International Journal of Medical Microbiology 309 (2019) 344–350

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Bacterial adhesion and host cell factors leading to effector protein injection by type III secretion system

Erwin Bohn*, Michael Sonnabend, Kristina Klein, Ingo B. Autenrieth

Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen (IMIT), Institut für Medizinische Mikrobiologie und Hygiene, Universität Tübingen, Tübingen, Germany

ARTICLE INFO

Keywords:
T3SS
Host cell targets
Integrins
Immune system
Virulence
Bacteria-host cell interaction

ABSTRACT

Type III secretion systems (T3SS) play a crucial role for virulence in many Gram-negative bacteria. After tight bacterial contact to host cells, the T3SS injects effector proteins into the host cells, which leads to cell invasion, tissue destruction and/or immune evasion. Over the last decade several attempts were made to characterize the host-cell interactions which precede and determine effector protein injection during infection. The development of the TEM-β-lactamase reporter was an important breakthrough to achieve this goal. By this means it was demonstrated that during infection with many Gram-negative pathogens such as *Salmonella*, *Pseudomonas* or *Yersinia* the main targets of T3SS are leukocytes of the myeloid lineage such as neutrophils, macrophages or dendritic cells. This is due to the recruitment of these cells to the site of infection, but also due to the specific interplay between bacterial and host cells. Comprehensive studies on *Yersinia pestis*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* effector translocation show that adhesins such as Invasin (Inv), *Yersinia* adhesin A (YadA) and attachment and invasion locus (Ail) are critical for effector translocation. Here, mainly the complex interaction of YadA and Ail with various host cell receptor repertoires on leukocytes and the modulatory effects of serum factors direct effector translocation predominantly towards myeloid cells. The current understanding suggests that mostly protein based interactions between bacteria and host determine host cell specific effector translocation during *Yersinia* infection. However, for *Shigella dysenteriae* infection it was shown that glycan-glycan interactions can also play a critical role for the adhesion preceding effector translocation. In addition, the *Shigella* infection model revealed that the activation status of cells is a further criterium directing effector translocation into a distinct cell population. In this review the current understanding of the complex and species-specific interaction between bacteria and host cells leading to type III secretion is discussed.

1. Introduction

For many pathogenic Gram-negative bacteria type III secretion systems (T3SS) are critical for virulence (Deng et al., 2017; Egan et al., 2014; Pinaud et al., 2018). Depending on the various virulence strategies, pathogens use the T3SS for invasion into and survival in host cells (e.g. *Salmonella* and *Shigella*) (Agbor and McCormick, 2011; de Jong and Alto, 2018; Jennings et al., 2017; Johnson et al., 2018; Picking and Picking, 2016; Yang et al., 2015), to assure tight adhesion (EPEC) (Platenkamp and Mellies, 2018) or to maintain an extracellular life style (*Yersinia*) (Bliska et al., 2013; Pha and Navarro, 2016; Plano and Schesser, 2013). A common objective of all T3SS is the establishment of a habitat in infected hosts including the evasion of the immune response. Therefore, targets of T3SS are often epithelial cells and leukocytes. Specifically the interaction between bacteria and leukocytes is a decisive step for the outcome of infection. Here the interaction can take place with cells of the myeloid lineage involved in innate immunity comprising macrophages, dendritic cells, granulocytes and NK cells or the lymphocytes comprising B and T cells. Identification of T3SS target cells and immune evasion strategies reflect the evolutionary crucial host defence mechanisms against bacterial pathogens.

One question over the last decade was which cells are efficiently targeted and how further bacterial factors beside the T3SS and host factors contribute to this process. Despite many other attempts (Briones et al., 2006; Jacobi et al., 1998) the breakthrough for investigating effector translocation was the development of the β-lactamase (bla)-

---

Abbreviations: Ail, attachment and invasion locus; bla, β-lactamase; CNF, Cytotoxic necrotizing factor; CR3, complement receptor 3; DCs, dendritic cells; ECM, extracellular matrix protein; FRET, fluorescence resonance energy transfer; Inv, invasin; NK cells, natural killer cells; LPS, lipopolysaccharide; Pla, plasminogen activator; T3SS, type III secretion system; Vn, vitronectin; YadA, *Yersinia* adhesin A; Yop, *Yersinia* outer protein

* Corresponding author at: Institut für Medizinische Mikrobiologie und Hygiene, Elfriede-Aulhorn-Str., 672076 Tübingen, Germany.
E-mail address: erwin.bohn@med.uni-tuebingen.de (E. Bohn).

https://doi.org/10.1016/j.ijmm.2019.05.008
Received 9 January 2019; Received in revised form 2 May 2019; Accepted 31 May 2019
1438-4221/

reporter assay system developed by Charpentier and Oswald (Charpentier and Oswald, 2004), which allowed studies of the T3SS during *in vivo* infection (Marketon et al., 2005). The assay bases on the principle that vectors expressing fusion proteins consisting of a T3SS effector and a β-lactamase as reporter are introduced into the bacteria. After effector translocation the cells are stained with CCF2-AM or CCF4-AM which after excitation by FRET-transfer leads to emission of green fluorescence. The β-lactamase cleaves CCF2/4 and one of the educts represents a coumarin derivate which leads to emission of blue fluorescence (Charpentier and Oswald, 2004). Meanwhile, many researchers use this system not only for basic research but also for drug screening (Harmon et al., 2010; Morgan et al., 2018).

2. The preferential effector translocation into myeloid cells

Using the bla-reporter system effector translocation was investigated not only in cell culture experiments but also in mouse infection. Although translocation of T3SS effectors can occur in most cell types, there are clearly cell type specific differences which may influence injection.

After intraperitoneal *Salmonella* infection, various effector proteins are preferentially translocated into neutrophils and to a lesser extent into macrophages (Geddes et al., 2007). Similar observations were made for *Pseudomonas aeruginosa* (Rangel et al., 2015). After lung infection with *P. aeruginosa* most cells translocated with the effector ExoS were neutrophils, while the frequency of translocation into other leukocytes was rather low. In the later phase of infection translocation was also observed in specific pneumocyte subpopulations (Rangel et al., 2015). Studies with various *Yersinia* species using mouse infection revealed also in general a preferential higher targeting of myeloid cells (Deuschle et al., 2016; Durand et al., 2010; Köberle et al., 2009; Maldonado-Arocho et al., 2013; Marketon et al., 2005; Merritt et al., 2015) which applies to targeting in lungs, Peyer´s patches and spleen (Paczosa et al., 2014; Pechous et al., 2013). More detailed studies of *Yersinia enterocolitica (Ye)* infection suggest that specific properties of cells influence whether they are more or less appropriate targets for effector translocation. For instance, amongst dendritic cells $CD8^+$ DCs and amongst B cells follicular B cells (Autenrieth et al., 2010; Köberle et al., 2009) are the preferred targets. The preferential targeting of myeloid cells is not surprising because specifically neutrophils but also other myeloid cells are recruited to the site of inflammation. Thus, tissue architecture, cell composition, cell influx, cell migration and chemotaxis and host cell surface structures (tissue remodeling) may indeed strongly influence with which (immune) cells bacteria get in contact. However, cell tropism can also be observed in *in vitro* studies and is therefore not only determined by tissue architecture and recruitment of immune cells but also by the specific properties of the cell surface (Deuschle et al., 2016; Maldonado-Arocho et al., 2013; Merritt et al., 2015). Specifically, the interaction between bacterial factors, the host cell receptor repertoire and serum factors plays a decisive role for effector translocation. This leads to the questions which bacterial and host cell factors are critical for effector translocation.

