



Review

Immunomodulation of Macrophages by Pathogenic *Yersinia* Species

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Abstract. The interaction between macrophages and bacterial pathogens plays a crucial role in the pathogenesis of infectious diseases. Pathogenic species of the Gram-negative bacterium *Yersinia* deploy complex strategies to disarm macrophages and to disrupt their response to infection. For this purpose, *Yersinia* sp. engage a type III protein secretion system that mediates the polarized translocation of *Yersinia* virulence factors, the so-called Yops (*Yersinia* outer proteins), into the host cell cytoplasm. There, the Yops act on different cellular levels to neutralize a sequence of programmed phagocyte effector functions. *Yersinia* initially impair the phagocytic machinery and block the generation of the bactericidal oxidative burst. Furthermore, *Yersinia* uncouple an array of fine-tuned signals of innate immunity, which leads to suppression of macrophage TNF- α production and to macrophage apoptosis. The impairment of cellular functions results in a scenario by which *Yersinia* efficiently resists the attack of the macrophage and finally kills the macrophage by activating its intrinsic cell suicide mechanism. This review highlights the aspects of *Yersinia*-macrophage interaction that determine the fate of the infected cell.

Key words: *Yersinia*; macrophage; apoptosis; YopP/YopJ; NF- κ B.

Introduction

Disease caused by a bacterial pathogen is the outcome of a complex interaction between the bacterium and the host immune system. This “crosstalk” results in the activation of an array of signals and reactions on either side which first enable disease. The host, facing a bacterial pathogen, raises a series of defense mechanisms that involve both innate and adaptive immunity. The bacterial pathogen, on the other hand, tries to evade

or to neutralize host immune responses with the aim to multiply and to move to a new host. Particular attention has been paid in the last few years to the exploration of this bidirectional crosstalk, and this has led to the uncovering of many fascinating aspects of host-pathogen interactions. A prototypical example of the complexity of strategies a bacterial pathogen can evolve to modulate the host immune response is given by the Gram-negative bacterium *Yersinia*.

Abbreviations used: ERK – extracellular signal-regulated kinase, FADD – Fas-associated death domain protein, GAP – GTPase-activating protein, IAP – inhibitor of apoptosis protein, I κ B – inhibitory NF- κ B, IKK – I κ B kinase, JNK – c-Jun N-terminal kinase, LPS – lipopolysaccharide, MAPK – mitogen-activated protein kinase, MKK – MAPK kinase, MyD88 – myeloid differentiation factor 88, NF- κ B – nuclear factor κ B, TLR – Toll-like receptor, TNF – tumor necrosis factor, TRAF – TNF-receptor-associated factor, TUNEL – terminal dUTP nick end labeling, Yops – *Yersinia* outer proteins.

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Subversion of Phagocytes by the *Yersinia* Type III Protein Secretion System

Considering the lifestyle of *Yersinia* sp., professional phagocytes, in particular resident macrophages and polymorphonuclear leukocytes, appear to be the primary target cells for *Yersinia*-induced immunomodulation^{3, 12}. These phagocytic cells largely constitute the cellular components of innate immunity. They directly attack the invading pathogen and initiate a local as well as systemic defense response by the secretion of proinflammatory cytokines. In addition to these immediate antibacterial activities, phagocytes facilitate the maturation of the adaptive immune system. Accordingly, they represent a decisive part of the primary immune defense against bacterial pathogens, including pathogenic *Yersinia* sp.

There are three *Yersinia* sp. that can cause disease in rodents or humans. *Y. pestis* is the etiological agent of plague, whereas *Y. pseudotuberculosis* and *Y. enterocolitica* are enteric pathogens mediating gastrointestinal syndroms, lymphadenitis, and septicemia^{3, 12}. Despite following different routes of infection, the three pathogenic *Yersinia* sp. exhibit a common tropism for lymphatic tissue. After oral ingestion via contaminated food or water, enteropathogenic yersiniae invade the intestinal mucosa in the terminal ileum through lymphoid follicle-associated M cells and subsequently penetrate the lymphoid tissue of the Peyer's patches^{3, 12}. This represents a critical step in the lifecycle of *Yersinia*. The bacteria come in contact with phagocytic cells and challenge the first-line host defense. The onset of host defense mechanisms recruits additional phagocytes to the infectious site, which results in the formation of microabscesses in the submucosa. To escape this hostile microenvironment, pathogenic *Yersinia* sp. follow a panel of distinct strategies. A so-called type III protein secretion system represents the major virulence determinant of pathogenic *Yersinia* sp. and is central within the *Yersinia* antihost response¹². Related type III protein secretion systems are found in a broad range of pathogenic Gram-negative bacteria. These protein secretion systems act as powerful tools for modulation of host defense mechanisms^{13, 14}. They specifically mediate the polarized delivery of bacterial virulence proteins directly inside eukaryotic cells, where they perturb key cellular processes.

