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REVIEW

Pathogenic mechanisms of invasive group A *Streptococcus* infections by influenza virus–group A *Streptococcus* superinfection

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ABSTRACT

Group A *Streptococcus* (GAS) are pathogenic bacteria of the genus *Streptococcus* and cause severe invasive infections that comprise a wide range of diverse diseases, including acute respiratory distress syndrome, renal failure, toxic shock-like syndrome, sepsis, cellulitis and necrotizing fasciitis. The essential virulence, infected host and external environmental factors required for invasive GAS infections have not yet been determined. Superinfection with influenza virus and GAS induced invasive GAS infections was demonstrated by our team in a mouse model, after which clinical cases of invasive GAS infections secondary to influenza virus infection were reported by other investigators in Japan, USA, Canada, UK China, and other countries. However, the pathogenic mechanisms underlying influenza virus-GAS superinfection are not yet fully understood. The present review describes the current knowledge about invasive GAS infections by superinfection. Topics addressed include the bacteriological, virological and immunological mechanisms impacting invasion upon superinfection on top of underlying influenza virus infection by GAS and other bacteria (i.e., *Streptococcus pneumoniae* and *Staphylococcus aureus*). Future prospects are also discussed.

Key words group A *Streptococcus* (GAS), influenza virus, invasive GAS infections, superinfection.

INVASIVE GROUP A STREPTOCOCCAL INFECTIONS

Group A *Streptococcus* are pathogenic bacteria of the genus *Streptococcus* that infect the pharynx and skin of humans (1). GAS cause a number of diseases, including uncomplicated pharyngitis, impetigo and acute rheumatic fever. A number of severe invasive GAS infections have been reported in North America, Europe and Japan (1–5). Because infection can occasionally progress to necrosis of an entire limb and lethal shock within hours, GAS has come to be known as the "flesh-eating bug." (5–8). The 700 million GAS infections worldwide annually include an estimated 1.8 million severe infections with a mortality rate as high as 25% (9). The first case of invasive GAS infections in Japan was reported in 1992 and the incidence of invasive infections by GAS and other hemolytic streptococci has increased progressively in recent years (3, 10). The number of reported cases of invasive hemolytic streptococcal infections caused by SDSE has also increased rapidly (11–15) worldwide; however, the reason for this is unknown.

Invasive GAS infections lead to a wide range of diseases, including necrotizing fasciitis (streptococcal

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List of Abbreviations: *emm*, M protein gene; GAS, group A *Streptococcus*; GP96, glycoprotein 96; HA, hemagglutinin; M3, type 3 M protein; SDSE, *Streptococcus dysgalactiae* subspecies *equisimilis*; SLO, streptolysin O; SLS, streptolysin S; SPEB, streptococcal pyrogenic exotoxin B; TSLS, toxic shock-like syndrome.

gangrene), TSLS, sepsis and cellulitis (5–8, 16); necrotizing fasciitis and TSLS are the most severe of their manifestations. Invasive GAS infections are thought to be caused by highly pathogenic GAS strains (17). GAS strains are subtyped by their M protein genes (*emm*), and *emm* subtypes correlate with disease types (17–19). Severe invasive GAS infections are mainly caused by subtypes *emm*1, *emm*3, *emm*12, *emm*28, *emm*89 and *emm*90 (10, 17, 20). Further, the *emm*1 and *emm*3 GAS strains are most often found in invasive infections (10, 16, 21–23). Many studies have suggested that GAS virulence factors, such as adhesin, invasin, enzymes that destroy host tissues and exotoxins are associated with development of the disease. SLO (24), SLS (24), SPEB (25, 26), streptococcal C5a peptidase of GAS (27), fibronectin-binding proteins (28, 29), laminin-binding proteins (30), collagen-like proteins (31) and hyaluronan capsules (32) are candidates for development of invasive GAS infections. Mutations of *covS* and *rgg*, which encode negative transcriptional regulators in GAS, are also associated with invasive GAS infections (17, 33, 34).