2.1. The interplay between Yersinia and host cells leading to effector translocation

2.1.1. Role of adhesins for Yersinia effector translocation

The most comprehensive understanding how the interplay of bacterial and host factors leads to a targeting of distinct cell populations is available for *Yersinia* species. It could be demonstrated that *Yersinia* adhesins are the most important factors acting as prerequisite for effector translocation. The most prominent adhesins for *Ye* and *Yersinia pseudotuberculosis* (*Yps*) are the adhesins Inv, YadA and Ail. Inv belongs to the type Ve secretion systems and plays a role in pathogenicity in the early phase of infection (Grassl et al., 2003; Leo and Skurnik, 2011). However, at least in *Ye* infection Inv is not essential for virulence in experimental mouse infection (Pepe et al., 1994; Pepe and Miller, 1993).

YadA is a trimeric autotransporter of the type Vc secretion system and highly important for virulence according to its pleiotropic role involving adhesion to extracellular matrix (ECM) proteins, complement resistance and autoagglutination (Leo and Skurnik, 2011). The Ail adhesin belongs to a family of outer membrane proteins which includes PagC and Rck of *Salmonella enterica* as well as OmpX of *Escherichia coli*. Ail plays a role in serum resistance in all three human pathogens, especially in *Yersinia pestis (Yp)*, where deletion of Ail leads to almost complete loss of serum resistance (Thomson et al., 2019). Both YadA and Ail confer serum resistance by recruiting the complement-regulatory proteins factor H, complement component 4-binding protein C4BP and vitronectin (Ho et al., 2012, 2014; Mühlenkamp et al., 2017; Schindler et al., 2012; Thomson et al., 2019).

Due to the small size of Ail its activity is usually masked by the lipopolysaccharide (LPS) outer-core oligosaccharide and O-antigen in *Ye* O:3 (Biedzka-Sarek et al., 2008) or O-antigen in *Yps* YPIII (Tsang et al., 2013). Therefore Ail only displays full biological activity in strains with rough LPS, such as *Yp*. While YadA and Ail are predominantly expressed at 37 °C, Inv is fully expressed at 27 °C and expression declines due to regulatory processes at 37 °C. *Yp* lacks Inv and YadA and mediates adhesion and subsequent effector translocation predominantly by Ail.

In contrast to enteric *Yersinia* other factors seem to be involved in effector translocation by *Yp*, namely the pH 6 antigen PsaA and plasminogen activator Pla (Merritt et al., 2015). Pla (which is absent in enteric *Yersinia*) is an outer membrane adhesin and protease that facilitates degradation of extracellular matrix proteins and promotes bacterial dissemination. The *psa* operon encodes a fimbrial structure which binds to two receptors: PsaA, also called MyfA or pH 6 antigen, is present in all three *Yersinia* species, but its expression is restricted to slightly acidic conditions at temperatures above 36 °C (Chauhan et al., 2016). PsaA of *Yp* consists of fimbriae that bind to two receptors: β1-linked galactosyl residues in glycosphingolipids and the phosphocholine group in phospholipids (Bao et al., 2013).

Studies on both epithelial cells as well as splenocytes showed that the presence of either Inv or YadA on *Ye* is sufficient to mediate adhesion in a way to achieve optimal effector translocation and that deletion of both adhesins abrogates adhesion totally, indicating that other factors including the adhesin Ail may play only a minor or no role for effector translocation during *Ye* infection (Deuschle et al., 2016; Keller et al., 2015) while for *Yps,* Ail additionally seems to play a role for effector translocation. Maldonado-Arocho et al. reported that the contribution of Inv, YadA and Ail to effector translocation seems to show strain-specific variations in which effector translocation depends either more on Inv, YadA, or on a combination of Ail and YadA (Maldonado-Arocho et al., 2013). In addition, it was observed that after mouse infection with a high infection dose of a $\Delta inv/\Delta yadA/\Delta ail$ mutant effector translocation can still be achieved showing a similar host cell tropism like after infection with WT, indicating an involvement of additional adhesin-independent mechanisms shaping effector translocation (Maldonado-Arocho et al., 2013). These studies show that (i) adhesin expression levels, (ii) sequence differences of individual adhesins (Heise and Dersch, 2006; Mühlenkamp et al., 2017), (iii) LPS structure (e.g. masking of Ail), and (iv) probably also other so far unknown bacteria-cell interactions may contribute to adhesion and effector translocation.

2.1.2. The role of β1 integrins for effector translocation into Ye

While *Yersinia* Inv is well known to interact directly with members of the β1 integrin family such as α3β1, α4β1, α5β1, α6β1 and αvβ1 (Hamburger et al., 1999; Isberg and Leong, 1990; Leong et al., 1993), YadA seems to be bound indirectly via ECM proteins such as fibronectin, laminin and collagen to β1 integrin family members (Heise and Dersch, 2006; Leo and Skurnik, 2011; Mühlenkamp et al., 2015). Similarly to YadA, investigation of *Yp* Ail also showed interaction with fibronectin and laminin (Tsang et al., 2012, 2010; Tsang et al., 2017), which leads to the speculation that Ail might also indirectly interact with β1 integrins. However, this was not proven so far.

Studies addressing the interaction between Inv/YadA and β1 integrin deficient fibroblasts and epithelial cells revealed that Inv-dependent translocation is strictly β1 integrin dependent albeit Inv-deficiency allows to some extent adhesion of *Ye* to fibroblasts lacking β1 integrins, suggesting that the quality of adhesion is important for subsequent effector translocation (Keller et al., 2015). EM studies demonstrate that a tight contact between *Ye* and host cells seems to be critical for effector translocation. In contrast to Inv, YadA uses a broader repertoire of interaction partners according to the indirect interaction via ECM proteins. Thus, β1 integrin deficient epithelial cells are still able to interact via YadA to mediate adhesion and subsequent translocation. However, additional depletion of integrins of the αV integrin family (including eg. αVβ3, αVβ6, αVβ1) using a shRNA approach strongly reduced adhesion and translocation. Thus, one major difference between fibroblasts and epithelial cells seems to be the different integrin receptor repertoire which influences the quality of adhesion and the subsequent effector translocation (Keller et al., 2015). The importance of β1 integrins for effector translocation could also be demonstrated after *in vivo* infection with *Ye* using β1 integrin depleted mice (Deuschle et al., 2016; Keller et al., 2015). One limiting problem to address the contribution of integrins is that both β1 and αV integrin deficient mice are embryonic lethal (Fässler and Meyer, 1995). This can be partially circumvented by using conditional knockout mice, which allows depleting almost completely β1 integrins exposed by leukocytes. In this case depletion was achieved by polyI:C treatment which induces expression of Cre-recombinase under control of a Mx1 promoter and subsequent deletion of the floxed β1 integrin genes in leukocytes (Bauer et al., 2009; Brakebusch et al., 2000).

