

Immunology of *Helicobacter pylori* Infection

Dominique Velin Pierre Michetti

Service de Gastro-Entérologie et d'Hépatologie, CHUV, Lausanne, Suisse

Key Words

Helicobacter pylori • Immune response • Mast cells

Abstract

Helicobacter pylori is a Gram-negative, spiral bacterium that colonizes the gastric mucosa of at least 50% of the world's population and plays a causative role in the development of chronic gastritis as well as in gastric and duodenal ulcers. *H. pylori* triggers vigorous humoral and cellular immune responses in both systemic and mucosal compartments. In spite of this response, the vast majority of infected hosts are unable to clear the infection, and it persists for decades. Although *Helicobacter* is tolerated by a naïve host organism, preclinical studies have demonstrated that prophylactic or therapeutic vaccinations efficiently clear *Helicobacter* from the stomach. The understanding of the mechanisms leading to the *Helicobacter* persistence or the vaccine-induced eradication of *Helicobacter* in animal models will help to define optimal immunization strategies for future anti-*Helicobacter* vaccination clinical trials.

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Introduction

Since the first culture of *Helicobacter pylori* 26 years ago, the diagnosis and treatment of upper gastrointestinal disease have changed dramatically. Peptic ulcer dis-

ease is now approached as an infectious disease, in which elimination of the causative agent cures the condition. Infection with *H. pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country [1]. The overall prevalence of *H. pylori* infection is strongly correlated with socioeconomic conditions [2]. The prevalence among middle-aged adults is >80% in many developing countries, as compared with 20–50% in industrialized countries. The infection is acquired by oral ingestion of the bacterium and is mainly transmitted within families in early childhood [1, 3]. It seems likely that in industrialized countries, direct transmission from person to person by vomitus, saliva or feces predominates; additional transmission routes, such as water, may be important in developing countries [4, 5].

H. pylori is now recognized as the major trigger for a sequence of phenotypic changes in the gastric mucosa, progressing from inflammation to superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally, carcinoma [6]. Most infected individuals experience asymptomatic gastritis, although recurrent gastroduodenal ulceration may occur in 10–15% of the infected population. The incidence of gastric cancer is lower, with approximately 1% of infected individuals developing gastric adenocarcinoma and even fewer experiencing gastric mucosa-associated lymphoid tissue lymphoma [7–9].

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Dr. Dominique Velin
Service de Gastro-Entérologie et d'Hépatologie, CHUV, BH18-521
Rue du Bugnon 46
CH-1011 Lausanne (Suisse)
Tel. +41 21 314 06 85, Fax +41 21 314 06 84, E-Mail dominique.velin@chuv.ch

The Discovery of *H. pylori*

In 1984, Marshall and Warren [10] suggested that gastric 'curved bacilli' were associated with gastritis and peptic ulceration. Soon after, it was shown that intentional infection of Dr. Marshall led to gastritis that resolved after antibiotic therapy [11]. For this discovery, Marshall and Warren were awarded, in 2005, the Nobel Prize in Physiology and Medicine [12]. The classical biological approaches demonstrated that *H. pylori* is a Gram-negative, flagellated organism that produces a number of enzymes, including catalase and urease, that help to neutralize host responses and favor colonization [13, 14]. *H. pylori* shows a strict tropism for the gastric mucosa or sites in which there is gastric metaplasia. *H. pylori* infection resides predominantly in the lumen of the stomach and attaches to host cells. Once attached to the cells, *H. pylori* has the capacity to inject into the epithelial cells some bacterial components. A segment of bacterial DNA referred to as the *cag* pathogenic island encodes proteins that provide a type IV secretion apparatus (*cagE*), which allows bacterial macromolecules to translocate into the host cell (*cagA*) [15]. The translocation of bacterial products is believed to have consequences for the pathogenesis of the disease. Indeed, naturally occurring or experimentally induced mutations of the *H. pylori cag* region were associated with decreased gastric mucosal inflammation in vivo, and this correlated to reduced activation of interleukin (IL)-8 gene expression or apoptosis in vitro [16].