Necrotizing fasciitis manifests as extensive and rapidly spreading necrosis of the skin and underlying structures that occurs very quickly as a result of GAS infection of the deeper subcutaneous tissues. In 1924, before any conclusive reports on necrotizing fasciitis caused by invasive GAS infections had been published, Meleney reported cases with similar features (35). However, the current mortality rate of necrotizing fasciitis is more than 70%–80%, which is much greater than that described by Meleney (20%) (16, 36). Bryant *et al.* demonstrated that injury to cultured human skeletal muscle cells increases binding of GAS and that the ubiquitous intermediate filament protein vimentin may be the principal GAS adhesion molecule on injured muscle cells (37).

TSLS, also called streptococcal toxic shock syndrome, refers to any streptococcal infection associated with the sudden onset of shock and organ failure (16). TSLS outbreaks have occurred in closed environments, such as nursing homes and hospitals, and also by transmission to family members (38–40). In their review, Stevens *et al.* (16) proposed that development of severe TSLS is associated with strains that stimulate immune cells to produce inflammatory cytokines. For example, streptococcal pyrogenic exotoxins play a role in superantigen stimulation. Superantigens stimulate a large number of T cells by crosslinking MHC class II molecules on antigen presenting cells and specific Vβ chains on T cells (41), resulting in excessive production of inflammatory cytokines, such as TNF-α and −β, IL-1β, −2, and −6, and IFN-γ. In addition, SPEB (which is not a category of superantigen), SLO, peptidoglycan and lipoteichoic acid also induce mononuclear cells to produce proinflammatory cytokines (42–44). Several reports have indicated that these proteins contribute to TSLS by excessive inflammatory cytokine production (16).

Other factors that promote invasive GAS infections, excluding the pathogen's virulence factors, are also being investigated. For example, experiments in mice have shown that for necrotizing soft tissue infections, host genetic variations and differences in sex contribute significantly to differences in susceptibility and clinical outcomes, such as survival, weight change and lesion size, of invasive GAS infections (45). Another study found that 125 IL-1β network mouse genes are involved in modulating the differential susceptibility to GAS necrotizing soft tissue infections (46). In addition, another study demonstrated that patients who are immunocompromised are more likely to develop invasive GAS infections, including necrotizing fasciitis and septic shock, and have a higher mortality rate than those who are not immunocompromised (16). The use of nonsteroidal anti-inflammatory drugs is reportedly independently associated with a three-fold increased risk for development of TSLS (47–49). Nonsteroidal anti-inflammatory drugs mask fever and reduce pain, suggesting that they do more than merely mask the signs and symptoms of developing invasive GAS infections (16).

INFLUENZA VIRUS INFECTIONS SUPERINFECTED WITH GAS RESULT IN INVASION

The highest incidence of GAS infections is during winter (50, 51): this seasonality has been well documented in many countries, with influenza epidemics generally occurring from December to March in the northern hemisphere, and from June to September in the southern hemisphere (52). Influenza A virus infection alone is rarely lethal; however, it can promote secondary bacterial infections that are often fatal (53). Bacteria causing frequent secondary infections include members of the genus *Streptococcus*, such as Group B *Streptococcus*, *Streptococcus pneumoniae*, and others. Based on these data, we examined whether nonlethal influenza A virus infection in mice affects the outcome of GAS superinfection with the aim of better understanding the pathogenesis of severe invasive GAS infections. In our study, intranasal infection with a nonlethal dose of influenza A virus 2 to 4 days prior to intranasal inoculation of mice with a nonlethal dose of invasive

GAS strains led to a death rate of more than 90%, 10% of which showed necrotizing fasciitis (8). Superinfection with other invasive types of GAS strains, but not with non-invasive types, also caused the deaths of more than 80% of mice within 2 weeks of GAS infection. These results indicate that underlying influenza A virus infection in mice induces a lethal synergism, helping GAS bacteria to invade mice.

After the publication of our superinfection study in 2003, clinical cases of invasive GAS infections secondary to influenza started being reported one after the other in Japan, USA, Canada, UK, China and other countries (54–60). Many of the symptoms accompanying pneumonia, septicemia, and necrotizing fasciitis in humans are similar to those we had found using the mouse experimental system, suggesting that superinfection by invasive GAS bacteria is promoted by external environmental factors.

