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Review Article

Nonprofessional Phagocytic Cell Receptors Involved in *Staphylococcus aureus* Internalization

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Staphylococcus aureus is a successful human and animal pathogen. The majority of infections caused by this pathogen are life threatening, primarily because *S. aureus* has developed multiple evasion strategies, possesses intracellular persistence for long periods, and targets the skin and soft tissues. Therefore, it is very important to understand the mechanisms employed by *S. aureus* to colonize and proliferate in these cells. The aim of this review is to describe the recent discoveries concerning the host receptors of nonprofessional phagocytes involved in *S. aureus* internalization. Most of the knowledge related to the interaction of *S. aureus* with its host cells has been described in professional phagocytic cells such as macrophages. Here, we showed that in nonprofessional phagocytes the $\alpha 5\beta 1$ integrin host receptor, chaperons, and the scavenger receptor CD36 are the main receptors employed during *S. aureus* internalization. The characterization and identification of new bacterial effectors and the host cell receptors involved will undoubtedly lead to new discoveries with beneficial purposes.

1. Introduction

Staphylococcus is a Gram-positive commensal and opportunistic human pathogen that causes serious community-acquired and nosocomial infections, including abscess formation, wound infection, endocarditis, osteomyelitis, pneumonia, and sepsis/septic shock [1, 2]. Additionally, strains of *S. aureus* cause diseases in cattle (mastitis), poultry, pigs, and horses [3, 4]. Treatment of these infections has become difficult because of the emergence of antibiotic-resistant strains [5].

Evidence exists that several strains of *S. aureus* have the ability to invade and persist within nonprofessional phagocytic cells (NPPCs), such as epithelial [6–8], endothelial [9, 10], osteoblast [11, 12], fibroblast [13, 14], and kidney cells [15, 16]. This ability enables the bacteria to evade the host innate immune system and to survive inside a wide variety of mammalian cells. Bacteria initially adhere to the cell membrane and extracellular matrix substrates through surface proteins (adhesins) [17, 18] and are then internalized by different NPPCs.

Several reviews have discussed the intracellular persistence of this bacterium [19], the role of small colony variants (SCVs) [20], and the fate of the infected phagosome in professional phagocytes as well as in different NPPCs [21]. In this review, we will focus on the host NPPC receptors that are involved in the molecular interaction with *S. aureus* to accomplish bacterial internalization. Finally, we will discuss the medical implications derived from this knowledge and show a summary of the host receptors related to *S. aureus* internalization in NPPCs in Figure 1.

2. Bacterial Adhesion and Internalization

Bacterial internalization is a strategy that allows bacteria to evade the host immune response and to survive in the host cells. Several bacteria require initial adhesion to the host cell before the internalization process. Therefore, the adhesion and invasion into eukaryotic cells are major steps in bacterial pathogenesis [18].

Bacteria are capable of adhering to extracellular matrix components (i.e., collagen, vitronectin, fibrinogen, and especially fibronectin (Fn)) through protein-protein interactions mediated by "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs) or "secreted