Infection experiments using such mice clearly demonstrated that β1 integrins play a critical role for effector translocation (Deuschle et al., 2016). However, β1 integrin depletion diminishes effector translocation into all investigated leukocyte populations. This means that during *in vivo* infection β1 integrins are of general importance for effector translocation by *Ye* into leukocytes. Interestingly, depletion of β1 integrins diminishes the amount of targeted cells in a similar manner in both lymphocytes and myeloid cells (Deuschle et al., 2016). The experiments also suggest that other receptors beside integrins of the β1 family are critical for shaping host cell tropism towards myeloid cells such as neutrophils in *in vivo* infection.

2.1.3. The role of serum factors for effector translocation into Yersinia species

Further components which are critical to shape effector translocation are serum factors. Infection of splenocytes with *Yps* or *Ye* in the absence of serum results in a comparable frequency of effector translocated cells for various splenocyte subpopulations. (Deuschle et al., 2016; Maldonado-Arocho et al., 2013; Merritt et al., 2015). However, in case of both *Yps* and *Ye* infection of splenocytes, heat-inactivated fetal calf serum inhibited predominantly effector translocation into B and T cells but to a lesser extent into myeloid cells (Deuschle et al., 2016; Maldonado-Arocho et al., 2013). Thus, serum components are clearly important to shift effector translocation in direction of myeloid cells. In case of *Ye* infection the interplay of serum factors and Inv as well as YadA was also investigated. Surprisingly, a mutant expressing Inv but not YadA showed higher translocation into splenocytes compared to wildtype and no preferred cell tropism (Deuschle et al., 2016). Thus, in the presence of both YadA and Inv, effector translocation seems to be dominated by YadA, shaping the cell tropism towards myeloid cells. One plausible explanation is that the longer YadA (23 nm) (Hoiczyk and Blobel, 2001) prevents the binding of the shorter Inv (14 nm) (Dersch and Isberg, 2000; Hamburger et al., 1999) to host cells because of sterical reasons and therefore dominates adhesion and subsequent effector translocation.

Despite the absence of Inv and YadA, serum also shapes effector translocation during *Yp* infection. It was observed that effector translocation into splenocytes cultured in the presence of heat-inactived fetal calf serum (as a typical component of cell culture media) was not affected by deletion of *ail*, *pla/psa* or *ail/pla/psa*. However, in the presence of both heat-inactived fetal calf serum and active mouse serum (but not heat inactivated mouse serum), effector translocation into all leukocytes was diminished with exception of neutrophils, which as a net effect led to a preferential targeting of neutrophils (Merritt et al., 2015). Under these conditions, in the absence of *ail*, effector translocation was further diminished and host cell tropism towards neutrophils further increased. These and other studies identify Ail as the most important adhesin involved in effector translocation (Felek and Krukonis, 2009; Merritt et al., 2015. Nevertheless, the additional adhesins PsaA and Pla and obviously also adhesin-independent interactions contribute to effector translocation and more importantly are also involved in determining host cell tropism towards neutrophils and to a lesser extent also towards other myeloid cells.

Thus, for *Ye*, *Yps* and *Yp* both heat-sensitive and heat-stable serum factors seem to play a decisive role by influencing adhesion to host cells and in consequence directing effector translocation into distinct subpopulations (Deuschle et al., 2016; Maldonado-Arocho et al., 2013; Merritt et al., 2015). Studies demonstrate that serum comprises virtually all extracellular matrix proteins of the body in very different amounts (Chan et al., 2004). Many of these factors readily absorb to and can coat surfaces like bacteria (Singh et al., 2012). When matrix-coated bacteria come into contact with host cells harboring adhesion receptors for the respective matrix proteins a considerable array of adhesive bacteria-host cell interaction can arise. Furthermore, most cells can bind and organize extracellular matrix proteins, depending on their integrin receptors and associated signalling. This again constitutes a wealth of possibilities of how matrix binding adhesins can associate with cells. The serum factors which actually modulate *Yersinia* effector translocation, however, are not identified so far. Nevertheless some studies as well as unpublished own observations point to (e.g. C3bi, vitronectin) and exclude (e.g. fibronectin) distinct candidates involved in modulation of effector translocation.

For instance, studies with *Yp* indicate that αMβ2 integrins (also termed as CR3, CD11b/CD18) (Small et al., 2016) contribute to this host cell tropism. In one study the interaction between *Yp* and predominantly neutrophils could be partially blocked by antibodies against CD11b (Merritt et al., 2015). The integrin αMβ2 is mainly expressed by neutrophils, macrophages and NK cells (Ross, 2002). For Ail and YadA (*Ye* and *Yps*) it was described that they bind factor H which subsequently binds and inactivates the complement component C3 to C3bi. C3bi then interacts with CR3 (αMβ2) on neutrophils and macrophages (Schindler et al., 2012; Thomson et al., 2019). This interaction may now specifically support the adhesion to and effector translocation into neutrophils and macrophages. However, as Merritt et al. pointed out the situation seems to be even more complex because the remaining effector translocation after deletion of all *Yp* adhesins still showed a host cell tropism towards neutrophils, which may argue for a deposition of C3bi on the bacterial surface (Merritt et al., 2015).

A role of the complement system for effector translocation in an adhesin-independent manner was also provided during *Yps* infection and inhibition of the complement system using cobra venom factor (Maldonado-Arocho et al., 2013). Further host cell receptors were described which may modulate host cell tropism, namely CD14, which is highly expressed on myeloid cells and B cells and may interact via the LPS binding protein with LPS on the surface (Merritt et al., 2015). All these players may provide plausible explanations for the distinct interaction of *Yersinia* with neutrophils and other myeloid cells.

However, the presence of serum during infection of splenocytes with any *Yersinia* species reduces effector translocation, arguing for the presence of serum factors interfering with adhesion and effector translocation. The only candidate which could be excluded as a serum factor modulating effector translocation is fibronectin (Maldonado-Arocho et al., 2013). One possibility could be soluble vitronectin, which is highly present in serum and binds to YadA (Mühlenkamp et al., 2017). A possible scenario could be that soluble vitronectin interacts with YadA and acts as a competitive inhibitor of the YadA interaction with already cell-bound insoluble ECM proteins (including laminin, fibronectin and vitronectin) on the host cell. This hypothetical scenario is supported by so far unpublished own observations. Such a scenario could also occur in *Yp* because Ail was also shown to bind vitronectin (Thomson et al., 2019).

2.1.4. *Cell activation as a critical step for effector translocation*

Besides tight bacteria-host cell interaction, host cell signalling events seem to play an important role to facilitate effector translocation. One important step for type III secretion is the secretion of proteins which build a pore complex in the host cells membrane serving as an entry port for the subsequently secreted effector proteins. In case of *Yersinia* these pores consist of YopB and YopD. Several studies pointed out that activation of Rho-GTPases such as Rac and RhoA are critical for effector translocation predominantly by facilitating YopB/D pore formation (Mejia et al., 2008). Activation of Rho-GTPases can be achieved by Inv- or YadA-induced β1 integrin signalling or by YopB itself (Keller et al., 2015; Köberle et al., 2009; Mejia et al., 2008). In addition, factors like the *Yps* toxin CNF-Y amplify effector translocation by Rac1 activation (Schweer et al., 2013; Wolters et al., 2013). A screening approach by Sheahan et al. identified several host cell factors which modulate effector translocation or specifically YopB/D pore formation like RhoA and its downstream effector ROCK2 (Sheahan and Isberg, 2015). It could also be demonstrated that proteins involved in membrane trafficking and tubulin formation influence pore formation and effector translocation (e.g. COPβ1, ARF1, ARF6) (Sheahan and Isberg, 2015). Additionally, activation of the chemokine receptor CCR5 may specifically facilitate pore formation and effector translocation in a cell-type specific manner into neutrophils and macrophages (Sheahan and Isberg, 2015).