The *Yersinia* type III protein secretion system is encoded by a 70 kb virulence plasmid that is common to pathogenic *Yersinia* sp.¹². The *Yersinia* secretion apparatus decisively determines the outcome of *Yersinia* infection. Yersiniae cured of the virulence plasmid are

rapidly killed and eliminated by phagocytes in the Peyer's patches of infected mice, whereas wild-type plasmid-bearing yersiniae resist the phagocytic attack and survive¹⁶. The *Yersinia* type III secretion machinery is activated when the bacteria contact the host cell. It mediates the translocation of a set of at least 6 effector *Yersinia* virulence proteins, the so-called Yops ("Yersinia outer proteins"; YopE, YopH, YopT, YopM, YopO/YpkA, YopP/J), into the cell cytoplasm^{3, 12}. There, the diverse Yops act on distinct cellular levels to neutralize a sequence of programmed phagocyte effector functions (Fig. 1).

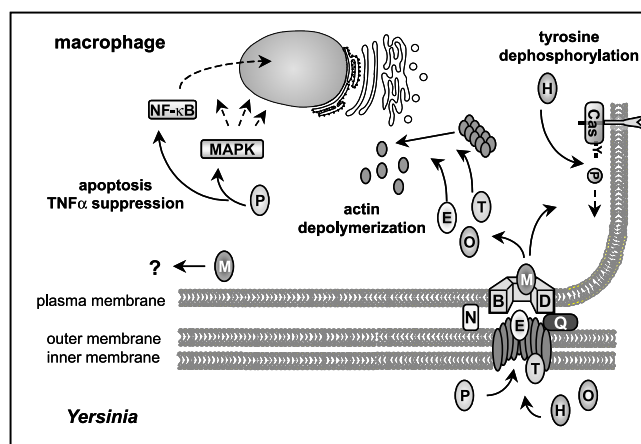


Fig. 1. Scheme on the effector functions of *Yersinia* virulence proteins in the macrophage. Effector Yops: YopH, YopE, YopT, YopM, YopO/YpkA, YopP/J. The target of YopM is still unknown. Translocator Yops: YopB, YopD. YopB and YopD mediate pore formation in the host cell plasma membrane, by which the effector Yops are translocated inside the host cell. Regulator Yops: YopN, YopQ/K. YopN and YopQ/K control the polarized Yop translocation and the size of the pore, respectively

Escape of Killing by Phagocytes

In contrast to various other enteropathogenic bacteria, for instance *Salmonella* and *Shigella* sp. which engage their type III protein secretion systems to invade the host cell and to replicate intracellularly, *Yersinia* sp. prevent ingestion by phagocytes¹²⁻¹⁴. By rapid injection of YopH, YopE, YopT, and YopO/YpkA, yersiniae paralyze the phagocytic machinery, which inhibits the uptake of the bacteria by macrophages and polymorphonuclear leukocytes^{3, 12}. The activities of these Yops modulate signal transduction pathways that control the actin cytoskeleton dynamics of the host cell, leading to disruption of the actin microfilament structure. This effect is mediated by particular targeting of members of the Rho-GTPase family by YopE, YopT, and YopO/YpkA^{2, 12}. The Rho-GTPases Rho, Rac, and Cdc42 critically regulate actin cytoskeleton rearrangements².

