizing factor-1 elicit a controlled inflammatory response. J Biol Chem 2004;279(34):35849–35857. doi:10.1074/jbc.M401580200, PMID:15152002.
[93] Augspach A, List JH, Wolf P, Bielek H, Schwan C, Elsässer-Beile U, *et al*. Activation of RhoA,B,C by *Yersinia* Cytotoxic Necrotizing Factor (CNFy) induces apoptosis in LNCaP prostate cancer cells. Toxins (Basel) 2013;5(11):2241–2257. doi:10.3390/toxins5112241, PMID:24284827.
[94] Timchenko NF, Psareva EK, Ermolaeva SA. The cytotoxic necrotizing factor *Yersinia pseudotuberculosis*. Molecular Genetics, Microbiology and Virology 2019;37(4):158–164. doi:10.17116/molgen201937041158.
[95] Somova LM, Plekhova NG, Drobot EI, Nedashkovskaya EP, Timchenko NF. Pathomorphological changes in case of experimental toxemia caused by thermolabile toxin of *Yersinia pseudotuberculosis*. Pacific Med J 2010;3:67–72.
[96] Timchenko NF, Popov AF, Andryukov BG. Current issues of microbiology, clinical picture and diagnosis of Far East scarlet-like fever caused by *Yersinia pseudotuberculosis*. Health Medical Ecology Science 2015;2(60):94–100.
[97] Lyapun IN, Drobot EI, Somova LM, Psareva EK, Timchenko NF. Effect of *Yersinia pseudotuberculosis* toxins on the reactivity of innate immunity cells. Pacific Med J 2018;4:53–56. doi:10.17238/PmJ1609-1175.2018.4.53-56.
[98] Chesnokova MV, Klimov VT, Maramovich AS. Genotyping of *Yersinia pseudotuberculosis* isolated in Siberia and Far East. Zh Mikrobiol Epidemiol Immunobiol 2006;6:20–25. PMID:17163133.
[99] Carnoy C, Müller-Alouf H, Desreumaux P, Mullet C, Grangette C, Simonet M. The superantigenic toxin of *Yersinia pseudotuberculosis*: a novel virulence factor? Int J Med Microbiol 2000;290(4-5):477–482. doi:10.1016/S1438-4221(00)80069-7, PMID:11111929.
[100] Carniel E. The *Yersinia* high-pathogenicity island: an iron-uptake island. Microbes Infect 2001;3(7):561–569. doi:10.1016/s1286-4579(01)01412-5, PMID:11418330.
[101] Fukushima H, Matsuda Y, Seki R, Tsubokura M, Takeda N, Shubin FN, *et al*. Geographical heterogeneity between Far Eastern and Western countries in prevalence of the virulence plasmid, the superantigen *Yersinia pseudotuberculosis*-derived mitogen, and the high-pathogenicity island among *Yersinia pseudotuberculosis* strains. J Clin Microbiol 2001;39(10):3541–3547. doi:10.1128/JCM.39.10.3541-3547.2001, PMID:11574570.
[102] Abe J, Onimaru M, Matsumoto S, Noma S, Baba K, Ito Y, *et al*. Clinical role for a superantigen in *Yersinia pseudotuberculosis* infection. J Clin Invest 1997;99(8):1823–1830. doi:10.1172/JCI119349, PMID:9109426.
[103] Thal E. Untersuchungen uber *Pasteurella pseudotuberculosis* unter besondrer Berucksichtigung ihres immunologischen Verhalten. Nord Med 1954;6:829–832.
[104] Nedashkovskaya EP, Timchenko NF, Besednov AL, Vertiev YV. Heat-stable toxin of *Yersinia pseudotuberculosis*. J Microbiol (Zh Mikrobiol Epidemiol Immunobiol) 1995;4:5–9.
[105] Jain R, Tuteja U, Batra HV. Characterization and utilization of monoclonal antibodies reactive to *Yersinia pseudotuberculosis*. Southeast Asian J Trop Med Public Health 2003;34(4):839–844. PMID:15115097.
[106] Raev MB, Bochkova MS, Khramtsov PV, Timganova VP. Immunochromatographic system for diagnostics of pseudotuberculosis. Bull Exp Biol Med 2015;158(6):833–835. doi:10.1007/s10517-015-2873-1, PMID:25894787.
[107] Dolmatova LS, Zaika OA, Nedashkovskaya EP, Timchenko NF. Studying mechanisms of apoptosis-modulating activity of thermostable toxin *Yersinia pseudotuberculosis* and corrective effect of Far-Eastern holothurians-derived extract in rat neutrophils in vitro. Pacific Med J 2010;3:76–80.