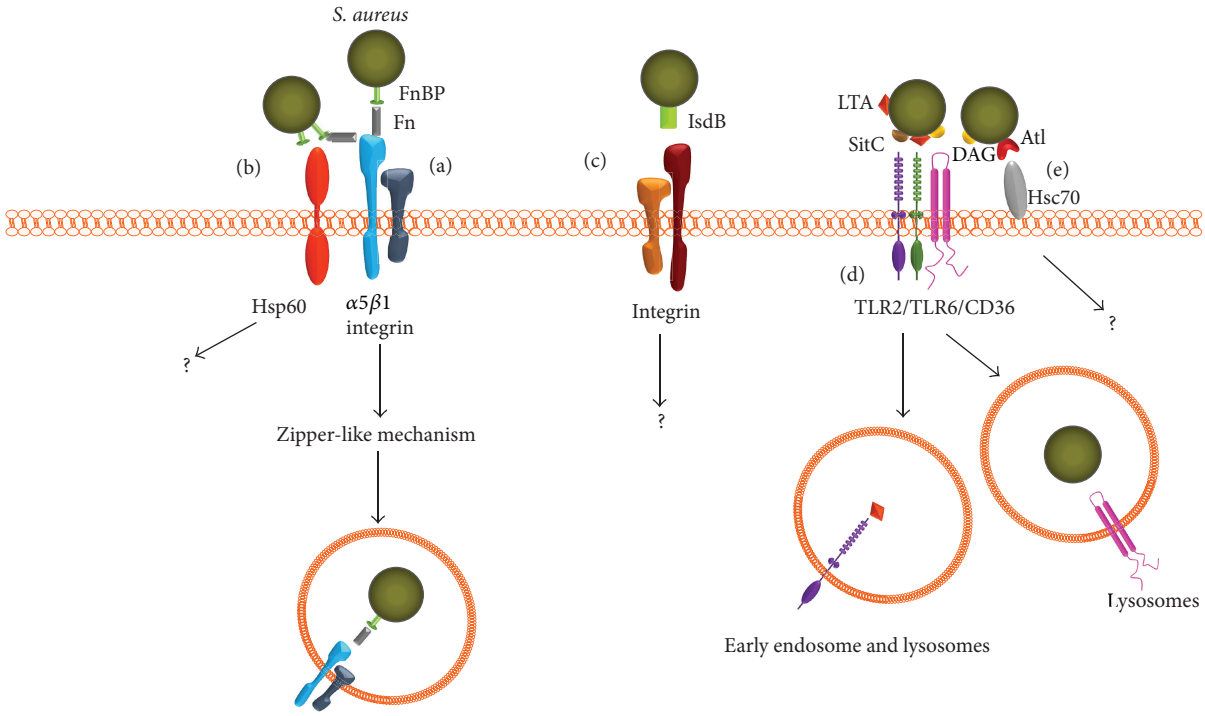

Figure 1: Different receptors and mechanisms involved in *S. aureus* internalization into nonprofessional phagocytic cells. (a) The first mechanism described for *S. aureus* internalization involved the $\alpha 5\beta 1$ integrin host receptor and is mediated by bacterial FnBPs via Fn as a linking molecule; bacterial endocytosis is accomplished through a zipper-like mechanism [9, 16, 22, 23]. (b) FnBPs interact directly with host Hsp60 or with integrin and Hsp60 as a coreceptor through a Fn bridge [24], but the mechanism of endocytosis remains unknown. (c) The *S. aureus* iron-regulated surface determinant-B (IsdB) contributed to invasion, and IsdB most likely interacts with integrins that bind ligands with the RGD motif [25]; however, the endocytic pathway has not been determined. (d) TLR2 is involved in *S. aureus* internalization. CD36 acts as a coreceptor and is capable of recognizing diacylglycerides, whereas TLR2/TLR6 dimers recognize different PAMPs, such as LTA and SitC [26–28]. In monocytes TLR2 colocalizes with LTA in early endosomes and lysosomes [29]. In HeLa cells, internalized *S. aureus* colocalizes with CD36 [30]. (e) The host chaperone Hsc70 binds directly to autolysin (Atl) and mediates *S. aureus* internalization [31], but the endocytic routes remain uncharacterized.

expanded repertoire adhesive molecules." Additionally, bacterial adhesins recognize host cell surface elements such as integrins, cadherins, and selectins [18]. Pathogen adhesion occurs in two ways: (1) adhesins directly engage the host cell surface receptor, that is, *Listeria* spp. [37], *Yersinia* spp. [38, 39], and *Neisseria gonorrhoeae* [39, 40], and (2) bacterial connections form indirectly with the host receptor via the recruitment of extracellular matrix proteins (e.g., *S. aureus*) [16, 41].

The bacterial engagement of eukaryotic receptors such as integrins often triggers a receptor-mediated internalization process that facilitates access to a protected intracellular niche, promoting bacterial replication [6, 42].