Immune Responses to *H. pylori* Infection

Epithelial and Innate Immune Responses

H. pylori causes continuous gastric inflammation in virtually all infected persons [17]. This inflammatory response initially consists of the recruitment of neutrophils, followed by T and B lymphocytes, plasma cells and macrophages, along with epithelial cell damage [18]. Since *H. pylori* rarely, if ever, invades the cells of the gastric mucosa, the host response is triggered primarily by the attachment of bacteria to epithelial cells. The epithelial cell responses include changes in epithelial cell morphology [19], disruption of the tight junctional complexes [20], production of cytokines [21], increased epithelial cell proliferation [22], increased rates of epithelial cell death via apoptosis [23] and induction of numerous genes associated with the stress due to infection [24]. The gastric epithelium of *H. pylori*-infected persons has enhanced levels of IL-1 β , IL-2, IL-6, IL-8 and tumor necro-

sis factor- α [25–28]. Importantly, it has been described that the magnitude of inflammation (IL-1 β production) is associated with cancer linked to *H. pylori* infection [29]. Some bacteria may contact epithelial cells, while significant amounts of bacterial material may 'leak' around epithelial cells and reach the lamina propria, where it can activate underlying phagocytes, including neutrophils and macrophages. Recruitment and activation of macrophages and neutrophils cause the release of other inflammatory mediators such as nitric oxide and superoxide [30].

Specific Immune Responses

H. pylori infection induces a vigorous systemic and mucosal humoral response [31]. During infection with *H. pylori*, the number of immunoglobulin (Ig)A-producing cells increases. IgG- and IgM-producing cells are also detected [32], along with activated complement [33]. The presence of anti-*H. pylori* IgG antibodies in human sera remains one of the simplest methods for the detection of bacterial infection. This antibody production does not lead to eradication of the infection but may contribute to tissue damage. Some *H. pylori*-infected patients have an autoantibody response directed against the H⁺/K⁺-ATPase of gastric parietal cells that correlates with increased atrophy of the corpus [34].

A strong infiltration of CD4⁺ $\alpha\beta$ T cell receptor-positive cells with an activated/memory phenotype (CD45RO⁺) is commonly found in infected individuals [35, 36]. In the normal course of specific immune responses, different subgroups of T cells emerge. Immature T helper 0 (Th0) cells can differentiate into two functional subtypes: Th1 cells, secreting IL-2 and interferon- γ , and Th2 cells, secreting IL-4, IL-5 and IL-10. Whereas Th2 cells stimulate B cells in response to extracellular pathogens, Th1 cells are mostly induced in response to intracellular pathogens. Because *H. pylori* is noninvasive and induces a strong humoral response, a Th2 cell response would be expected. Paradoxically, *H. pylori*-specific gastric mucosal T cells generally present a Th1 phenotype [35–37].

How Can *Helicobacter* Infection Persist for Decades?

Although *H. pylori* infection elicits innate and adaptive immune responses, the bacteria elude these protective mechanisms and persist for decades in our stomach. Several mechanisms of immune evasion used by *H. pylori* have been described. For instance, in contrast to the