PATHOGENETIC MECHANISMS OF INVASIVE GAS INFECTIONS CAUSED BY INFLUENZA VIRUS–GAS SUPERINFECTION

Few studies have addressed the mechanisms of invasive GAS infections introduced by intranasal superinfection in patients with underlying influenza virus infections; however, results of research on other intranasal superinfections with *S. pneumoniae* and *Staphylococcus aureus* have shown how viral infection may facilitate invasive bacterial infections. Virally-infected host cells show enhanced adhesion and invasion by bacteria. Viral pathogenetic factors and changes in protective immune responses caused by viral infections in host epithelial cells are both associated with severe invasive infections as described below (Fig. 1).

Enhancement of bacterial adhesion and invasion of host epithelial cells infected with influenza virus

We have shown that intranasal influenza virus infection somehow enhances host cell–GAS adhesion and invasion (8) (Fig. 2): infection of alveolar epithelial cells with influenza virus enhances GAS adhesion and invasion by a factor of two to three. An electron microscopic study confirmed that GAS directly binds to virus-infected cells and viral particles (8). Enhancement of adhesion and invasion is suppressed by adding an antibody against the fusion functional domain of influenza virus HA; administering this antibody 12 hours before GAS infection suppresses GAS invasion. These findings suggest that enhancement of GAS adhesion and invasion of virus-infected cells is caused by viral HA (8).

GAS mutant strains with deleted pathogenic factors have been used to determine the factors involved in invasive infections (25, 61). Bacterial mutants with disruptions in synthesis of their capsules, such as multiple virulence gene regulator, SLO, SLS or SPEB, have been shown to significantly reduce the mortality of superinfected mice. These studies have helped to determine pathogen-associated factors required for invasion after superinfection. In particular, the number of GAS organisms adhering to influenza virus-infected alveolar epithelial cells is markedly reduced in capsule-depleted mutants (61). Wild-type GAS bacteria have been found to bind directly to influenza virus particle, whereas non-encapsulated mutants show much less ability to bind to particles, suggesting that the capsule plays a key role in invasion of host tissues in superinfections (61). Our studies have shown that influenza virus HA and GAS capsules are important for invasion in superinfections (8, 61); however, we have not as yet determined whether influenza virus HA and GAS capsules interact during enhancement of adhesion and invasion.

The number of influenza virus particles peaks in the host 2 to 3 days post-infection and decreases thereafter (62). However, even if GAS is inoculated several days after influenza virus infection, a considerable proportion of mice die from invasive GAS infections (8), indicating that factors other than HA and other viral surface proteins can also enhance bacterial invasion. M3 protein is an important pathogenic factor for invasive GAS infections in influenza virus–GAS superinfection (63). According to this study, GAS mutants lacking M3 protein cannot cause invasion. Secretion of fibronectin and albumin increases in the respiratory tract several days after influenza virus infection and M3 protein binds to these proteins, suggesting that this pathogenic mechanism is active several days after influenza virus infection.

A review of superinfection models by influenza virus and *S. pneumoniae* or *S. aureus* has shown how the bacteria display improved adhesion and invasion of influenza virus-infected cells (64). Molecules associated with enhancing adhesion and invasion of *S. pneumoniae* include pneumococcal surface protein A, choline-binding protein A and pneumococcal serine-rich repeat protein. Those in *S. aureus* include microbial surface components that recognize adhesive matrix molecules and other members of the serine-aspartate dipeptide repeat-containing family. These molecules are involved in enhanced adhesion to the basement membrane or to elements of the extracellular matrix of the host epithelial cells, such as fibrin, fibrinogen and collagen, resulting in

