Review Article

Increasing evidence of the role of *Helicobacter pylori* SabA in the pathogenesis of gastroduodenal disease

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Abstract

Although infection with *Helicobacter pylori* always results in chronic active gastritis, only a fraction of those infected develop severe clinical disease. In addition, certain populations with high incidences of *H. pylori* infection, such as those in East Asian countries, have high incidences of gastric cancer, while other highly infected populations, such as those in Africa and South Asia, do not. This phenomenon might be partially explained by differences in the genotypes of *H. pylori*; however, currently no definite *H. pylori* factors can clearly explain it. Recently, the importance of sialic acid binding adhesin (SabA), a novel outer membrane protein in gastroduodenal pathogenesis, has become increasingly apparent. Binding of blood group antigen binding adhesin (BabA) to Lewis b antigen and related fucosylated ABO blood group antigens is probably important in the initial stage of infection. However, when host inflammation increases, expression of sialyl-Lewis x increases, and *H. pylori* is likely to adhere to the gastric mucosa with SabA. Many of the genes encoding outer membrane proteins undergo phase variation such that not all strains will produce functional proteins, and SabA expression is frequently switched "on" or "off", suggesting that SabA expression can rapidly respond to changing conditions in the stomach or in different regions of the stomach. SabA production is indeed reported to be associated with severe intestinal metaplasia, gastric atrophy, and the development of gastric cancer in both developed and developing countries, confirming the importance of investigating SabA in developing countries.
Key Words: *Helicobacter pylori*; SabA, gastroduodenal diseases, sialyl-Lewis x/a antigens.

Introduction

Helicobacter pylori is a gram negative bacterium that colonises the gastric mucosa and causes gastritis, peptic ulcers and gastric malignancy. Although infection with *H. pylori* always results in chronic active gastritis, only a fraction of those infected develop severe clinical disease [1]. In addition, some populations with high incidences of *H. pylori* infection, such as those in East Asian countries, have high incidences of gastric cancer, while other highly infected populations, such as populations in Africa (African enigma) and South Asia (Asian enigma), do not. While these phenomena remain unexplained, host genetics, host immune response, and the relationship of the host response to bacterial virulence factors appear to play critical roles. A number of studies are investigating the roles of putative virulence factors of *H. pylori*, in particular CagA and VacA. CagA and/or VacA producing strains are reported to be related to severe clinical outcomes, especially in Western countries [2-7]. However, the importance of CagA and VacA has not been observed in East Asian countries, where most strains produce CagA and VacA irrespective of the disease [8,9], or in many developing countries, including Colombia [9], Thailand [10] and India [11].

It has been proposed that any *H. pylori* virulence factor should 1) have a disease or other *in vivo* correlation; 2) be epidemiologically consistent across populations and regions; and 3) be biologically plausible, with an effect that is reduced or eliminated by gene deletion [12]. Based on the second criterion, CagA and VacA might not be the true virulence factors, and therefore, recent searches for other putative virulence factors of *H. pylori* are of great interest.

Epithelial adherence may benefit *H. pylori* by providing better access to epithelial surfaces and may contribute to delivery of bacterial toxins and various effector molecules through the type IV

secretion mechanisms. Many *H. pylori* strains express adhesin proteins that bind to specific host-cell macromolecule receptors, most of which are members of the outer membrane protein (OMP) family and include the blood group antigen binding adhesin (BabA), outer membrane inflammatory protein (OipA) and sialic acid binding adhesin (SabA). Both BabA and OipA conform to the first and third virulence factors described above and BabA and/or OipA producing strains are also reported to be related to severe clinical outcomes, especially in Western countries [13,14]. However they are again independent of clinical outcomes in several geographic regions including East Asia [13,15].