lipopolysaccharides and flagellins of other Gram-negative bacteria, the lipopolysaccharides and flagellins of *H. pylori* do not adequately activate the antigen-presenting cells via the Toll-like receptors [38, 39]. In addition, Akhiani et al. [40] recently demonstrated that bacteria were completely cleared from B-cell-deficient mutant mice within the context of severe gastric inflammation, although initial colonization was comparable with the one observed in wildtype mice. This suggests that the presence of antibodies directed toward the bacteria results in less severe inflammation and allows chronic infection. In addition, to subvert the activation of the antigen-presenting cells and antibody response, it has been described that *H. pylori* infection also leads to the generation of regulatory T cells (T_{reg}). Indeed, it has been documented that *H. pylori*-induced T cell response is actively downregulated, partly by immunosuppressive CD25+ T cells or T_{reg} [41–43]. Lundgren et al. [42] have shown that the memory T cell responses to *H. pylori* antigens in the peripheral blood are under the control of T_{reg} in *H. pylori*-infected asymptomatic individuals. Removal of T_{reg} , specifically from the memory T cell population, increased the proliferative responses to *H. pylori* antigens, and, importantly, the readdition of T_{reg} to the memory T cells suppressed the *H. pylori*-specific responses but failed to suppress responses to unrelated antigens. Moreover, CD4+ CD25^{high} T cells (putative T_{reg}) isolated from the gastric and duodenal mucosa of *H. pylori*-infected asymptomatic carriers express the specific T_{reg} marker *FOXP3* [44]. Although widely acknowledged to play a role in the maintenance of self-tolerance, recent studies indicate that T_{reg} can be activated and expanded against bacterial, viral and parasite antigens in vivo. Such pathogen-specific T_{reg} can prevent infection-induced immunopathology but may also increase the load of infection and prolong pathogen persistence by suppressing protective immune responses. Therefore, it can be anticipated that these *H. pylori*-specific T_{reg} maintain a balance between bacterial infection chronicity and development of tissue damage affecting the gastric mucosa.

Is *H. pylori* Viewed by Our Immune System as a Commensal?

It is well known that most individuals have lifelong clinical and immunological tolerance to both food antigens and their gut flora [45]. Several mechanisms have been proposed for the development of oral tolerance, ranging from the deletion of antigen-specific T cells to

immune deviation, induction of anergy and suppression by T_{reg} [45]. Oral tolerance to food antigens and gut flora is mainly acquired during early childhood [45]. During this time, the immature mucosal immune system is educated to tolerate novel diet and colonization by novel strains and species of bacteria. Oral tolerance and *H. pylori* infection seem to be acquired during the same period of time [1, 3, 45], and thus, it can be postulated that our mucosal immune system learned to tolerate *H. pylori* colonization. Therefore, it might be possible that *H. pylori* is seen as a component of the gut flora allowing its persistence in the stomach.

Although *H. pylori* mimics a commensal bacterium, its persistence can lead to alterations in the gastric mucosa [7–9]. Hence, gastroenterologists are used to eradicate *H. pylori* infection with a combination of antisecretory and antimicrobial agents [46]. As it is the case for other antimicrobial treatments, the therapy selects resistant *H. pylori* strains [46]. Therefore, there is a need to develop alternative therapies to eradicate *H. pylori* infection.

Development of a Vaccine against *H. pylori*

Although *Helicobacter* is tolerated by a naïve host, prophylactic or therapeutic vaccinations clear *Helicobacter* from the stomach. We and others have shown that oral [47], nasal [48] or rectal [49] immunization with *Helicobacter* urease B subunit and the mucosal adjuvants cholera toxin (CT) or heat-labile toxin of *Escherichia coli* was sufficient to confer protection against *Helicobacter* infection in mice and ferret [50]. When administered with heat-labile toxin adjuvant, urease was also shown to be immunogenic and protective in rhesus monkeys [51–53].

In theory, mucosal vaccination is best suited to elicit potent mucosal immune responses and proved to be effective in animals [46, 54]. Therefore, the safety, immunogenicity and efficacy of mucosal vaccination were initially explored in humans. To date, four randomized, double-blind, placebo-controlled clinical trials have been conducted with orally administered recombinant urease in healthy asymptomatic volunteers [55–58]. Although these trials confirmed the safety of urease, its immunogenicity remained weak. On the other hand, the recent demonstration in animal models that parenteral vaccination was as effective as mucosal vaccination was unexpected [59]. This clearly highlighted the need to complete our understanding of the mechanisms leading to the *He-*