3. The Interaction between Nonprofessional Phagocyte Cell Receptors and *Staphylococcus aureus* Virulence Factors Promotes Internalization

S. aureus possesses a wide arsenal of virulence factors (adhesins, invasins, enzymes, toxins, and surface components) that contribute to the pathogenesis of infection (reviewed in Zecconi and Scali, 2013) [43]. These components promote the bacterial evasion of the host immune system as well as the colonization, dissemination, tissue damage, and transmission [1, 43]. *S. aureus* expresses adhesins such as fibronectin-binding proteins (FnBPs), fibrinogen-binding proteins, elastin-binding proteins, collagen-binding proteins, clumping factor, extracellular adhesion protein (Eap), and protein A [43–45]. *S. aureus* also possesses other cell-associated components such as capsular polysaccharide, peptidoglycan (PGN), and lipoteichoic acid (LTA) and secretes components such as enzymes (coagulase, lipase, hyaluronidase, and protease) and toxins (enterotoxins, toxic shock syndrome, hemolysins, and leukocidin), which are very important for the establishment of infection [1, 43, 46]. In the next sections, we will describe the *S. aureus* components and their cognate receptors in NPPCs that lead to bacterial internalization.

4. $\alpha 5\beta 1$ Integrin and Fibronectin Receptors

Integrins are cation-dependent glycoprotein transmembrane receptors containing noncovalently associated α- and β-subunits [47]. In vertebrates, at least 18 α- and 8 β-subunits have been described [48]. Integrins have an extracellular

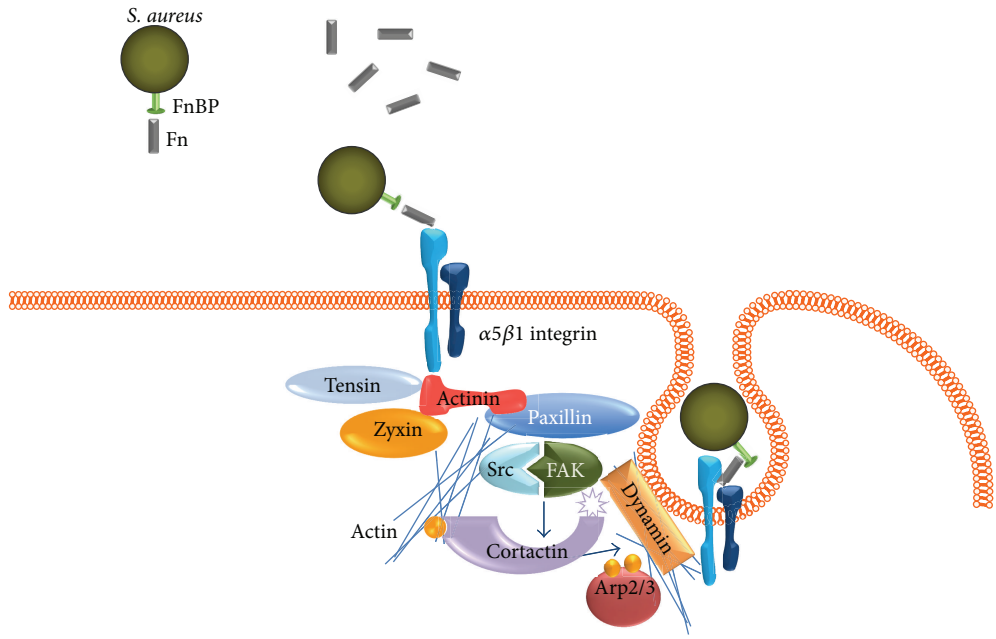

Figure 2: Summary of $\alpha5\beta1$ integrin-mediated internalization of *S. aureus* into NPPCs. The RGD motif in fibronectin (Fn) is the crucial attachment site for fibronectin receptors, such as integrins. The activation and clustering of $\alpha5\beta1$ integrin trigger particular signaling pathways and the accumulation of a focal adhesion-like protein complex in the vicinity of attached bacteria, as characterized by the recruitment of actinin, paxillin, zyxin, tensin, focal adhesion kinase (FAK), and Src kinase [32–34]. A crucial step in these signaling events is the reorganization of the actin cytoskeleton. Cortactin, an actin-binding protein, has been identified as one of the effectors of activated FAK and Src kinases, which associates with Arp2/3 complex to promote actin polymerization and binds to dynamin-2, a regulator of endocytosis [33, 35, 36].