The sialyl-Lewis x/a antigens (sLe^x and sLe^a) serve as the functional receptors for *H. pylori* adherence, and sLe^x and sLe^a are recognized by SabA [16]. Sialylated glycoconjugates are scant in healthy gastric mucosa; however, the persistent colonization of the human stomach with *H. pylori* leads to dramatic gastric mucosal inflammation, accompanied by the replacement of naturally produced Lewis antigens by sialylated glycans (sLe^x and sLe^a) [16,17]. The expression of sialylated antigens, in particular sLe^a, has been reported to be elevated in gastric carcinoma [18]. These data suggest that SabA could play important roles in gastroduodenal pathogenesis, and, indeed, SabA production is associated with severe intestinal metaplasia, gastric atrophy, and the development of gastric cancer in both a developed country (United States) and in a developing country (Colombia) [19], suggesting that studies of SabA should be broadened in developing countries. This review summarizes recent information regarding SabA and gives insight into its role in pathogenesis and adaptation to the host.

Discovery of SabA

Lewis b antigen (Le^b) and related fucosylated ABO blood group antigens are recognized by BabA [20], and BabA has been believed to be the primary molecule involved in adherence to the gastric mucosa. Significantly, however, a *babA* deletion mutant that could not bind ABO/Le^b blood group antigens still bound to the gastric epithelium in humans and in a Rhesus monkey [16]. Moreover, binding of the *babA* mutant could not be inhibited by pretreatment of bacteria with soluble Le^b antigen. Further analysis showed that the *babA* mutants preferentially bound to inflamed gastric mucosa. These results implicated the presence of another adhesin, possibly specific to *H. pylori*-infected inflamed tissue, which binds to a receptor distinct from that of BabA.

Using thin layer chromatography, mass spectrometry and nuclear magnetic resonance, Mahdavi *et al*. identified sialyl-dimeric-Le^x (sdiLe^x) glycosphingolipid as a receptor for *H. pylori* and showed that *H. pylori* infection induced formation of sLe^x antigens in gastric epithelium in humans and in a Rhesus monkey [16]. Using an affinity purification method, this sialyl-dimeric-Le^x-binding bacterial adhesin was identified as a 70 K OMP called SabA. Importantly, a paralogous protein, called SabB, was also identified. However, while both sLe^x and sLe^a antigen-binding activity was abolished in the *sabA* mutant, neither were affected in the *sabB* mutant [16].

Regulation of SabA

Based on the comparison of the three completed genomes of *H. pylori*, six *hop* genes (*oipA, sabA, sabB, babB, babC* and *hopZ*) are thought to undergo phase variation such that not all strains will produce functional proteins [21-23]. Recent studies also showed that phase variation is a method of regulating BabA production in some strains [24-26]. Functionality is regulated by a slipped strand mispairing mechanism and is mediated by the number of Cysteine-Threonine (CT) dinucleotide repeats in the 5' region of the gene (switch "on" = functional and "off" = non-functional).

The genome sequences of *H. pylori* J99 deposited in GenBank show that *sabA* is out of frame with nine CT repeats. Nonetheless, Mahdavi *et al*. used J99 as the sLe^x-binding strain to identify *sabA*. However, their isolate possessed 10 CT repeats, which would produce a functional SabA protein and could thus explain the sLe^x-binding [16]. These results show the flexibility of the locus that confers the "on/off" binding properties. The metastability of the SabA adhesin might enable a subpopulation of *H. pylori* to detach from the gastric cells and thereby escape from the most vigorous immune response. This equilibrium between close attachment and detachment might support the establishment of a chronic infection.

During *in vivo* passage in a murine model of *H. pylori* infection, both the *sabA* and *sabB* genes switched from "on" to "off" (27). At four weeks post-inoculation, the switch status was changed in strains recovered from one of three mice infected with strain JK101 (two of nine colonies were changed from *sabB* "on" to "off" and one of nine colonies was changed from *sabA* "on" to "off") and one of three mice infected with strain JK64 (two of nine colonies were changed from *sabA* "on" to "off"). Interestingly, the bacterial density in mice infected with *H. pylori* strains that were switched "off" was reduced compared with those of mice infected with *H. pylori* strains that showed no change in the switch status. In addition, strains JK101 and JK64 could not keep infecting mice after 12 weeks of inoculation

When multiple colonies derived from one human biopsy specimen were examined for mixed expression, the frequency was highest with SabA, less with BabB and BabA, and very rare with OipA [19]. In addition, the stability of expression of the OMPs changed during in vitro passages (up to 20 passages) such that OipA > BabA > BabB > SabA. SabA expression frequently switched "on" or "off" suggesting that SabA expression can rapidly respond to changing conditions in the stomach or in different regions of the stomach.