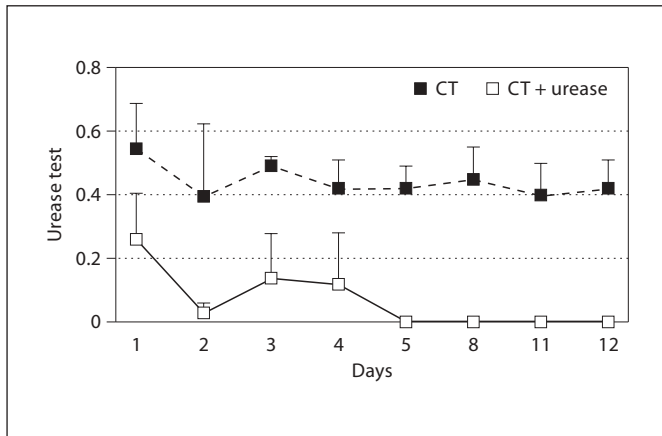


Fig. 1. Kinetics of *Helicobacter* clearance after vaccination. Balb/c mice were treated intranasally (at days 0, 7, 14 and 28) with either 30 μ g of urease + 10 μ g of CT as adjuvant, or CT alone. At day 42, the mice were challenged with *H. felis* (5×10^7) and sacrificed at different time points after challenge. At sacrifice, stomachs were recovered for urease testing.

licobacter clearance following vaccination in animal models to define optimal immunization strategies for future clinical trials.

Development of an Efficient Anti-*H. pylori* Vaccine Needs Additional Preclinical Studies

In animal models, vaccination-induced protection against *H. pylori* requires major histocompatibility complex class-II-restricted CD4+ T cells [54, 60, 61], and both Th1 and Th2 CD4+ T cell responses have been shown to mediate protection [62, 63]. B cells (antibodies) are not required for protection [61], although they may be beneficial [64]. $\alpha_4\beta_7$ -Integrin-mediated homing processes are also critical for host protection [65]. Even if CD4+ T cells appear as key players in the *Helicobacter* clearance [61], their mechanisms of action remain open. Indeed, although CD4+ T cells can migrate into the stomach mucosa [65], they do not pass through the mucosa, and therefore, will never be in direct contact with the bacteria. Therefore, the role of CD4+ T cells in the bacterial eradication is certainly indirect, and the cellular mechanisms leading to *Helicobacter* clearance following vaccination still need to be defined.

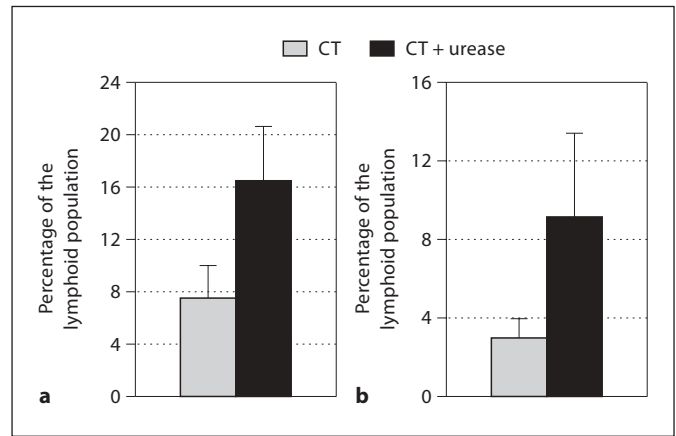


Fig. 2. Mast cell hyperplasia in the stomach of urease + CT-vaccinated mice during the *Helicobacter* clearance. Flow cytometry analysis of lymphoid cell populations recovered from the stomachs of vaccinated (CT + urease) or CT control mice (CT) at day 4 after *H. felis* challenge. **a** Percentages of CD4+ cells. **b** Percentages of mast cells (CD3- CD117+ cells).

CD4+ T Cells and Mast Cells Are Enriched in the Gastric Mucosa during Vaccine-Induced *Helicobacter* Clearance