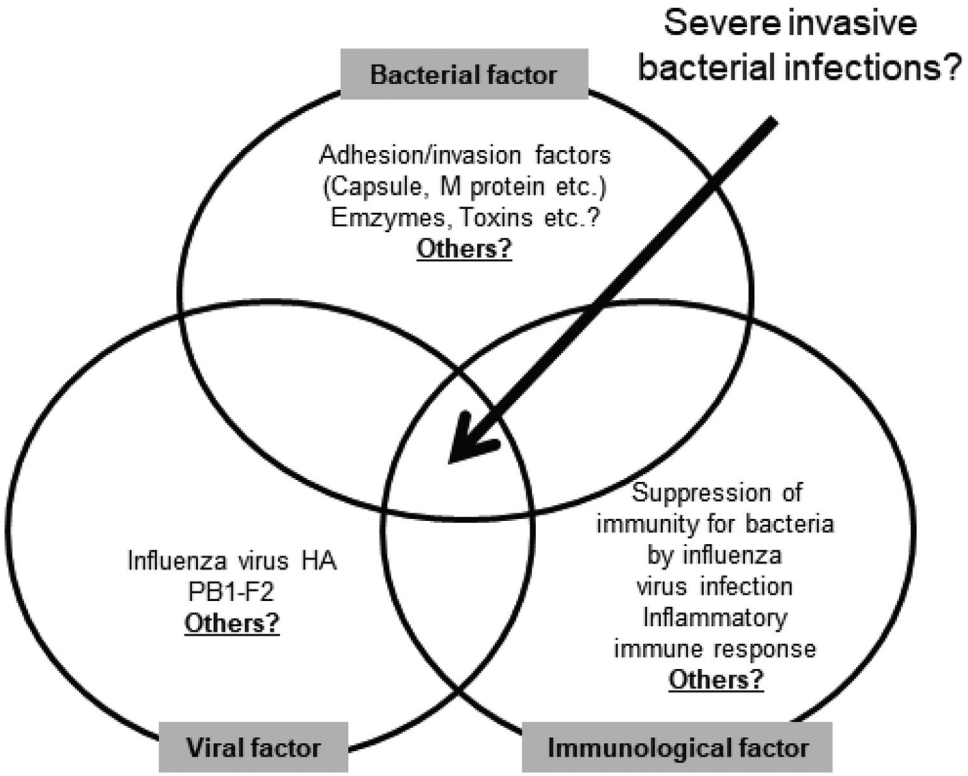

Fig. 1. Hypothetical causes of invasive GAS or other bacterial infections mediated by superinfection with influenza virus and bacteria. Invasive GAS or other bacterial infections by superinfection with influenza virus and bacteria is thought to develop by interaction of bacterial, viral and immunological factors. Because few factors in bacteria, viruses or the host immune system have as yet been identified, we speculate that many other unknown factors are associated with invasive GAS infections by superinfection.

enhanced invasion of influenza virus-damaged epithelial cells (65, 66). We predicted that proteins commonly found in *S. pneumoniae*, *S. aureus* and GAS would be involved in adhesion and invasion to influenza virus-infected cells; we therefore investigated whether their homologs are present in the GAS strain but did not find such homologs.

Various kinds of protein are expressed on the surfaces of virus-infected cells and are involved in the enhancement of bacterial adhesion and invasion. One such protein is GP96, which is a type of stress protein (67). GP96, also known as glucose regulated protein 94 (68), is expressed on the surface of cells infected with hepatitis C virus or human herpesvirus-6 and induces enhanced adhesion and invasion by *Neisseria gonorrhoeae* and *Listeria monocytogenes* (69–71). Although there are no reports of influenza virus-infected cells expressing cell surface proteins such as GP96, it is possible that some proteins expressed during stress are also involved in bacterial adhesion and invasion.

Almost 50% of patients with invasive GAS infections, including necrotizing fasciitis and TSLS, have a defined portal of entry, whereas the other 50% do not (16). In general, soft tissues are the most common primary sites of infection; pneumonia, meningitis, endophthalmitis, peritonitis, myocarditis, joint infection and intrauterine infection have been reported in the remaining cases (16). In cases of invasive GAS infection with no defined portal of entry, the GAS infection may begin to develop deep in the tissue, proliferate inside the body, and then spread via the blood. In the superinfection model, we found that intranasal influenza virus infection enhances alveolar epithelial cell–GAS adhesion and invasion, which is followed by lung tissue destruction, intravascular invasion of GAS, and the accompanying systemic spreading and proliferation of GAS (8). Thus, our finding of influenza virus–GAS superinfection may provide a clue to such cases.

Influenza virus pathogenic factors

Pathogenic factors of influenza virus in invasive GAS superinfections include influenza HA (8). However, it is as yet unknown which bacterial surface protein binds to HA or how HA associates with the bacteria to enhance their invasiveness.