Recently, CT repeats of the *sabA* gene in Taiwanese strains were analyzed (28). Interestingly, among the 145 *H. pylori* isolates examined, 116 (80%) were sabA gene positive by PCR, but only 45 (31%) expressed SabA as detected by immunoblot. Importantly, the sequence of *sabA* gene positive PCR products from 92 isolates identified that 60% (n = 55) had regular CT repeats whereas the other 40% (n = 37) had imperfect CT repeats (i.e., CTTTCTCTATCC) in the 5' region of the *sabA* gene. Among those with a regular CT repeat tract, 18 strains had an in-frame translatable sequence ("on" subtype), but only 13 of these had predicted full-size protein translation. Strains with imperfect CT repeats (37 strains) uniformly had a predicted *sabA* "on" status, except one isolate, which had a stop codon that interfered with protein translation. However, only 16 of these strains produced detectable SabA protein by immunoblot. Overall, neither the imperfect CT repeats nor the different CT repeats in the *sabA* region correlated with SabA production defined by immunoblot.

H. pylori strains with 7 ± 3 CT repeats in the 5' region of the *sabA* gene are theoretically regarded as "on." However, recent reports from France have shown that *sabA* expression can be altered by nucleotide changes after the dinucleotide repeats [29]. In addition, there are poly T tracts upstream of the *sabA* gene, and SabA expression might be also regulated at transcriptional levels, as observed in BabA expression. Therefore, the expression of *sabA* is not completely reliant on the number of CT dinucleotide repeats in the 5' region of the *sabA* gene and SabA status based simply on the number of CT repeats should be interpreted with caution.

The presence of the *sabA* gene does not appear to be universal among *H. pylori* isolates. Although microarray analyses of 15 isolates showed that all possessed the *sabA* gene [30], 20% (29/145) of Taiwanese strains (28), 14% (6/43) of French strains (29) and 7% (7/96) of Dutch strains (31) did not possess the gene. While the *sabA*-negative genotype might be attributed to false PCR negatives due to subtle mutations among strains in the primer regions, the use of several primer pairs in the Taiwanese and Dutch studies mitigates that risk. Further studies using other methods (e.g., Southern hybridization) will be necessary to determine whether *sabA* gene negative strains are rare in clinical outcomes.

Major Function of SabA

SabA was identified as an adhesin, with sLe^x and sLe^a serving as additional functional receptors for *H. pylori* adherence. Aspholm *et al.* recently reported that clinical *H. pylori* isolates showed "polymorphism" in their ability to bind various sialylated glycans including sdiLe^x, sLe^a and dialyl-lactosamine (sLn) (32). They examined 99 Swedish clinical isolates and found that 39 (39%) could bind to sialylated glycans, and the majority (87%) of these isolates could bind all three glycans. Preferential binding to sdiLe^x was the most common (24 of 39 isolates, or approximately 60% of strains tested), but the binding patterns were not uniform among strains (e.g., some strains bound to sLe^a beter than sdiLe^x and sLn). Further analyses using complementation of *sabA* mutants confirmed that variation in sialyl binding affinity is an inherent property of the SabA protein. It is likely that the variation is the result of subtle

differences in sequence, although the specific motifs are yet to be elucidated.

The findings that SabA mediates binding to sLe[x] and sLe[a] are important since *H. pylori*-induced gastric inflammation and gastric carcinoma are associated with the replacement of nonsialylated Lewis antigens by sLe[x] and sLe[a] [16-18]. Levels of sialylated glycoconjugates are low in healthy gastric mucosa, but *H. pylori* infection elicits gastric mucosal sialylation as part of the chronic inflammatory response. This sialylation correlated with the capacity for SabA-dependent, but not BabA-dependent, binding in situ [16]. Accordingly, high levels of sialylated glycoconjugates have been found in *H. pylori*-infected individuals, and those levels decreased after eradication of infection and resolution of gastritis.