[108] Jeong CH, Joo SH. Downregulation of reactive oxygen species in apoptosis. J Cancer Prev 2016;21(1):13–20. doi:10.15430/JCP.2016.21.1.13, PMID:27051644.
[109] Homa J, Stalmach M, Wilczek G, Kolaczkowska E. Effective activation of antioxidant system by immune-relevant factors reversely correlates with apoptosis of *Eisenia andrei* coelomocytes. J Comp Physiol B 2016;186(4):417–430. doi:10.1007/s00360-016-0973-5, PMID:26922789.
[110] Heine WA. The influence of the cytotoxic necrotizing factor toxin of *Yersinia pseudotuberculosis* on pathogenesis: Doctoral thesis, Life Sciences. Braunschweig: Technical University of Carolo-Wilhelmina

[Dissertation]. Braunschweig: Technical University of Carolo-Wilhelmina; 2017. doi:10.24355/dbbs.084-201707060942.
[111] Lambert B, Aublin JL, Champion S, Haye B, Jacquemin C. Comparative effects of cholera and *Bordetella pertussis* toxins on cyclic AMP and GTP levels and on lipolysis in rat adipocytes incubated in vitro. FEBS Lett 1985;181(2):390–396. doi:10.1016/0014-5793(85)80299-4, PMID:2982667.
[112] Calabrese EJ. Biphasic dose responses in biology, toxicology and medicine: accounting for their generalizability and quantitative features. Environ Pollut 2013;182:452–460. doi:10.1016/j.envpol.2013.07.046, PMID:23992683.
[113] Wang Z, Wei X, Ji C, Yu W, Song C, Wang C. PGE2 inhibits neutrophil phagocytosis through the EP2R-cAMP-PTEN pathway. Immun Inflamm Dis 2022;10(7):e662. doi:10.1002/iid3.662, PMID:35759236.
[114] Parkkonen J, Hasala H, Moilanen E, Giembycz MA, Kankaanranta H. Phosphodiesterase 4 inhibitors delay human eosinophil and neutrophil apoptosis in the absence and presence of salbutamol. Pulm Pharmacol Ther 2008;21(3):499–506. doi:10.1016/j.pupt.2007.11.003, PMID:18282775.
[115] Green ER, Clark S, Crimmins GT, Mack M, Kumamoto CA, Mecsas J. Fis is essential for *Yersinia pseudotuberculosis* virulence and protects against reactive oxygen species produced by phagocytic cells during infection. PLoS Pathog 2016;12(9):e1005898. doi:10.1371/journal.ppat.1005898, PMID:27689357.
[116] Zaccolo M, Zerio A, Lobo MJ. Subcellular organization of the cAMP signaling pathway. Pharmacol Rev 2021;73(1):278–309. doi:10.1124/pharmrev.120.000086, PMID:33334857.
[117] Ren C, Zhou P, Zhang M, Yu Z, Zhang X, Tombran-Tink J, *et al*. Molecular mechanisms of oxidative stress relief by CAPE in ARPE-19 cells. Int J Mol Sci 2023;24(4):3565. doi:10.3390/ijms24043565, PMID:36834980.
[118] Bouchez C, Devin A. Mitochondrial biogenesis and mitochondrial reactive oxygen species (ROS): a complex relationship regulated by the cAMP/PKA signaling pathway. Cells 2019;8(4):287. doi:10.3390/cells8040287, PMID:30934711.
[119] Dey B, Bishai WR. Crosstalk between *Mycobacterium tuberculosis* and the host cell. Semin Immunol 2014;26(6):486–496. doi:10.1016/j.smim.2014.09.002, PMID:25303934.
[120] Brodsky IE, Palm NW, Sadanand S, Ryndak MB, Sutterwala FS, Flavell RA, *et al*. A *Yersinia* effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system. Cell Host Microbe 2010;7(5):376–387. doi:10.1016/j.chom.2010.04.009, PMID:20478539.
[121] Miao EA, Leaf IA, Treuting PM, Mao DP, Dors M, Sarkar A, *et al*. Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nat Immunol 2010;11(12):1136–1142. doi:10.1038/ni.1960, PMID:21057511.
[122] Cochrane R, Clark RB, Maulik N, Cordis G, Cone RE. cAMP-mediated suppression of a Th1 clone associated with an alteration of the intracellular redox environment. Cell Mol Biol (Noisy-le-grand) 2003;49(2):301–306. PMID:12892052.
[123] Tsubokura Tsubokura M, Otsuki K, Sato K, Tanaka M, Hongo T, Fukushima H, *et al*. Special features of distribution of *Yersinia pseudotuberculosis* in Japan. J Clin Microbiol 1989;27(4):790–791. doi:10.1128/jcm.27.4.790-791.1989, PMID:2656752.