binding domain that recognizes RGD or LVD sequences in ligands such as Fn, fibrinogen, vitronectin, and laminin [47, 48]. These receptors mediate a wide range of physiological and pathological processes, including cellular adhesion, migration, differentiation, apoptosis, phagocytosis, wound healing, and cancer. In addition, many integrins participate in pathogen recognition and host defense response in NPPCs; that is, $\beta1$ integrin mediates adhesion and endocytosis of *Yersinia* [39] and *S. aureus* [16, 41]. This event is mediated by a zipper-like process and depends on remodeling the actin cytoskeleton and membrane dynamics [49, 50]. The detailed mechanism for zipper-like-mediated internalization of *S. aureus* in NPPCs is shown in Figure 2.

Fn is a key dimeric glycoprotein in the extracellular matrix. The ability to bind to Fn is a characteristic of bacterial adhesion, which is a well-known mechanism described for many pathogens, including *S. aureus*. This bacterium expresses two closely related FnBPs encoded by the genes *fnbA* and *fnbB* [51], which are both contained in the majority of isolates with invasive properties [52].

Since the 1980s, it has been well recognized that *S. aureus* adhesion and internalization via a zipper-like process in NPPCs are mediated by integrins, Fn, and FnBPs. The role of FnBPs during *S. aureus* invasion has been established in endothelial cells [9, 10], osteoblasts [53], keratinocytes [54, 55], fibroblast [56], and epithelial cells [16, 22]. The events of internalization that occur via a zipper-like process were elucidated by experiments that included the following: (1) FnBP-deletion mutants of invasive strains; (2) noninvasive strains that express FnBPs; (3) the Fn-binding soluble domain isolated from FnBP; and (4) the blockage of receptors using anti-$\alpha5\beta1$ or anti-Fn antibodies. The results of these approaches showed that FnBPA has a relevant role in invasion because its deletion in the *S. aureus* Cowan strain diminished the level of invasiveness (~80%) into a human embryonic kidney cell line (HEK 293) [16]. Similarly, an isogenic mutant (DU5883) of *S. aureus* (8325-4) that does not express FnBPs showed reduced internalization into transformed bovine mammary epithelial cells (MAC-T cells) [22], osteoblasts [53], and keratinocytes [57]. The role of FnBPs in host invasion was confirmed using complementation assays in which noninvasive strains transformed with plasmid overexpressing FnBPs were able to invade NPPCs [16]. The presence of FnBPs on the surface of *S. aureus* confers the advantage for tissue colonization *in vivo*, as observed in mammary glands, and confers the induction of severe infection [58, 59]. In addition, Dziewanowska et al. (1999) showed that FnBP-mediated bacterial uptake by NPPCs requires actin polymerization and is dependent on tyrosine kinases [22].

In contrast, the role of Fn was initially elucidated in HEK 293 cells. The preincubation of these cells with a soluble recombinant protein fragment composed of the Fn-binding domain of FnBP completely abolished the invasion by *S. aureus* Cowan and P1 strains, presumably by competing with the *S. aureus* FnBP to interact with the host cell receptor [16]. The use of polyclonal anti-Fn antibodies corroborated the role of Fn during *S. aureus* internalization in other cell types, for example, endothelial cells [9, 16, 24]. These data

demonstrated that Fn mediates the interaction of *S. aureus* FnBPs with NPPCs.