In contrast to the Le[b] antigen on glycoproteins, sLe[x] and sLe[a] antigens in membrane glycolipids may protrude less from the cell surface (16). Thus, *H. pylori* adherence during chronic infection might involve two separate receptor-ligand interactions: one by Le[b]-mediated adherence through BabA, and a more intimate and weaker sLe[x]/sLe[a]-mediated adherence by SabA. Mahdavi *et al*. proposed a scenario for the receptor-ligand interactions [16]. During persistent infection and chronic inflammation (gastritis), *H. pylori* triggers the host tissue to up-regulate the inflammatory-associated sLe[x]/sLe[a] antigens. Then, SabA performs selectin-mimicry by binding the sLe[x]/sLe[a] glycosphingolipids. At sites of vigorous local inflammatory response, including activated neutrophils carrying sialylated carbohydrates on their surface, some *H. pylori* might lose sLe[x]-binding capacity due to the "on/off" switching of SabA. The "on/off" switching and the weakness of the sLe[x]/sLe[a]-mediated adherence may benefit *H. pylori* by allowing escape from sites where bactericidal host defense responses are most vigorous. The ability of *H. pylori* strains to adhere to sialylated glycoconjugates expressed during chronic inflammation might thus contribute to virulence and the extraordinary chronicity of *H. pylori* infection.

The minimal structure recognized by the SabA adhesin was determined to be α2-3-linked sialyllactose, irrespective of a terminal or subterminal fucosylation [33,34]. Fucosylation, sialylation, and length of the core chain are parameters that together form a high-affinity binding epitope for SabA. Recent studies using *sabA* mutants and sialidase treatment of glycoconjugate arrays showed that SabA is involved in the binding of the extracellular matrix protein laminin [33].

Function of SabA on Neutrophils and Erythrocytes

Until recently, *H. pylori* was considered a non-invasive enteropathogenic bacterium. However, on several occasions intracellular *H. pylori* have been spotted in cultured epithelial cells, animal models and human gastric biopsy specimens [35-39]. In addition, *H. pylori* was found, by *in situ* hybridization, on erythrocytes in capillaries and post-capillary venules in gastric mucosa of infected humans and Rhesus monkeys, indicating that the bacterium may disseminate into the circulation by way of gastric mucosal capillaries [32]. Therefore, it is meaningful to investigate *H. pylori*-neutrophil and erythrocyte interactions.

Infiltration of neutrophils into the gastric mucosa is a hallmark of chronic gastritis caused by *H. pylori*. Recently, Unemo *et al*. found that SabA adhesin has a pivotal role in the nonopsonic activation of human neutrophils [40]. The nonopsonic neutrophil oxidative burst induced by *H. pylori* and adherence of the bacteria is initiated by the binding of *H. pylori* SabA to sialic acid-carrying neutrophil receptors. SabA protein, but not BabA, specifically binds to neutrophils through sialylated carbohydrates and thereby induces an oxidative burst in these cells, causing oxidative damage of the gastric epithelium. The link between receptor binding and oxidative burst involves a G-protein-linked signaling pathway and downstream activation of phosphatidylinositol 3-kinase as shown by experiments using signal transduction inhibitors. SabA binding has been identified by flow cytometry, fluorescence microscopy and luminol-enhanced chemiluminescence as the key inducer of oxidative metabolism and as an essential component in the induction of phagocytosis [41]. Overall, SabA adhesin is the key molecule in the nonopsonic activation of human neutrophils and, therefore, is a virulence factor important for the pathogenesis of *H. pylori* infection.