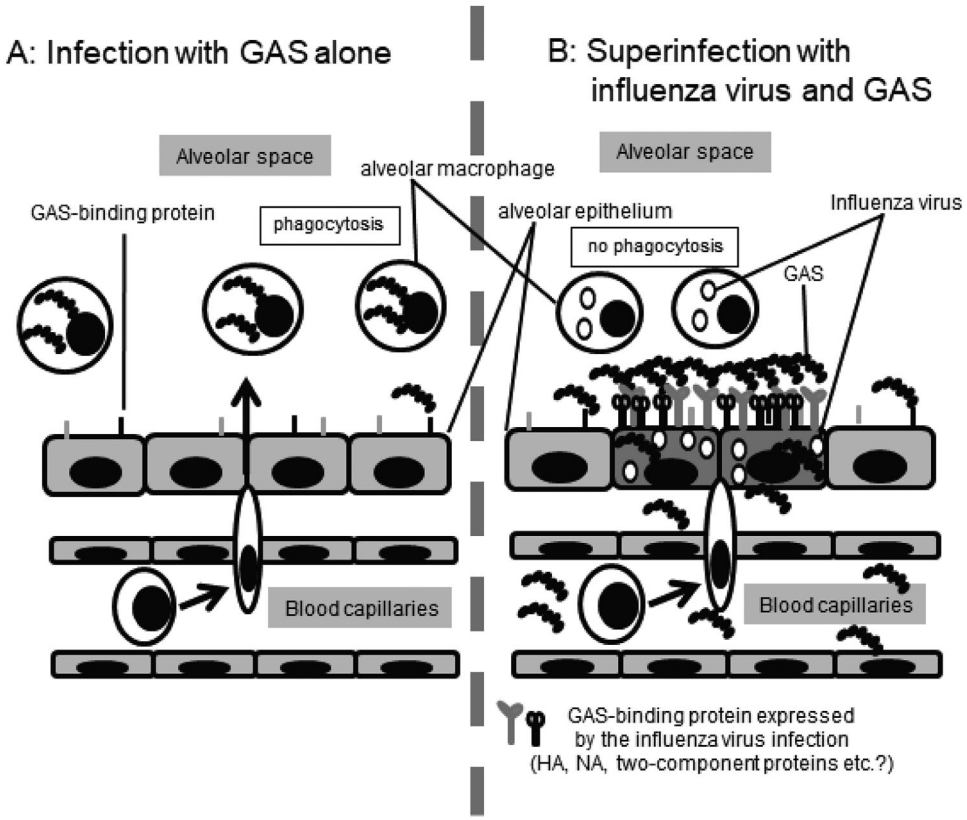

Fig. 2. Enhanced adhesion and invasion of GAS into influenza A virus-infected alveolar epithelial cells is associated with invasive GAS infection mediated by intranasal influenza A virus-GAS superinfection. (a) In intranasal infection with GAS alone, GAS binds to the alveolar epithelium surface proteins that are the ligands of GAS adhesion molecules. However, monocytes migrate into the infected site and differentiate into alveolar macrophages, which in turn eliminate GAS by phagocytosis and digestion. (b) In intranasal superinfection with influenza A virus and GAS, GAS adhesion/invasion to the epitheliums is enhanced. It has been shown that influenza virus HA and GAS capsules are important for adhesion and invasion in superinfections and that M3 protein is an important pathogenic factor for invasive GAS infection in influenza virus-GAS superinfection. Infection of macrophages with influenza virus results in suppression of phagocytosis and digestion and decreased expression of scavenger receptor macrophage receptor with collagenous structure in alveolar macrophages, diminishing their ability to ingest bacteria. Thus, GAS easily invades and proliferates in lung tissue and diffuses throughout the body through blood capillaries, leading to invasive GAS infections.