The role of integrins during *S. aureus* internalization into NPPCs has been demonstrated by blockage experiments with antibodies. The blockage of integrin $\alpha5\beta1$ by specific antibodies in HEK 293 [16], in HUVEC [60] cells, or in keratinocytes [57] demonstrated that these receptors have a relevant role during *S. aureus* internalization because their blockage leads to a significant reduction of internalized bacteria. Additionally, a monoclonal antibody specific for $\beta1$ integrins dramatically reduced *S. aureus* invasion into human Hep-2 cells [24]. In addition, a mutant mouse fibroblast line (GD25) lacking $\beta1$ integrin showed significantly reduced bacterial invasion [23]. Recent work by Ridley et al. (2012) showed that both the availability and functional state of integrin $\alpha5\beta1$ are crucial for *S. aureus* invasion in different epithelial cells [61]. The use of GRGDS, a competitive inhibitor of $\beta1$ integrin ligands, has demonstrated the role of integrin during the internalization of *S. aureus* into alveolar epithelial cells (A549) by reducing the number of CFU recovered. In this work, the siRNA-mediated knockdown of $\beta1$ integrin expression in A459 cells significantly reduced *S. aureus* internalization (~50%) [8]. In addition, indirect evidence from our group established that the blockage of this integrin with latex beads covered with Fn inhibits *S. aureus* internalization into primary bovine mammary epithelial cells [62].

Overall, these results strongly suggest that *S. aureus* FnBPs and $\alpha5\beta1$ integrin are necessary for efficient *S. aureus* internalization into NPPCs; however, other mechanisms are employed by this bacterium favoring its internalization that we will describe below.

5. Heat Shock Proteins

Heat shock proteins (Hsps) are a group of evolutionarily highly conserved molecules that are expressed by prokaryotic and eukaryotic cells. These proteins perform important intracellular functions regarding protein folding and transport [63].

The role of Hsps during *S. aureus* internalization into NPPCs was first reported by Dziewanowska et al. (2000) [24]. Using a ligand blotting assay, Dziewanowska and colleagues identified that Hsp60 interacts with FnBP and showed that the pretreatment of epithelial cells with a monoclonal antibody specific for eukaryotic Hsp60 significantly reduces *S. aureus* internalization. Another Hsp related to *S. aureus* internalization in NPPCs is Hsc70. This protein is associated with viral infections by acting as a receptor for human T-cell lymphotropic virus type 1 (HTLV-1) [64] or rotaviruses [65, 66]. Hsc70 interacts with *S. aureus* hydrolases such as autolysin (Atl) during the bacterial internalization process. Atl participates in biofilm formation and mediates binding to the extracellular matrix and plasma proteins [31, 67, 68]. Hirschhausen et al. (2010) analyzed the *atl*-deficient *S. aureus* mutant SA113*atl* strain for its capability to be internalized into endothelial cells, and they showed the impaired ability of this strain to be endocytosed by these host cells [31]. Additionally, they reported that Atl binds directly to endothelial Hsc70 without a bridging molecule such as Fn. In addition, antibody blockade of Hsc70 decreases *S. aureus* internalization in these cells, and this protein has also been involved during *Brucella abortus* invasion into trophoblast giant cells [69], which suggests that this receptor is used as a generalized pathway during bacterial internalization.

6. Toll-Like Receptors

TLRs offer an efficient and immediate response to bacterial, fungal, and viral infections by recognizing PAMPs. The TLR family consists of 13 mammalian members, and each member mediates an intrinsic signaling pathway and induces specific biological responses against microorganisms [70]. The cytoplasmic domain (Toll/IL-1 receptor domain) of TLRs is required for the signaling response leading to the activation of transcription factors such as NF-κB [70]. The leucine-rich repeat (LRR) extracellular motif is responsible for the recognition of PAMPs [71]. TLRs are activated by ligand-induced multimerization and act by cooperating with several proteins such as other TLRs or coreceptors.