YopE displays a GTPase-activating protein (GAP) activity, which mediates inactivation of the Rho-GTPases. YopT selectively modifies and inactivates RhoA by an unknown mechanism. YopO/YpkA contains sequence homology to eukaryotic serine/threonine protein kinases and causes autophosphorylation. It has been shown to interact with Rho and Rac, as well as with cellular actin. YopH is a tyrosine phosphatase which exerts its effects by dephosphorylating host cell proteins, such as p130Cas and the focal adhesion kinase. These signaling scaffolds regulate the association of actin filaments with the cytoplasmic part of integrin receptors and dephosphorylation of these molecules causes disruption of peripheral focal adhesion complexes^{2, 3}.

In addition to their antiphagocytic activities, YopH and YopE prevent the killing of yersiniae by the phagocyte oxidative burst^{3, 12}. The precise molecular mechanisms of this inhibition are not yet understood, but YopH and YopE have been shown to act in concert to suppress the generation of bactericidal reactive oxygen intermediates by macrophages and polymorphonuclear leukocytes. By action of these Yops, *Yersinia* sp. resist the initial attack of phagocytes, which prevents clearance of the pathogen from the lymphoid tissue and enables its extracellular survival and proliferation. From the Peyer's patches, the yersiniae may subsequently disseminate to the mesenteric lymph nodes, liver and spleen, and establish a systemic infection^{3, 12}.

However, the impact of yersiniae on phagocytes is more subtle than only mediating its primary survival during the first interaction. *Yersinia* sp. additionally modulate long-term phagocyte antibacterial activities, which accounts for the later stages of infection, especially for spread of the bacteria into deeper lymphatic tissues. This process is largely supported by the effector protein YopP (*Y. enterocolitica*) or its homologue YopJ (*Y. pseudotuberculosis*, *Y. pestis*) in a type III secretion-dependent manner.

Disruption of Innate Immunity Signaling by YopP/J

Specific microbial components, such as lipopolysaccharides (LPS), peptidoglycan, and bacterial lipoproteins, are powerful stimuli of innate immunity in the mammalian host^{1, 6, 11, 20, 37}. They rapidly induce a variety of reactions in immune effector cells, leading to the production of proinflammatory cytokines, which mount a protective inflammatory response. *Yersinia* sp. poss-

ess efficient capabilities to interfere with the signaling networks of innate immunity. Yersiniae target the Toll-like receptor (TLR)-NF- κ B signaling pathway, a host defense system that is remarkably conserved in vertebrates and invertebrates. The TLRs are a family of surface receptors of cells of innate immunity that serve to identify microbial pathogens^{1, 6, 11, 20, 37}. They exhibit specificity in the recognition of bacterial components. The LPS of Gram-negative bacteria preferentially activates TLR4, whereas the lipoteichoic acid and peptidoglycan of Gram-positive bacteria predominantly stimulate TLR2. Downstream from the receptor complexes, the TLR-dependent signals converge on the adapter molecule MyD88, which initiates a signal relay that ultimately leads to activation of the NF- κ B and mitogen-activated protein kinase (MAPK) pathways^{1, 6, 11, 20, 37}.

The NF- κ B and MAPK signaling cascades crucially determine host immunity functions. NF- κ B is a heterodimeric transcription factor that upregulates the synthesis of proinflammatory cytokines (i.e. TNF- α , IL-1, IL-6), chemokines (i.e. IL-8), adhesion molecules (i.e. VCAM-1, ICAM-1), and inducible enzymes (i.e. iNOS, Cox-2)^{9, 17, 37}. In addition, NF- κ B is implicated in mediating cellular survival by the prevention of apoptosis^{8, 29}. The MAPKs, including the kinases extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38, are global regulators of cellular homeostasis¹⁵. They play a role in the control of the cellular stress response, cell cycle machinery and cellular differentiation. By regulating the activity of proinflammatory transcription factors, such as AP-1 (c-Fos/c-Jun) and CREB, the MAPKs coordinate the cytokine production of the stimulated cell¹⁵.