H. pylori has been reported to agglutinate erythrocytes [42]. The activity could be dependent

on sialic acid since the hemagglutination (HA) activity was lost by prior sialidase treatment of erythrocytes [43]. Recently, Aspholm *et al*. found that adherence of *H. pylori* to erythrocytes may require molecular mechanisms similar to the sialic acid-dependent in vitro agglutination of erythrocytes (i.e., sialic acid-dependent hemagglutination) [32]. The SabA adhesin was identified as the sialic acid-dependent hemagglutinin based on sialidase-sensitive hemagglutination, binding assays with sialylated glycoconjugates, and analysis of a series of isogenic sabA deletion mutants. The NeuAcα2–3Gal-disaccharide constitutes the minimal sialylated binding epitope required for SabA binding in erythrocytes.

Relationship between SabA and other virulence factors

Mahadavi *et al*. initially examined a panel of 95 European clinical isolates for sLe^x binding [16]. Thirty-three of the 77 (43%) *cagA*-positive strains, but only 11% (2 out of 18) of *cagA*-negative strains (P <0.001) bound sLe^x. In addition, the great majority of the sLe^x-binding isolates (28 of 35: 80%) also bound the Le^b antigen. These results suggest that the presence of SabA is closely related to the presence of CagA (or *cag* PAI) and BabA. However, subsequent studies could not confirm those relationships. For example, CagA and BabA were present in 145 Taiwanese strains, but SabA was positive only for 31% [28], and no relationship was found between the presence of SabA and CagA (or *cag* PAI) or BabA in U.S. and Colombian populations [19]. Other reports examining SabA status in French [29] and Dutch [31] populations by PCR-based sequencing for the number of CT repeats in the *sabA* gene could not find a relationship between the *sabA* "on" status and the presence of the *cag* PAI and/or *babA*. Since SabA status is unstable in the stomach [19], it is reasonable that SabA status is not closely related to stable CagA expression.

SabA and pH

The stomach presents a variety of different microenvironments and the ability to switch various adhesins and surface proteins "on" and "off" may offer survival advantages to the bacteria. For example, deep in the pits the antrum bacteria would be exposed to a different population of cells than those on the luminal surface as well as much smaller fluctuations in pH. In fact, SabA expression was increased in strains obtained from patients with gastric cancer but reduced among those with duodenal ulcer, suggesting that alternating expression may be related to intragastric pH [19].

Studies of SabA expression support this hypothesis. A microarray study demonstrated that the expression levels of *sabA* mRNA decreased in acidic conditions [44]. In addition, *H. pylori* cultures grown under high acid conditions (pH 5.0) for 2 hours showed a dramatic decrease in SabA expression levels compared to those grown at pH 7.0 [19]. In contrast, the acid exposure did not result in any change in expression levels for BabA, BabB and OipA. When the strains were exposed to pH 5.5 for 20 to 24 hours, the SabA status changed from positive to negative in one strain and the expression of SabA decreased in the other two strains.

Overall, SabA-positive expression was inversely related to the ability of the stomach to secrete acid suggesting that its expression may be regulated by changes in acid secretion and/or in antigens expressed by the atrophic mucosa.

SabA, clinical outcomes and gastric inflammation

It has been proposed that SabA-positive status is associated with gastric cancer, intestinal metaplasia, and corpus atrophy and negatively associated with duodenal ulcer and neutrophil infiltration. A diverse cohort of strains from 200 patients including 120 from Colombia (40 with gastritis, 40 with duodenal ulcer and 40 with gastric cancer) and 80 from the United States (40 with gastritis and 40 with duodenal ulcer) was examined to determine the relationship between the expression of SabA, BabA, BabB, and OipA and clinical outcome [19]. The prevalence of SabA-positive isolates was 66% (53/80) in gastritis, 88% (70/80) in duodenal ulcer, and 89% (36/40) in gastric cancer. Multiple logistic regression analysis among these OMPs showed that only SabA status was a predictor of gastric cancer versus duodenal ulcer (odds ratio, 2.8; 95% confidence interval = 1.2-6.7). Furthermore, SabA-positive status was also closely associated with decreased neutrophil infiltration in both the

antrum and corpus and with severe intestinal metaplasia and gastric atrophy in the antrum [19].