Although the whole picture of the interaction of *Yersinia* species with host cells to promote effector translocation is still not complete according to its complexity, the basic principle for the preferential targeting of neutrophils and other myeloid cells seems to be clear leading to a simplified model as shown in Fig. 1:

1. There is a robust and redundant interaction network between *Yersinia* and host cell assuring effector translocation.
2. Effector translocation is predominantly mediated in an adhesin-dependent manner. In addition to classical adhesins (Inv, YadA and Ail) other factors such as LPS or soluble factors like CNF-Y contribute to effector translocation.
3. *Yersinia* preferentially adheres to and translocates effectors into myeloid cells and particularly into neutrophils.
4. Although other adhesin-dependent and adhesin-independent mechanism cannot be neglected YadA and Ail seem to play a central role for shaping host cell tropism towards neutrophils and other myeloid cells because of their pleiotropic interaction with many factors: (i) interaction with cell bound ECM matrix proteins attached to integrins of the β1 and αV families promoting adhesion and translocation, (ii) binding of soluble vitronectin present in plasma which we postulate acts as a competitive inhibitor of (i), and (iii) factor H binding leading to C3b cleavage and putative binding of C3bi to αMβ2 integrins (CR3) promoting predominantly adhesion to and effector translocation into myeloid cells and thus counteracting the inhibition of serum factors such as putatively vitronectin.
5. The preferential targeting to myeloid cells seems to be supported by additional receptors such as CD14 probably acting via the axes CD14-LBP-LPS and by the so far uncharacterized activation of CCR5.

3. The interplay between other Gram-negative bacteria and host cells leading to effector translocation

For many other Gram-negative bacteria the molecular mechanisms leading to binding and subsequent effector translocation are poorly understood. For instance, T3SS secretion by *Shigella dysenteriae* leads to invasion of *Shigella* into host cells (Belotserkovsky and Sansonetti, 2018). One critical step might be the activation of the T3SS by bile salts, which additionally leads to the upregulation of the autotransporter IcsA as one of the factors involved in host cell adhesion (Brotcke Zumsteg et al., 2014). In similar, the Multivalent Adhesion Molecule (MAM) was shown to play a role in type III secretion as a MAM deletion mutant in *Shigella sonnei* showed reduced adhesion, effector translocation and virulence *in vivo* (Mahmoud et al., 2016).

The early T3SS effector IpaB, which is part of the translocon, interacts with the proteoglycan CD44 and this step seems to be important to stabilize adhesion. In addition, several other T3SS effectors such as OspE1 and OspE2 seem to have a role in bacterium-cell interaction (Faherty et al., 2012). Thus, adhesion of *Shigella* can be divided into an initial adhesion which is subsequently followed by adhesion via T3SS effectors such as IpaB, leading to activation steps promoting invasion of *Shigella*. A more complete picture which explains initial adhesion of *Shigella* to host cells is so far not available. *Shigella* also achieves effector translocation and invasion into naïve B and T lymphocytes. Interestingly, interaction of *Shigella* with long-term activated lymphocytes led to effector translocation due to glycan-glycan interaction (Pinaud et al., 2017). Effector translocation into epithelial cells usually initiates invasion of *Shigella*. In contrast, effector translocation into activated T cells predominantly occurs without subsequent invasion, suggesting some sort of hit-and-run mechanism which inhibits the adaptive immune system. Changes occurring in T cells during long-term activation include modification of the plasma membrane composition with the accumulation of cholesterol and major changes in the ganglioside content (Belotserkovsky et al., 2018). In particular, a dramatic increase in the ganglioside GM1 content was observed, which suggests a direct glycan-glycan interaction between bacterial lipopolysaccharides and gangliosides leading to adhesion and subsequent effector translocation. This study demonstrated that alteration in ganglioside content is causative for the effector translocation (Belotserkovsky et al., 2018). These findings are in line with a recent study which showed that lipopolysaccharides/lipooligosaccharides of *Shigella*, *Salmonella*, *Campylobacter* and *Helicobacter* are able to directly interact with various glycans such as glycosaminoglycans, blood group antigens or Lewis antigens (Day et al., 2015). In addition, several adhesion factors are either glycoproteins or proteoglycans, for instance type I fimbriae of *E. coli* or *P. aeruginosa* lectins 1 and 2 that have specificity for galactose and fucose or PsaA which binds β1-linked galactosyl residues in glycosphingolipids and the phosphocholine group in phospholipids (Bao et al., 2013). Thus, besides the described glycan-glycan interaction shown for *Shigella* and activated T cells probably other glycan-glycan interactions might be of importance for host cell interaction leading to effector translocation. However, so far no further studies are available which confirm this assumption. Another lesson which can be learned by the *Shigella* system is that cell activation is an additional parameter which may alter host cell tropism for effector translocation.

For other Gram-negative bacteria using T3SS such as *Pseudomonas* or *Salmonella* there are only a few data available of factors which may be involved in effector translocation. In *P. aeruginosa*, the amount of the T3SS effector ExoS injected into host cells was reduced in a PilA-deficient strain, indicating that adhesion via type IV pili is important for efficient effector translocation (Hayashi et al., 2015). In *S. enterica*, invasion of polarized epithelium is dependent on the T1SS-secreted giant adhesin SiiE. SiiE is required for adhesion to polarized epithelial cells, for T3SS-mediated remodelling of the host cell cytoskeleton and therefore for proper epithelial barrier function. Thus, SiiE-promoted adhesion is crucial for efficient T3SS effector translocation (Barlag and