Yersinia sp. have developed an efficient strategy to modulate these signal transduction pathways, which leads to suppression of the host cytokine production. *In vivo* studies in infected mice first demonstrated that yersiniae impair the release of the proinflammatory cytokines TNF- α and IFN- γ ²⁴. The cytokine-suppressive effect essentially contributes to pathogenesis of yersiniosis, since additional administration of cytokine-neutralizing antibodies to yersiniae-infected mice seriously exacerbates disease⁷. *In vitro* studies revealed that *Yersinia* infection dampens the release of TNF- α and IL-8 in cultivated cells^{31, 35}. The cytokine-suppression is best characterized in macrophages, which are the major source for TNF- α in the compromised host. Yersiniae inhibit TNF- α mRNA expression and TNF- α synthesis as soon as 120 min after onset of macrophage infection³¹. The TNF- α production in macrophages is controlled by the synergistic activities of NF- κ B and MAPKs, which regulate the synthesis of TNF- α at the

transcriptional (NF- κ B, ERK, JNK) and translational levels (p38, JNK)¹⁵. Accordingly, the promoter of the TNF- α gene contains several enhancer sequences and maximal activation requires at least two *cis*-acting regulatory elements, such as NF- κ B and c-Jun³⁶.

Various studies revealed that yersiniae simultaneously interfere with NF- κ B activation as well as MAPK activities in infected macrophages^{10, 28, 30, 31, 34}. The activation of NF- κ B is conferred by a multisubunit protein kinase, the I κ B kinase (IKK) complex^{9, 15, 17, 37}. This complex is composed of the kinases IKK α and IKK β , and the noncatalytic regulatory IKK γ subunit. The IKK complex mediates phosphorylation of the NF- κ B inhibitory I κ B proteins, which sequester NF- κ B in the cytoplasm. Phosphorylated I κ Bs are degraded through the ubiquitin-proteasome pathway, thereby releasing NF- κ B, which translocates to the nucleus and activates transcription^{9, 15, 17, 37}. The MAPK members are activated by upstream kinase cascades through dual phosphorylation¹⁵. This, in turn, enables MAPK to phosphorylate and activate the relevant proinflammatory transcription factors. In the initial phase, macrophages respond with NF- κ B induction and MAPK activation to *Yersinia* infection^{10, 28, 30, 31, 34}. But after a lag time of 30–60 min, yersiniae block NF- κ B nuclear translocation and impair MAPK activities, which differs from the effects of LPS on macrophages. *Yersinia*-induced NF- κ B and MAPK deactivation correlates with upstream events, including inhibition of MAPK tyrosine phosphorylation and reduced I κ B degradation. A number of studies revealed that downregulation of NF- κ B and MAPK signaling as well as the accompanying cytokine suppressive effect are conferred by the translocated protein YopP (*Y. enterocolitica*), or its homologue YopJ (*Y. pseudotuberculosis*, *Y. pestis*)^{10, 28, 34}. The time necessary for direction of YopP/J to its intracellular targets explains the delay until the YopP/J effects are observed.

YopP/J engages multiple binding partners to exert its action on the distinct signaling pathways. YopP/J interacts with members of the MAPK kinase (MKK) superfamily, which leads to disruption of MAPK activities²⁶. MKKs are the direct upstream activators of MAPKs and, by simultaneous targeting of MKK1 to MKK5, YopP/J inhibits the ERK, JNK, and p38 pathways at the same time. Subversion of the NF- κ B cascade is accomplished by targeting YopP/J to the NF- κ B-activating IKK β ^{26, 32}. IKK β is the catalytic IKK subunit that mediates I κ B phosphorylation and NF- κ B activation in monocytes and macrophages upon LPS treatment²⁵. IKK β exhibits homology to the MKKs in the kinase activation loop, suggesting that YopP/J in-

terferes with IKK β and MKK activation by upstream signaling pathways. However, a recent report indicates that YopP/J may act as a cysteine protease on cellular proteins modified with the ubiquitin-like molecule SUMO-1²⁷. A link between reduced SUMO-1 conjugation of proteins and inhibition of IKK β or MKKs is hitherto unclear, but IKK β and MKKs do not appear to be direct targets for SUMO-1 cleavage by YopP/J²⁷. It is speculated that SUMO-1 conjugation may be an important posttranslational modification of signaling complexes, directing their intracellular processing²⁷. This may involve the regulation of IKK β - and MKK-dependent signaling pathways, which are then disrupted by YopP/J.