These results are in agreement with the pathogenesis scenario that Mahdavi *et al.* proposed previously [16]. Briefly, during persistent infection and chronic inflammation (gastritis), *H. pylori* triggers an alteration in the glycosylation patterns in the gastric mucosa including up-regulation of the inflammation-associated sLe^x antigens, and SabA binds the sialyl-(di)-$Le^{x/a}$ glycosphingolipids promoting membrane attachment and apposition. It is proposed that at sites of vigorous local inflammatory response (e.g. severe antral gastric inflammation in duodenal ulcer) *H. pylori* undergoes phase variation and turns "off" SabA-mediated sLe^x-binding capacity, which in turn enables *H. pylori* cells to escape intimate contact with sialylated lymphocytes or other defensive immune cells. The ability of *H. pylori* strains to adhere to sialylated glycoconjugates expressed during chronic inflammation might therefore contribute to virulence and the extraordinary chronicity of *H. pylori* infection.

However, one study of 145 *H. pylori*-infected patients from Taiwan reported that the prevalence rate of SabA-positive isolates is similar between patients with different endoscopic diagnoses (33% [10/30] in duodenal ulcer, 22% [10/30] in gastric ulcer and 29% [25/85] in non-ulcer dyspepsia) [28]. In addition, the prevalence rates of gastric atrophy and intestinal metaplasia were not statistically different between patients infected with SabA-positive and SabA -negative isolates. Whether there are geographical differences in the relationship between SabA and clinical outcomes or histological changes warrants further attention.

Interestingly, SabA expression affected *H. pylori* density in the gastric mucosa. Patients infected with SabA-positive *H. pylori* isolates (n = 45) had a higher *H. pylori* density, especially the density over the gastric body, than those (n = 100) infected with SabA-negative isolates (*P* <0.05). Since all Taiwanese patients studied were infected with *babA* gene positive *H. pylori* isolates, the *H. pylori* density of the infected host was largely determined by the intensity of Le^b expression on the gastric epithelium [45]. When the relationship between *H. pylori* density and the sLe^x or Le^b antigen was examined, *H. pylori* density was found to be higher only in patients expressing gastric

sLe^x antigen (which interacts with SabA) (*P* <0.001) but little or no gastric Le^b antigen (which would interact with BabA), and not in those patients with evident expression of gastric Le^b antigen. The data suggest that the interaction of SabA and Le^x should have little influence on the *H. pylori* density when Le^b expression is strong enough to interact with *H. pylori* BabA. This finding indicates that SabA interacting with the sialyl-Le^x antigen can enhance *H. pylori* colonization in those patients with weak or no Le^b expression.

Other studies indirectly evaluated SabA status by using PCR to assess whether the gene was likely "on" or "off." Lehours *et al.* [29] reported that combination of the *sabA* "on" status with *hopZ* "off" status and *iceA1* allele was related to an increased risk for mucosa-associated lymphoid tissue lymphoma. In contrast, de Jong *et al.* [31] evaluated SabA status by the number of CT repeats in a small study (e.g., 9 gastric cancers) and reported that *sabA* "on" status had no relation to clinical outcome. However, as described above, evaluation of SabA status using PCR-based sequencing should be interpreted with caution.

Conclusion

The importance of SabA on gastroduodenal pathogenesis is increasingly apparent. In the initial stage of infection, binding of BabA to Le^b/ABO is probably important; however, when host inflammation increases, expression of sLe^x increases, and *H. pylori* is likely to adhere to the gastric mucosa with SabA. Importantly, SabA expression most frequently switched "on" or "off", suggesting that SabA expression can rapidly respond to changing conditions in the stomach or in different regions of the stomach. SabA production is indeed reported to be associated with severe intestinal metaplasia, gastric atrophy, and the development of gastric cancer both in the U.S. and Colombia (19); however, there are currently no reports about the importance of SabA in developing countries outside of Colombia. Further studies will be necessary to determine whether SabA plays an important role in other geographic regions and to confirm that SabA is a true virulence factor, conforming to all three criteria for *H. pylori* virulence [12].

Acknowledgments

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