Reports have shown two influenza A virus types; one of which results in severe invasive bacterial superinfections whereas the other does not: PB1-F2 factor seems to influence this phenomenon (72, 73). Influenza A viruses contain an eight-segment, negative-strand RNA genome. PB1, segment 2 of most IAV strains, encodes a small (up to 90 amino acids) accessory PB1-F2 protein in the +1 open reading frame (74). A third product, N40, can also be produced from an upstream start site in the PB1 open reading frame (75). PB1-F2 has been studied as a potentially important viral virulence factor because of its links to the pathogenicity of strains, such as its presence in the highly pathogenic avian influenza viruses (76–79). Although the pathogenic mechanism of PB1-F2 has not been clarified, the *in vivo* effects of PB1-F2 appear to be largely mediated through interactions of this accessory protein with the immune system, either through potentiation of inflammatory responses or blockade of early type 1 INF pathways. Viral regions L62, R75, R79 and L82 of PB1-F2 are thought to be responsible for the pathogenicity of viral–*S. pneumoniae* superinfections (72). The PB1-F2 regions in influenza A virus have also been implicated in the pathogenicity of viral–*S. aureus* and GAS co-infections (73). These viral residues are responsible for the inflammatory immune response.

Changes in protective immune responses to bacterial infection attributable to virus co-infection in host epithelial cells

Both enhancement of the inflammatory immune response and suppression of pathogen exclusion mechanisms have been recognized in superinfection

with influenza virus and bacteria. PB1-F2 seems to be involved in enhancement of inflammatory immune responses.

Suppression of pathogen exclusion mechanisms during superinfection is described in an excellent review by Braciale *et al.* (80); however, there have been no published experiments on superinfection with influenza virus and GAS. As part of the innate immune system, alveolar macrophages eliminate bacteria invading the lungs by phagocytosis. However, infection of these macrophages with influenza virus suppresses phagocytosis and digestion, resulting in proliferation of bacteria in the lungs (80). Alveolar macrophages infected with influenza virus express CD200R, which inhibits phagocytosis of bacteria by binding to the CD200 expressed in virus-infected apoptotic alveolar epithelial cells (81). Another study has shown that expression of scavenger receptor macrophage receptor with collagenous structure is decreased in alveolar macrophages infected with influenza virus, diminishing their ability to ingest bacteria (82). Influenza virus-infected alveolar macrophages also show poor secretion of cytokines and chemokines as a result of decreased translocation of nuclear factor-κB (83). Influenza infection also induces inhibition of cell-mediated immunity by neutrophils, Th17 cells and NK cells, resulting in diminished bacterial clearance (80, 84–86). In addition, Ghoneim *et al.* have reported a murine model in which most resident alveolar macrophages are decreased by influenza infection. These authors also reported significant reduction in macrophage-mediated bacterial clearance (87). Several studies, including our study, have shown that inoculation of bacteria into influenza virus-infected mice kills them, whereas inoculation of influenza virus into bacteria-infected mice is not fatal (8, 72, 73). Suppression of the immune response to bacterial infection by prior influenza virus infection is the mechanism thought to responsible for this finding. However, this mechanism has only been studied in influenza virus–*S. pneumoniae* or −*S. aureus* superinfections; thus, we cannot be sure that the same applies to influenza virus–GAS superinfections.

PREVENTION FROM INVASIVE GAS INFECTIONS BY SUPERINFECTION, AND OTHERS

The incidence of clinical cases of invasive GAS infection is increasing in Japan. Although only a small proportion seem to be caused by superinfection of influenza virus-infected individuals, the presence of worldwide reports on the latter warrants implementation of prevention strategies. The most effective way to counteract influenza infections is vaccination. Some animal studies have found that vaccination with inactivated influenza whole virion vaccine, live attenuated influenza vaccine or inactivated influenza component vaccine suppresses invasive GAS infections (88–90). Future studies should determine which vaccine antigens and administration routes (i.m., intranasal or oral) safely confer the most effective protection from viral–bacterial co-infections. Additionally, the protective mechanism(s) after vaccination requires elucidation.

Many questions remain about the pathogenic mechanisms of invasive GAS infections after superinfection. It is unclear whether GAS pathogenic factors differ between strains causing invasion on their own and strains causing invasion after superinfection. It is also not known whether expression of tracheal and alveolar epithelial cell surface proteins induces invasive GAS infections in superinfection. Furthermore, while the incidence of clinical cases of invasive infection by SDSE has been increasing (17), we still do not know whether influenza virus-infected hosts are more prone to invasive SDSE infections or just to GAS invasion. Elucidation of the pathogenesis of invasive GAS infections caused by superinfection with influenza virus will help in development of improved methods for treating and preventing this disease.

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DISCLOSURE

The authors declare no conflicts of interest regarding this article.

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