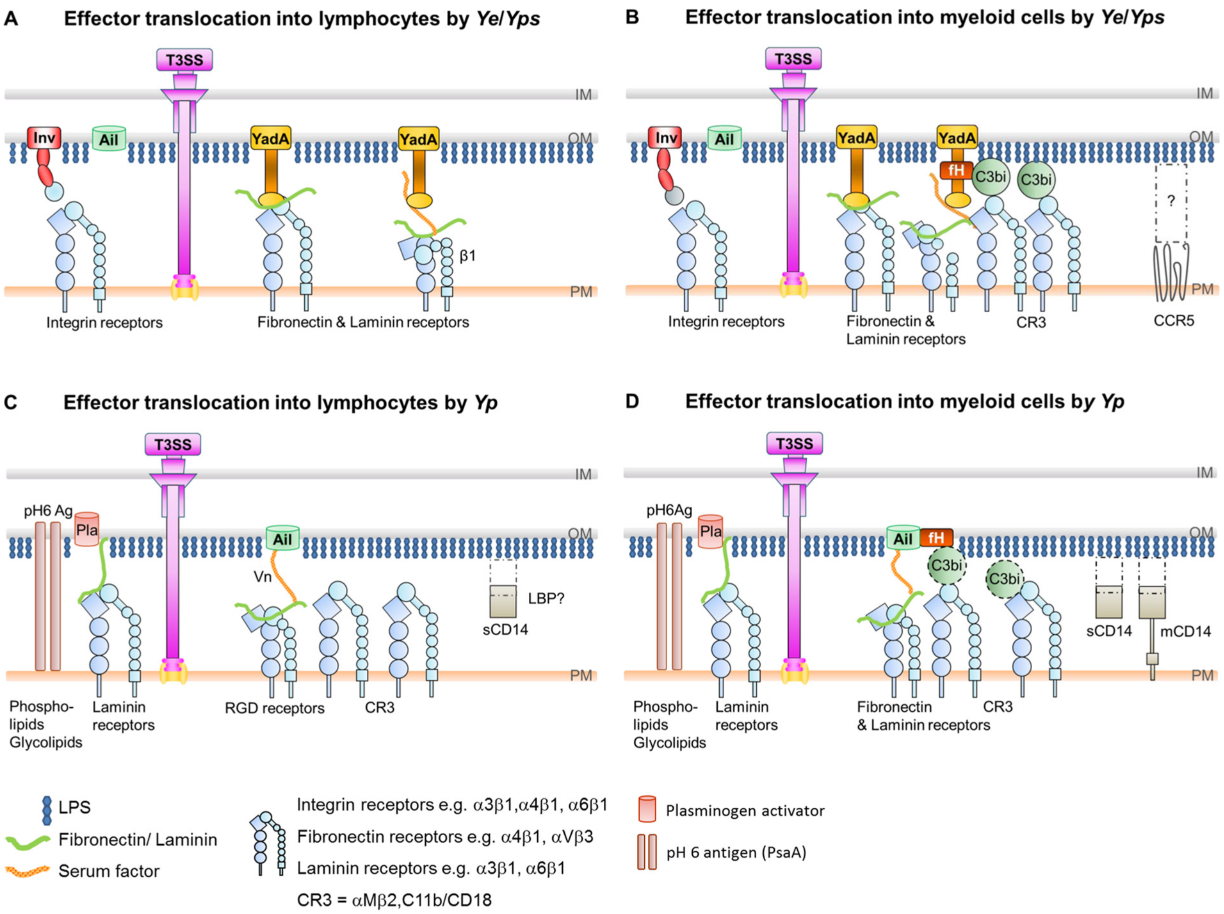

Fig. 1. Hypothetical models for adhesion to and effector translocation into *Yersinia*. (A) During *Ye/Yps* infection Inv and YadA can mediate adhesion leading to effector translocation. In the presence of YadA and Inv, adhesion via YadA is favoured according to the steric hindrance of Inv interaction. YadA interacts via ECM with both fibronectin binding integrin receptors (RGD receptors including α5β1 and αVβ3) and laminin binding integrin receptors (e.g. α3β1 and α6β1). Heat-stable serum factors inhibit the YadA mediated interaction. It is postulated that soluble Vn could be a factor binding to YadA and inhibiting interaction with insoluble ECM proteins. In the absence of YadA, Inv can take over the adhesion function and mediate effector translocation even more efficient because Inv mediated is not inhibited by serum factors. In the absence of YadA/Inv, Ail may contribute to effector translocation depending on the LPS structure, masking or not masking Ail. **(B)** In myeloid cells the inhibitory effect of heat-stable serum factors on YadA mediated adhesion via the YadA-ECM-integrin axes seems to be compensated due to myeloid cell-bacteria specific interactions. This might be achieved by predominantly myeloid specific receptors such as CCR5, CR3 and CD14 or so far unknown interactions. Hypothetically YadA may bind factorH which binds C3b and inactivates it to C3bi. C3bi deposited on the bacterial membrane may then interact with CR3. In addition an interaction by the LPS-LBP-CD14 can be postulated in the absence of adhesins. Adhesin independent interaction may be sufficient to mediate some effector translocation. **(C)** During *Yp* infection Ail replaces YadA. Ail binds ECM proteins which might also lead to integrin interaction. Serum factors then inhibit effector translocation in lymphocytes. Such factors might be again Vn interfering Ail-ECM interactions, but also heat-labile serum factors. **(D)** In myeloid cells the inhibitory effect of serum factors on translocation seems to be compensated in an Ail dependent manner. The remaining adhesion and effector translocation depends then predominantly on Ail. Whether in the presence of serum Pla and PsaA may contribute to effector translocation remains unclear, because in the absence of Ail effector translocation is strongly diminished but not in the absence of Pla and PsaA. Additional host cell receptors are expressed on myeloid cell such as CCR5, CR3 and CD14. Hypothetically Ail may bind factor H which binds C3b and inactivates it to C3bi. C3bi deposited on the bacterial membrane may then interact with CR3. In addition an interaction by the LPS-LBP-CD14 can be postulated. The importance of CR3 and CD14 into myeloid but not those of CCR5 for effector translocation was proven for *Yp*. Even in the absence of adhesins, adhesin independent may be sufficient to mediate some effector translocation. **IM**, Inner membrane; **OM**, Outer membrane; **PM**, Plasma membrane (eukaryotic).

Hensel, 2015; Gerlach et al., 2008).

4. Conclusion and perspectives

Studies of the T3SS effector translocation over the last decade not only presented a picture why and how pathogenic Gram-negative bacteria interact with host cells during infection but also unravelled at least for some pathogens the complex interweaving action of virulence mechanisms. However, not only the repertoire of virulence factors but also the properties of the host and specially cell-type specific differences determine why such pathogens interact preferentially with distinct cell types. Known host cell factors modulating effector translocation are summarized in Table 1.

Specifically infection with *Yersinia* species provides an example how different virulence mechanisms interlock in a complex network. YadA and Ail together with the specific LPS structure on bacteria strains and the T3SS form a sophisticated virulence network. While YadA and Ail are critical to provide serum resistance, they additionally bind compounds such as factor H cleaving C3 and C3bi as well as vitronectin which in turn modulate adhesion and shape host cell tropism.

Binding of YadA and hypothetically Ail indirectly via ECM proteins to β1 and αV family members also shapes adhesion to host cells. Thus, the lack of YadA and Ail does not only affect serum resistance but also adhesion, effector translocation and cell tropism. In addition, Ail can only contribute to all these processes in the presence of rough LPS. The rough stealth LPS used by *Yp* during infection of eukaryotes allows the unmasked Ail to be the most critical player confer serum resistance, adhesion and T3SS while YadA is dispensable. In the presence of

Table 1
Proof for cell tropism and host cell factors modulating T3SS injection.