For *S. aureus* infections, TLR2 is the most relevant receptor involved in this process. TLR2 recognizes different PAMPs such as lipopeptides from Gram-positive and Gram-negative bacteria, lipoarabinomannan, LTA, PGN, atypical lipopolysaccharide, a phenol-soluble modulin from *S. epidermidis*, and others [72]. Additionally, TLR2 interacts with TLR1 and TLR6 in the process of ligand recognition, and the TLR2/TLR6 heterodimer recognizes the PGN in the macrophage phagosome [73] and a diacylated mycoplasma lipoprotein [74], while the TLR2/TLR1 heterodimer recognizes triacylated lipopeptides [75]. Reports have described the participation of TLR2 during *S. aureus* internalization in NPPCs; however, the results are not conclusive because TLR2 participation in phagocytosis may be indirect. For example, Rocha-de-Souza et al. (2008) indicated that TLR2 is involved in *S. aureus* internalization into human cord blood-derived mast cells using neutralizing antibodies [26]. The blockage of TLR2 in these cells decreases the number of bacteria internalized. In our work, we observed a similar result in primary bovine mammary epithelial cells (data unpublished); however, it remains to be clarified whether TLR2-mediated internalization is the consequence of the signaling activity of this receptor or whether the recognition of bacterial PAMPs by TLR2 is a key step for endocytosis. Although TLRs are not phagocytic receptors *per se*, they are also internalized in the process and participate in the link between phagocytosis and inflammatory responses by triggering the production of cytokines [76]. In addition, TLR2 is located in phagosomes and colocalizes with different *S. aureus* PAMPs. In NPPCs, the predominant triacylated lipoprotein of *S. aureus*, SitC, is located intracellularly with TLR2 in murine keratinocytes and stimulates proinflammatory cytokine expression [77]; however, SitC is internalized in a TLR2-independent manner. The results described above suggest that although no clear role of TLR2 has been observed during *S. aureus* internalization, this process appears to be a prerequisite for full TLR2

activation in both professional phagocytic cells as well as in NPPCs [76].

7. Coreceptors for TLR2 Mediate *Staphylococcus aureus* Recognition

CD36 is a membrane glycoprotein that belongs to the class B scavenger receptor family that interacts with other membrane receptors such as TLRs. This receptor plays a role during tumor growth, inflammation, wound healing, and angiogenesis and is able to recognize PAMPs or pathogen-infected cells by acting as a phagocytic receptor [78, 79]. During the host recognition of *S. aureus* mediated by TLR2, CD36 may act as a facilitator or coreceptor for diacylglyceride recognition through the TLR2/6 complex mediating bacterial invasion primarily in phagocytic cells [27]. In the NPPC line HEK 293, the overexpression of CD36 confers binding and uptake of *S. aureus*, suggesting a role for CD36 during the endocytosis of Gram-positive bacteria [28]. In addition, Lee-lahavanichkul et al. (2012) have demonstrated that intracellular *S. aureus* colocalizes with CD36 in HeLa cells [30]. CD14, a glycosylphosphatidylinositol-anchored membrane protein, is another coreceptor that participates in bacterial recognition by TLRs and enhances PGN and LTA signal transmission through TLR2 [80]. CD14/TLR2 is an essential receptor complex involved in Panton-Valentine leukocidin recognition [81]. CD14 and CD36 play a prominent role in LTA binding and enhancing LTA-induced signaling in human monocytes [29]. The aforementioned involvement suggests that CD14 may have a similar role as CD36 in *S. aureus* internalization; however, this effect remains to be fully explored.

8. Other *Staphylococcus aureus* Virulence Factors that Participate in the Internalization Process Interact with Uncharacterized Host Cell Receptors

As we have described above, several host receptors are used by *S. aureus* to invade NPPCs (Figure 1). Nonetheless, reports have indicated that different uncharacterized host receptors may be involved in *S. aureus* internalization in NPPCs. In the next section, we will describe several bacteria virulence factors involved in internalization whose host receptors remain to be characterized.