Induction of Macrophage Apoptosis

Interestingly, the modulation of cellular signaling by YopP/J exerts another profound effect on macrophages besides inhibition of cytokine production. Macrophages infected by YopP/J-producing yersiniae enter an irreversible and final step in their lifecycle: after a prolonged incubation of 5–20 h, the infected cells display characteristic membrane blebbing, shrinkage of the cytoplasm, chromatin condensation and DNA fragmentation, and ultimately the macrophages die^{21, 23, 33}. These are the classic morphological features of programmed cell death and provide evidence that the infected macrophages undergo apoptosis (Fig. 2). Apoptosis as a physiological, active process of cell suicide mediates the ablation of unwanted cells from the organism without inducing inflammation or damage to contiguous cells. Its initiation is tightly controlled in eukaryotic cells. The cell integrates signals emanating from death-inducing and survival-promoting receptors, and secondary intracellular responses regulate the entry of apoptosis⁸. The precise regulation of cell proliferation and cell death by these complex signaling networks maintains the homeostasis of the multicellular organism. Recent studies indicate that the induction of apoptosis also plays a role in the pathogenesis of infectious diseases. In yersiniosis, macrophage apoptosis appears to facilitate the establishment of a systemic *Yersinia* infection²³.

Diverse studies revealed that suppression of the NF- κ B signaling pathway by YopP/J critically contributes to the mechanism of apoptosis induction by *Yersinia*^{30, 32}. This phenomenon can be attributed to the dual function of NF- κ B, on the one hand acting as a key regulator of the inflammatory response, on the other hand conferring cellular survival by the prevention of apoptosis^{8, 17, 29}.

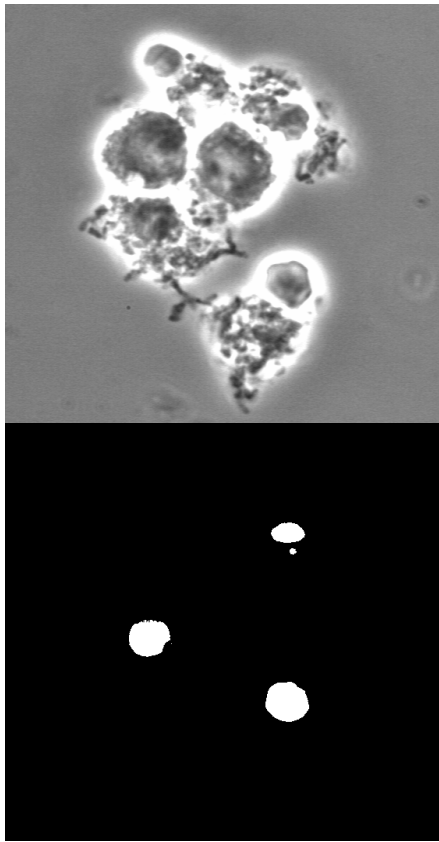


Fig. 2. J774A. 1 macrophages undergoing apoptosis 6 h after onset of *Y. enterocolitica* infection. In the phase-contrast microscope, the infected macrophages display a heterogenous, apoptotic morphology (top). DNA fragmentation is detected by the terminal dUTP nick end labeling (TUNEL) reaction in a portion of the dying cells. The TUNEL assay labels free ends of apoptotic DNA fragments with fluorescein, thus providing green fluorescence to condensed apoptotic nuclei (bottom)

NF- κ B activation provides protection against apoptosis under multiple stress-induced conditions. NF- κ B functions to up-regulate the synthesis of anti-apoptotic proteins, such as (inhibitors of apoptosis) IAP, TNF-receptor-associated factor (TRAF), and Bcl-2 family members^{8, 29}. These proteins counteract pro-apoptotic signals elicited by diverse extracellular stimuli, such as TNF- α and ionizing radiation. By this mechanism, NF- κ B activation suppresses apoptotic killing otherwise induced by these stimuli.