	Yersinia	Salmonella	Shigella	Pseudomonas
Cell tropism				
towards myeloid cells	Deuschle et al., 2016; Durand et al., 2010; Köberle et al., 2009; Maldonado-Arocho et al., 2013; Merritt et al., 2015	Geddes et al., 2007		Rangel et al., 2015
towards dendritic cell subpopulations	Autenrieth et al., 2010			
towards B cell subpopulations	Köberle et al., 2009			
toward activated T cells			Belotserkovsky et al., 2018; Pinaud et al., 2017	
Host cell receptors				
β1 integrins	Deuschle et al., 2016; Keller et al., 2015; Köberle et al., 2009; Mejia et al., 2008			
αV integrins	Keller et al., 2015			
αMβ2 integrins (CR3)	Merritt et al., 2015			
CD14	Merritt et al., 2015			
Gangliosides			Belotserkovsky et al., 2018	
Extracellular matrix proteins	Heise and Dersch, 2006; Keller et al., 2015; Leo and Skurnik, 2011; Mühlenkamp et al., 2017; Tsang et al., 2012, 2010; Tsang et al., 2017			
Serum factors				
Heat sensitive	Merritt et al., 2015			
Heat stable	Deuschle et al., 2016; Maldonado-Arocho et al., 2013			
Cell activation and downstream signalling events leading to pore formation and/or translocation				
Actin cytoskeleton rearrangement	Deuschle et al., 2016; Mejia et al., 2008			
Rho GTPase activity	Keller et al., 2015; Schweer et al., 2013; Sheahan and Isberg, 2015; Wolters et al., 2013			
CCR5	Sheahan and Isberg, 2015			
Membrane vesicle trafficking	Sheahan and Isberg, 2015			

smooth LPS, the much longer YadA takes all the functions which otherwise would be fulfilled by Ail in *Yp*. However, the presented models describing the host cell specific effector translocation still include a lot of unproven hypotheses and questions: What exactly are the adhesin-independent mechanisms contributing to effector translocation? Are LBP and vitronectin indeed involved in modulating effector translocation? While many protein-protein interactions leading to adhesion and effector translocation are known, the importance of glycan-glycan interaction for adhesion and translocation is not so well understood. There are only a few examples such as the interaction of *Shigella* with activated lymphocytes which clearly present that glycan-glycan interaction and cell type specific differences in glycan composition on the cell surface can modulate bacteria-host cell interaction. Whether the general understanding of effector translocation helps us to achieve new therapeutical approaches is so far elusive. At least the development of the bla-reporter system provides a tool to easily screen for drugs acting against T3SS as a support to develop new anti-infectives.

Acknowledgments

The work was supported by grants of the German research council (SFB766)

References

Agbor, T.A., McCormick, B.A., 2011. *Salmonella* effectors: important players modulating host cell function during infection. Cell. Microbiol. 13, 1858–1869.

Autenrieth, S.E., Linzer, T.R., Hiller, C., Keller, B., Warnke, P., Koberle, M., Bohn, E., Biedermann, T., Bühring, H.J., Hämmerling, G.J., Rammensee, H.G., Autenrieth, I.B., 2010. Immune evasion by *Yersinia enterocolitica*: differential targeting of dendritic cell subpopulations in vivo. PLoS Pathog. 6, e1001212.

Bao, R., Nair, M.K., Tang, W.K., Esser, L., Sadhukhan, A., Holland, R.L., Xia, D., Schifferli, D.M., 2013. Structural basis for the specific recognition of dual receptors by the homopolymeric pH 6 antigen (Psa) fimbriae of Yersinia pestis. Proc. Natl. Acad. Sci. U. S. A. 110, 1065–1070.

Barlag, B., Hensel, M., 2015. The giant adhesin SiiE of Salmonella enterica. Molecules 20, 1134–1150.

Bauer, M., Brakebusch, C., Coisne, C., Sixt, M., Wekerle, H., Engelhardt, B., Fässler, R., 2009. Beta1 integrins differentially control extravasation of inflammatory cell subsets into the CNS during autoimmunity. Proc. Natl. Acad. Sci. U. S. A. 106, 1920–1925.

Belotserkovsky, I., Brunner, K., Pinaud, L., Rouvinski, A., Dellarole, M., Baron, B., Dubey, G., Samassa, F., Parsot, C., Sansonetti, P., Phalipon, A., 2018. Glycan-Glycan interaction determines *Shigella* tropism toward human t lymphocytes. MBio 9, e02309–02317.

Belotserkovsky, I., Sansonetti, P.J., 2018. *Shigella* and Enteroinvasive *Escherichia coli*. Curr. Top. Microbiol. Immunol. https://doi.org/10.1007/82_2018_104.

Biedzka-Sarek, M., Salmenlinna, S., Gruber, M., Lupas, A.N., Meri, S., Skurnik, M., 2008. Functional mapping of YadA- and Ail-mediated binding of human factor H to *Yersinia enterocolitica* serotype O:3. Infect. Immun. 76, 5016–5027.

Bliska, J.B., Wang, X., Viboud, G.I., Brodsky, I.E., 2013. Modulation of innate immune responses by *Yersinia* type III secretion system translocators and effectors. Cell. Microbiol. 15, 1622–1631.

Brakebusch, C., Grose, R., Quondamatteo, F., Ramirez, A., Jorcano, J.L., Pirro, A., Svensson, M., Herken, R., Sasaki, T., Timpl, R., Werner, S., Fässler, R., 2000. Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. EMBO J. 19, 3990–4003.

Briones, G., Hofreuter, D., Galan, J.E., 2006. Cre reporter system to monitor the translocation of type III secreted proteins into host cells. Infect. Immun. 74, 1084–1090.

Brotcke Zumsteg, A., Goosmann, C., Brinkmann, V., Morona, R., Zychlinsky, A., 2014. IcsA is a *Shigella flexneri* adhesin regulated by the type III secretion system and required for pathogenesis. Cell Host Microbe 15, 435–445.

Chan, K.C., Lucas, D.A., Hise, D., Schaefer, C.F., Xiao, Z., Janini, G.M., Buetow, K.H., Issaq, H.J., Veenstra, T.D., Conrads, T.P., 2004. Analysis of the human serum proteome. Clin. Proteomics 2, 101–225.

Charpentier, X., Oswald, E., 2004. Identification of the secretion and translocation domain of the enteropathogenic and enterohemorrhagic *Escherichia coli* effector Cif, using TEM-1 beta-lactamase as a new fluorescence-based reporter. J. Bacteriol. 186, 5486–5495.

Chauhan, N., Wrobel, A., Skurnik, M., Leo, J.C., 2016. *Yersinia* adhesins: an arsenal for infection. Proteomics Clin. Appl. 10, 949–963.

Day, C.J., Tran, E.N., Semchenko, E.A., Tram, G., Hartley-Tassell, L.E., Ng, P.S., King, R.M., Ulanovsky, R., McAtamney, S., Apicella, M.A., Tiralongo, J., Morona, R., Korolik, V., Jennings, M.P., 2015. Glycan:glycan interactions: high affinity biomolecular interactions that can mediate binding of pathogenic bacteria to host cells. Proc. Natl. Acad. Sci. U. S. A. 112, E7266–7275.

de Jong, M.F., Alto, N.M., 2018. Cooperative immune suppression by *Escherichia coli* and *Shigella* effector proteins. Infect. Immun. 86. https://doi.org/10.1128/IAI.00560-17.

Deng, W., Marshall, N.C., Rowland, J.L., McCoy, J.M., Worrall, L.J., Santos, A.S., Strynadka, N.C.J., Finlay, B.B., 2017. Assembly, structure, function and regulation of type III secretion systems. Nature reviews. Microbiology 15, 323–337.

Dersch, P., Isberg, R.R., 2000. An immunoglobulin superfamily-like domain unique to the *Yersinia pseudotuberculosis* invasin protein is required for stimulation of bacterial uptake via integrin receptors. Infect. Immun. 68, 2930–2938.