9. The Extracellular Adherence Protein

The extracellular adherence protein (Eap) in *S. aureus* binds to matrix extracellular components, inhibits leukocyte adhesion to endothelial cells, acts like an anti-inflammatory factor [82], and causes *S. aureus* agglutination [83]. This protein stimulates the adherence of *S. aureus* to epithelial cells [83] and fibroblasts [84]. Eap also participates during the bacterial internalization process because its absence reduces the adherence and internalization of *S. aureus* into fibroblast and epithelial cells [14], while the addition of exogenous Eap increases *S. aureus* internalization [85, 86]. This invasion process may be influenced by the 32 kDa neutral phosphatase that is located on the bacterial surface that binds to Eap [87]; however, no reports have yet described the identification of a host receptor for Eap.

10. Glyceraldehyde-3-Phosphate Dehydrogenase-C

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme, and several GAPDH homologs present in bacteria are able to bind to Fn, lysozyme, plasminogen, and the cytoskeletal proteins myosin and actin. Therefore, this enzyme plays a role during *S. aureus* colonization and internalization [88, 89]. *S. aureus* has two GAPDH homologs termed *gapA* (also known as *gapC* in a bovine mastitis isolate) and *gapB* [90], and both proteins are important in the pathogenesis of *S. aureus* in a *Galleria mellonella* model of infection [91].

GapC plays an important role during *S. aureus* internalization into MAC-T cells [92]. The number of CFUs recovered from an isogenic *gapC* mutant H330 strain that were adhered and internalized into MAC-T cells was lower than the number corresponding to the WT strain. Nevertheless, the absence of *gapC* does not completely abolish the attachment and internalization of the bacteria, which is most likely due to the presence of other bacterial adhesins [92]. No reports have yet described the identification of a host receptor that recognizes *gapC*.

11. Iron-Regulated Surface Determinant-B

S. aureus acquires iron from host hemoglobin due to the bacterial expression of iron-regulated surface determinants (Isd) [93]. Zapotoczna et al. (2013) reported that iron-regulated surface determinant-B (IsdB) promotes the invasion of *S. aureus* into 293T and HeLa cells [25]. Additionally, they proposed that soluble *S. aureus* IsdB binds to and stabilizes the active conformation of integrins, enabling them to interact with RGD-containing ligands, which leads to bacterial internalization in an integrin-dependent pathway. In addition, IsdB adheres to platelets through the integrin receptor GPIIb/IIIa (aIIIbb3) [94]; however, this receptor has not been implicated in bacterial internalization.

12. Conclusions

Phagocytosis is an essential component of innate and adaptive immune responses. In NPPCs, phagocytosis plays major roles in tissue maintenance, regeneration, and remodeling. However, pathogenic bacteria also employ many of the receptors involved in phagocytosis during the interplay between the host cell defense response and tissue colonization. Thus, phagocytosis, endocytosis, and intracellular trafficking can be exploited for therapeutic objectives such as intracellular drug delivery (for a wide and detailed description of these beneficial strategies, see Duncan and Richardson, 2012) [95]. In addition, the manipulation of the host cell membrane affects numerous

events, including actin remodeling and phagocytosis. The characterization and identification of new bacterial effectors and the host cell receptors involved will undoubtedly lead to new discoveries with beneficial purposes. Many of the pathways operating during the intracellular trafficking of bacteria (e.g., autophagosome formation) may have roles in multiple pathologies such as cancer, metabolic diseases, or neurological disorders (reviewed in Rubinsztein et al. 2012) [96]. Furthermore, a very important role of integrins during apoptosis clearance has been established, which may be related to autoimmune disorders, atherosclerosis, cancer, or human age-related macular degeneration (reviewed in Sayedyahossein and Dagnino, 2013) [97]. All of these medical implications highlight the relevance of the study of phagocytic receptors in the infection of NPPCs by *S. aureus* (Figure 1) because diseases related to intracellular strains (e.g., *S. aureus*) are chronic and recurrent, and many of them are life threatening.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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