[124] Dolmatova LS, Smolina TP. Morphofunctional features of two types of phagocytes in the holothurian *Eupentacta fraudatrix* (Djakonov et Baranova, 1958). J Evol Biochem Physiol 2022;58(4):955–970. doi:10.1134/S0022093022040020.
[125] Dolmatova LS, Ulanova OA, Timchenko NF. *Yersinia pseudotuberculosis* thermostable toxin dysregulates the functional activity of two types of phagocytes in the holothurian *Eupentacta fraudatrix*. Biol Bull (Moscow) 2019;46(2):117–127. doi:10.1134/S1062359019020043.
[126] Rana RR, Zhang M, Spear AM, Atkins HS, Byrne B. Bacterial TIR-containing proteins and host innate immune system evasion. Med Microbiol Immunol 2013;202(1):1–10. doi:10.1007/s00430-012-0253-2, PMID:22772799.
[127] Coronas-Serna JM, Louche A, Rodríguez-Escudero M, Roussin M, Imbert PRC, Rodríguez-Escudero I, *et al*. The TIR-domain containing effectors BtpA and BtpB from *Brucella abortus* impact NAD metabolism. PLoS Pathog 2020;16(4):e1007979. doi:10.1371/journal.ppat.1007979, PMID:32298382.
[128] Wan L. TIR enzymatic functions: signaling molecules and receptor mechanisms. aBIOTECH 2023;4(2):172–175. doi:10.1007/s42994-023-00104-w, PMID:37581018.
[129] Pujol C, Bliska JB. Turning *Yersinia* pathogenesis outside in: subversion of macrophage function by intracellular yersiniae. Clin Immunol 2005;114(3):216–226. doi:10.1016/j.clim.2004.07.013, PMID:15721832.
[130] Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol 2001;19:47–64. doi:10.1146/annurev.immunol.19.1.47, PMID:11244030.
[131] Sarhan J, Liu BC, Muendlein HI, Li P, Nilson R, Tang AY, *et al*. Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection. Proc Natl Acad Sci U S A 2018;115(46):E10888–E10897. doi:10.1073/pnas.1809548115, PMID:30381458.
[132] Mittal R, Peak-Chew SY, McMahon HT. Acetylation of MEK2 and I kappa B kinase (IKK) activation loop residues by YopJ inhibits signaling. Proc Natl Acad Sci U S A 2006;103(49):18574–18579. doi:10.1073/pnas.0608995103, PMID:17116858.
[133] Atri C, Guerfali FZ, Laouini D. Role of human macrophage polarization in inflammation during infectious diseases. Int J Mol Sci 2018;19(6):1801. doi:10.3390/ijms19061801, PMID:29921749.
[134] Mily A, Kalsum S, Loreti MG, Rekha RS, Muvva JR, Lourda M, *et al*. Polarization of M1 and M2 human monocyte-derived cells and analysis with flow cytometry upon *Mycobacterium tuberculosis* infection. J Vis Exp 2020;163:e61807. doi:10.3791/61807, PMID:33016941.
[135] Wu S, Liao J, Hu G, Yan L, Su X, Ye J, *et al*. Corilagin alleviates LPS-induced sepsis through inhibiting pyroptosis via targeting TIR domain of MyD88 and binding CARD of ASC in macrophages. Biochem Pharmacol 2023;217:115806. doi:10.1016/j.bcp.2023.115806, PMID:37714273.
[136] Sakurada J, Tajima A, Shinji H, Seki K, Masuda S. Apoptosis observed in murine peritoneal macrophages treated with interferon gamma through staphylococcal enterotoxin-dependent cell-mediated cytotoxicity. Microbiol Immunol 2000;44(12):1063–1066. doi:10.1111/j.1348-0421.2000.tb02603.x, PMID:11220680.
[137] Spinner JL, Seo KS, O'Loughlin JL, Cundiff JA, Minnich SA, Bohach GA, *et al*. Neutrophils are resistant to *Yersinia* YopJ/P-induced apoptosis and are protected from ROS-mediated cell death by the type III secretion system. PLoS One 2010;5(2):e9279. doi:10.1371/journal.pone.0009279, PMID:20174624.
[138] Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol 2009;27:147–163. doi:10.1146/annurev.immunol.021908.132629, PMID:19302037.
[139] Sundin C, Zetterström CE, Vo DD, Brkljača R, Urban S, Elofsson M. Exploring resveratrol dimers as virulence blocking agents - Attenuation of type III secretion in *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. Sci Rep 2020;10(1):2103. doi:10.1038/s41598-020-58872-0, PMID:32034212.
[140] Gu L, Zhou S, Zhu L, Liang C, Chen X. Small-molecule inhibitors of the type III secretion system. Molecules 2015;20(9):17659–17674. doi:10.3390/molecules200917659, PMID:26404233.