Deuschle, E., Keller, B., Siegfried, A., Manncke, B., Spaeth, T., Köberle, M., Drechsler-Hake, D., Reber, J., Böttcher, R.T., Autenrieth, S.E., Autenrieth, I.B., Bohn, E., Schütz, M., 2016. Role of beta1 integrins and bacterial adhesins for Yop injection into leukocytes in *Yersinia enterocolitica* systemic mouse infection. Int. J. Med. Microbiol.

306, 77–88.

Durand, E.A., Maldonado-Arocho, F.J., Castillo, C., Walsh, R.L., Mecsas, J., 2010. The presence of professional phagocytes dictates the number of host cells targeted for Yop translocation during infection. Cell. Microbiol. 12, 1064–1082.

Egan, F., Barret, M., O'Gara, F., 2014. The SPI-1-like Type III secretion system: more roles than you think. Front. Plant Sci. 5, 34.

Faherty, C.S., Redman, J.C., Rasko, D.A., Barry, E.M., Nataro, J.P., 2012. *Shigella flexneri* effectors OspE1 and OspE2 mediate induced adherence to the colonic epithelium following bile salts exposure. Mol. Microbiol. 85, 107–121.

Fässler, R., Meyer, M., 1995. Consequences of lack of beta 1 integrin gene expression in mice. Genes Dev. 9, 1896–1908.

Felek, S., Krukonis, E.S., 2009. The *Yersinia pestis* Ail protein mediates binding and Yop delivery to host cells required for plague virulence. Infect. Immun. 77, 825–836.

Geddes, K., Cruz, F., Heffron, F., 2007. Analysis of cells targeted by *Salmonella* type III secretion *in vivo*. PLoS Pathog. 3, e196.

Gerlach, R.G., Claudio, N., Rohde, M., Jackel, D., Wagner, C., Hensel, M., 2008. Cooperation of *Salmonella* pathogenicity islands 1 and 4 is required to breach epithelial barriers. Cell. Microbiol. 10, 2364–2376.

Grassl, G.A., Bohn, E., Müller, Y., Bühler, O.T., Autenrieth, I.B., 2003. Interaction of *Yersinia enterocolitica* with epithelial cells: invasin beyond invasion. Int. J. Med. Microbiol. 293, 41–54.

Hamburger, Z.A., Brown, M.S., Isberg, R.R., Bjorkman, P.J., 1999. Crystal structure of invasin: a bacterial integrin-binding protein. Science 286, 291–295.

Harmon, D.E., Davis, A.J., Castillo, C., Mecsas, J., 2010. Identification and characterization of small-molecule inhibitors of Yop translocation in Yersinia pseudotuberculosis. Antimicrob. Agents Chemother. 54, 3241–3254.

Hayashi, N., Nishizawa, H., Kitao, S., Deguchi, S., Nakamura, T., Fujimoto, A., Shikata, M., Gotoh, N., 2015. *Pseudomonas aeruginosa* injects type III effector ExoS into epithelial cells through the function of type IV pili. FEBS Lett. 589, 890–896.

Heise, T., Dersch, P., 2006. Identification of a domain in *Yersinia* virulence factor YadA that is crucial for extracellular matrix-specific cell adhesion and uptake. Proc. Natl. Acad. Sci. U. S. A. 103, 3375–3380.

Ho, D.K., Riva, R., Kirjavainen, V., Jarva, H., Ginstrom, E., Blom, A.M., Skurnik, M., Meri, S., 2012. Functional recruitment of the human complement inhibitor C4BP to *Yersinia pseudotuberculosis* outer membrane protein Ail. J. Immunol. 188, 4450–4459.

Ho, D.K., Skurnik, M., Blom, A.M., Meri, S., 2014. *Yersinia pestis* Ail recruitment of C4b-binding protein leads to factor I-mediated inactivation of covalently and non-covalently bound C4b. Eur. J. Immunol. 44, 742–751.

Hoiczyk, E., Blobel, G., 2001. Polymerization of a single protein of the pathogen *Yersinia enterocolitica* into needles punctures eukaryotic cells. Proc. Natl. Acad. Sci. U. S. A. 98, 4669–4674.

Isberg, R.R., Leong, J.M., 1990. Multiple beta 1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. Cell 60, 861–871.

Jacobi, C.A., Roggenkamp, A., Rakin, A., Zumbihl, R., Leitritz, L., Heesemann, J., 1998. In vitro and in vivo expression studies of yopE from *Yersinia enterocolitica* using the gfp reporter gene. Mol. Microbiol. 30, 865–882.

Jennings, E., Thurston, T.L.M., Holden, D.W., 2017. *Salmonella* SPI-2 type III secretion system effectors: molecular mechanisms and physiological consequences. Cell Host Microbe 22, 217–231.

Johnson, R., Mylona, E., Frankel, G., 2018. Typhoidal *Salmonella*: distinctive virulence factors and pathogenesis. Cell. Microbiol. 20, e12939.

Keller, B., Mühlenkamp, M., Deuschle, E., Siegfried, A., Mössner, S., Schade, J., Griesinger, T., Katava, N., Braunsdorf, C., Fehrenbacher, B., Jimenez-Soto, L.F., Schaller, M., Haas, R., Genth, H., Retta, S.F., Meyer, H., Böttcher, R.T., Zent, R., Schütz, M., Autenrieth, I.B., Bohn, E., 2015. *Yersinia enterocolitic*a exploits different pathways to accomplish adhesion and toxin injection into host cells. Cell. Microbiol. 17, 1179–1204.

Köberle, M., Klein-Günther, A., Schütz, M., Fritz, M., Berchtold, S., Tolosa, E., Autenrieth, I.B., Bohn, E., 2009. *Yersinia enterocolitica* targets cells of the innate and adaptive immune system by injection of Yops in a mouse infection model. PLoS Pathog. 5, e1000551.

Leo, J.C., Skurnik, M., 2011. Adhesins of human pathogens from the genus Yersinia. Adv. Exp. Med. Biol. 715, 1–15.

Leong, J.M., Morrissey, P.E., Isberg, R.R., 1993. A 76-amino acid disulfide loop in the *Yersinia pseudotuberculosis* invasin protein is required for integrin receptor recognition. J. Biol. Chem. 268, 20524–20532.

Mahmoud, R.Y., Stones, D.H., Li, W., Emara, M., El-Domany, R.A., Wang, D., Wang, Y., Krachler, A.M., Yu, J., 2016. The Multivalent Adhesion Molecule SSO1327 plays a key role in *Shigella sonnei* pathogenesis. Mol. Microbiol. 99, 658–673.

Maldonado-Arocho, F.J., Green, C., Fisher, M.L., Paczosa, M.K., Mecsas, J., 2013. Adhesins and host serum factors drive yop translocation by *Yersinia* into professional phagocytes during animal infection. PLoS Pathog. 9, e1003415.

Marketon, M.M., DePaolo, R.W., DeBord, K.L., Jabri, B., Schneewind, O., 2005. Plague bacteria target immune cells during infection. Science 309, 1739–1741.

Mejia, E., Bliska, J.B., Viboud, G.I., 2008. *Yersinia* controls type III effector delivery into host cells by modulating Rho activity. PLoS Pathog. 4, e3.