In line with the antiapoptotic functions of NF- κ B, overexpression of the transcriptionally active p65 subunit of NF- κ B exerts a protective effect against YopP/J-induced apoptosis in *Yersinia*-infected macrophages³². This points out that subversion of the NF- κ B survival pathways by YopP/J is a crucial part of the strategy of yersiniae to mediate apoptosis. However, NF- κ B inhibition alone is in general not sufficient

to trigger apoptosis. It rather requires a secondary pro-apoptotic stimulus, such as TNF- α , which activates a cytotoxic pathway, leading to apoptosis when NF- κ B activation is suppressed^{8, 29}. In fact, transfection of macrophages with an eukaryotic YopP expression vector and the accompanying NF- κ B suppressive effect are ineffective in mediating efficient apoptosis³². Epithelial HeLa cells do not undergo apoptosis upon *Yersinia* infection, although yersiniae block NF- κ B activation in HeLa cells similarly as in macrophages³⁰. Interestingly, *Yersinia* infection mediates HeLa cell apoptosis when the cells are subsequently treated with TNF- α ³⁰. As the macrophage is directly compelled to undergo apoptosis upon *Yersinia* infection, this suggests activation of an indispensable pro-apoptotic signal by *Yersinia* specifically in macrophages. This signal apparently mediates macrophage apoptosis together with the YopP/J NF- κ B suppressive effect. It was recently demonstrated that LPS of Gram-negative bacteria can provide the necessary apoptotic signal that cooperates with the action of YopP/J to induce macrophage cell death³². This indicates a strategy by which *Yersinia* targets the NF- κ B pathway by injection of YopP/J and, simultaneously, takes advantage of pro-apoptotic LPS signaling to kill the macrophage³². Under these conditions, the initiation of LPS signaling, which is originally thought to mount a protective response against an invading organism, triggers the macrophage to undergo apoptosis.

In agreement with these findings, there is increasing evidence that bacteria and microbial components indeed activate pro-apoptotic signaling pathways in macrophages^{19, 30}. NF- κ B activation functions to counteract these cytotoxic signals and mediates macrophage survival in response to these components. As the respective pro-apoptotic reactions are elicited by conserved microbial components such as LPS, the initiation of cytotoxic signaling pathways appears to be a general mechanism of innate immunity. In line with these suggestions, two reports indicate a crucial role of TLR2 in signaling apoptosis upon stimulation with bacterial lipoproteins^{4, 5}. Thereby, the activation of TLR2 is coupled to the death-promoting pathway through activation of caspase-8, which initiates the proteolytic apoptotic cascade⁵. The pro- and anti-apoptotic signals downstream from TLR2 bifurcate at the level of the adaptor protein MyD88. MyD88 binds the Fas-associated death domain protein (FADD), mediating caspase-8 activation⁵. Interestingly, the MyD88-FADD cytotoxic pathway is similarly preserved in *Drosophila*¹⁸. The evolutionary conservation of this pathway confirms a functional, yet unknown role of caspase activation by TLR signaling within the innate immune response.

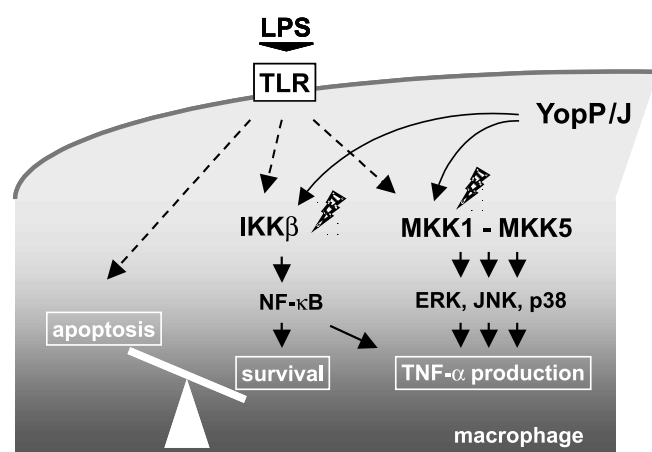


Fig. 3. The proposed model for the effects of YopP/J on macrophage signal transduction pathways. By inhibiting NF-κB and MAPK signaling cascades, YopP/J simultaneously mediates suppression of the macrophage TNF-α production and macrophage apoptosis

Together, these findings demonstrate that, by injection of YopP/J, *Yersinia* directly strikes the heart of host innate immunity (Fig. 3). YopP/J disrupts several key effector pathways of innate immunity that control cytokine production and cellular viability. This renders the macrophage unable to respond adequately to bacterial infection. The cytotoxic signals generated within the innate immune reaction subsequently compel the macrophage to undergo apoptosis. This reflects the end point of a powerful multi-step strategy by which this bacterial pathogen subverts the immune response of the host.

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