Merritt, P.M., Nero, T., Bohman, L., Felek, S., Krukonis, E.S., Marketon, M.M., 2015. *Yersinia pestis* targets neutrophils via complement receptor 3. Cell. Microbiol. 17, 666–687.

Morgan, J.M., Lam, H.N., Delgado, J., Luu, J., Mohammadi, S., Isberg, R.R., Wang, H., Auerbuch, V., 2018. An experimental pipeline for initial characterization of bacterial type iii secretion system inhibitor mode of action using enteropathogenic yersinia. Front. Cell. Infect. Microbiol. 8, 404.

Mühlenkamp, M., Oberhettinger, P., Leo, J.C., Linke, D., Schütz, M.S., 2015. *Yersinia* adhesin A (YadA)—beauty & beast. Int. J. Med. Microbiol. 305, 252–258.

Mühlenkamp, M.C., Hallström, T., Autenrieth, I.B., Bohn, E., Linke, D., Rinker, J., Riesbeck, K., Singh, B., Leo, J.C., Hammerschmidt, S., Zipfel, P.F., Schütz, M.S., 2017. Vitronectin binds to a specific stretch within the head region of *Yersinia* adhesin a and thereby modulates *Yersinia enterocolitica* host interaction. J. Innate Immun. 9, 33–51.

Paczosa, M.K., Fisher, M.L., Maldonado-Arocho, F.J., Mecsas, J., 2014. *Yersinia pseudotuberculosis* uses Ail and YadA to circumvent neutrophils by directing Yop translocation during lung infection. Cell. Microbiol. 16, 247–268.

Pechous, R.D., Sivaraman, V., Price, P.A., Stasulli, N.M., Goldman, W.E., 2013. Early host cell targets of *Yersinia pestis* during primary pneumonic plague. PLoS Pathog. 9, e1003679.

Pepe, J.C., Badger, J.L., Miller, V.L., 1994. Growth phase and low pH affect the thermal regulation of the *Yersinia enterocolitica* inv gene. Mol. Microbiol. 11, 123–135.

Pepe, J.C., Miller, V.L., 1993. *Yersinia enterocolitica* invasin: a primary role in the initiation of infection. Proc. Natl. Acad. Sci. U. S. A 90, 6473–6477.

Pha, K., Navarro, L., 2016. *Yersinia* type III effectors perturb host innate immune responses. World J. Biol. Chem. 7, 1–13.

Picking, W.L., Picking, W.D., 2016. The many faces of IpaB. Front. Cell. Infect. Microbiol. 6, 12.

Pinaud, L., Samassa, F., Porat, Z., Ferrari, M.L., Belotserkovsky, I., Parsot, C., Sansonetti, P.J., Campbell-Valois, F.X., Phalipon, A., 2017. Injection of T3SS effectors not resulting in invasion is the main targeting mechanism of Shigella toward human lymphocytes. Proc. Natl. Acad. Sci. U. S. A. 114, 9954–9959.

Pinaud, L., Sansonetti, P.J., Phalipon, A., 2018. Host cell targeting by enteropathogenic Bacteria t3ss effectors. Trends Microbiol. 26, 266–283.

Plano, G.V., Schesser, K., 2013. The *Yersinia pestis* type III secretion system: expression, assembly and role in the evasion of host defenses. Immunol. Res. 57, 237–245.

Platenkamp, A., Mellies, J.L., 2018. Environment Controls LEE Regulation in Enteropathogenic Escherichia coli. Front. Microbiol. 9, 1694.

Rangel, S.M., Diaz, M.H., Knoten, C.A., Zhang, A., Hauser, A.R., 2015. The role of ExoS in dissemination of *Pseudomonas aeruginosa* during pneumonia. PLoS Pathog. 11, e1004945.

Ross, G.D., 2002. Role of the lectin domain of Mac-1/CR3 (CD11b/CD18) in regulating intercellular adhesion. Immunol. Res. 25, 219–227.

Schindler, M.K., Schütz, M.S., Mühlenkamp, M.C., Rooijakkers, S.H., Hallström, T., Zipfel, P.F., Autenrieth, I.B., 2012. *Yersinia enterocolitica* YadA mediates complement evasion by recruitment and inactivation of C3 products. J. Immunol. 189, 4900–4908.

Schweer, J., Kulkarni, D., Kochut, A., Pezoldt, J., Pisano, F., Pils, M.C., Genth, H., Huehn, J., Dersch, P., 2013. The cytotoxic necrotizing factor of *Yersinia pseudotuberculosis* (CNFY) enhances inflammation and Yop delivery during infection by activation of Rho GTPases. PLoS Pathog. 9, e1003746.

Sheahan, K.L., Isberg, R.R., 2015. Identification of mammalian proteins that collaborate with type III secretion system function: involvement of a chemokine receptor in supporting translocon activity. MBio 6, e02023–02014.

Singh, B., Fleury, C., Jalalvand, F., Riesbeck, K., 2012. Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of the host. FEMS Microbiol. Rev. 36, 1122–1180.

Small, A.G., Al-Baghdadi, M., Quach, A., Hii, C., Ferrante, A., 2016. Complement receptor immunoglobulin: a control point in infection and immunity, inflammation and cancer. Swiss Med. 146, w14301.

Thomson, J.J., Plecha, S.C., Krukonis, E.S., 2019. Ail provides multiple mechanisms of serum resistance to Yersinia pestis. Mol. Microbiol. 111, 82–95.

Tsang, T.M., Annis, D.S., Kronshage, M., Fenno, J.T., Usselman, L.D., Mosher, D.F., Krukonis, E.S., 2012. Ail protein binds ninth type III fibronectin repeat (9FNIII) within central 120-kDa region of fibronectin to facilitate cell binding by Yersinia pestis. J. Biol. Chem. 287, 16759–16767.

Tsang, T.M., Felek, S., Krukonis, E.S., 2010. Ail binding to fibronectin facilitates *Yersinia pestis* binding to host cells and Yop delivery. Infect. Immun. 78, 3358–3368.

Tsang, T.M., Wiese, J.S., Alhabeil, J.A., Usselman, L.D., Thomson, J.J., Matti, R., Kronshage, M., Maricic, N., Williams, S., Sleiman, N.H., Felek, S., Krukonis, E.S., 2017. Defining the ail ligand-binding surface: hydrophobic residues in two extracellular loops mediate cell and extracellular matrix binding to facilitate yop delivery. Infect. Immun. 85, e01047–15.

Tsang, T.M., Wiese, J.S., Felek, S., Kronshage, M., Krukonis, E.S., 2013. Ail proteins of *Yersinia pestis* and *Y. pseudotuberculosis* have different cell binding and invasion activities. PLoS One 8, e83621.

Wolters, M., Boyle, E.C., Lardong, K., Trulzsch, K., Steffen, A., Rottner, K., Ruckdeschel, K., Aepfelbacher, M., 2013. Cytotoxic necrotizing factor-Y boosts *Yersinia* effector translocation by activating Rac protein. J. Biol. Chem. 288, 23543–23553.

Yang, S.C., Hung, C.F., Aljuffali, I.A., Fang, J.Y., 2015. The roles of the virulence factor IpaB in *Shigella* spp. in the escape from immune cells and invasion of epithelial cells. Microbiol. Res. 181, 43–51.