We explored the cellular events associated with *Helicobacter* clearance from the stomach following intranasal immunization of mice with urease and CT. We first studied the kinetics of *Helicobacter* clearance in vaccinated wildtype mice (fig. 1). At day 5 after challenge, only 2 out of 10 vaccinated mice showed positive urease tests, whereas 10 out of 10 control mice administered with CT alone were positive. These kinetic studies indicate that vaccination-mediated clearance of *Helicobacter* begins on day 4–5 after bacterial challenge [66]. Based on the kinetics of bacterial clearance, we analyzed the lymphoid cell populations recovered from the gastric mucosa at day 4 after challenge by flow cytometry using monoclonal antibodies directed towards CD3, CD4, CD8, CD19, CD117 and Fc ϵ RI [66]. Percentages of CD4+ cells were increased in the gastric lymphoid population of vaccinated mice at day 4 after challenge, as compared with controls (fig. 2), in agreement with the known role of CD4+ T cells in vaccination-mediated protection against *Helicobacter* spp. [60, 61, 66]. Interestingly, we also found a significant increase in gastric mast cell populations (CD3- CD117+) in vaccinated mice at day 4, as compared with controls (fig. 2). To confirm the observation that gastric mast cell populations increased upon urease vaccination and bac-

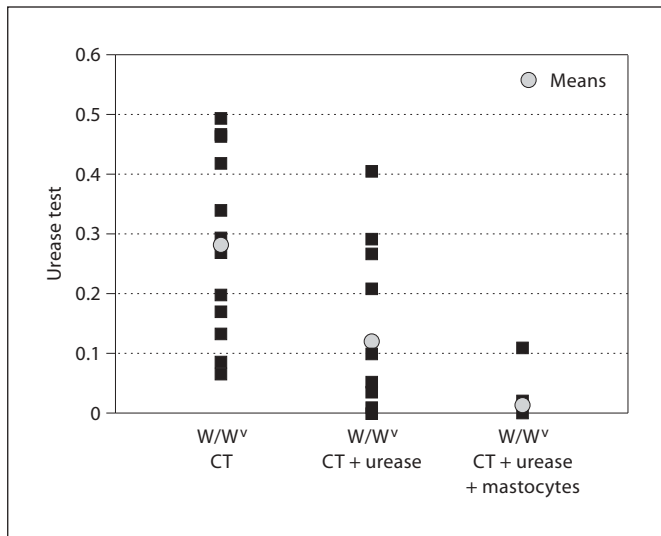


Fig. 3. Critical role of mast cells in anti-*Helicobacter* vaccination. W/W^v mast cell-deficient mice were vaccinated (at days 0, 7, 14 and 28) either with urease + CT or CT alone. At day 42, mice were challenged with *H. felis* (5×10^7). At sacrifice (day 56), urease tests were performed on gastric samples. In another set of experiments, W/W^v mast cell-deficient mice were vaccinated as described above and were reconstituted at day 35 with bone marrow-derived mast cells. At day 42, mice were challenged with *H. felis* and sacrificed at day 56 to perform urease tests on gastric specimens.

terial challenge, we measured mRNA expression levels of the mast cell proteases (mMCP)-1 and mMCP-2. mMCP-1 and mMCP-2 are components of the cytoplasmic granules of mast cells located in the gastric and intestinal mucosa in mice [67, 68]. Using quantitative real-time PCR, expression levels of both mMCP-1 and mMCP-2 were found highly increased in the stomach of vaccinated mice at day 5 after challenge, as compared with CT-vaccinated controls. In addition, serum mMCP-1 protein levels were significantly increased in vaccinated mice at day 5 after challenge, in comparison with CT-administered mice, suggesting that degranulation of mast cells only occurred in vaccinated mice [66].

Mast Cells Are Critical Mediators of Vaccine-Induced *Helicobacter* Clearance

As for CD4⁺ T cells, whose presence is known to be critical in vaccination-induced protection against *Helicobacter* spp., we undertook to determine whether mast cells were also required for immune mediated protection

following *Helicobacter* vaccination. Therefore, we immunized and challenged mast cell-deficient Kit(W)/Kit(W^v) double-mutant mice (W/W^v) [69]. W/W^v mice were not protected from *Helicobacter felis* colonization after vaccination. Indeed, only 1 out of 12 vaccinated W/W^v mice had a negative urease test (fig. 3), while nonmast cell-deficient mice (+/+, +/W or +/W^v), or wildtype F1, were fully protected from bacterial challenge by vaccination [66]. This result shows that mast cells are necessary to clear *Helicobacter* following vaccination. To challenge this interpretation, we injected cultured wildtype F1 bone marrow-derived mast cells (BMMC) to vaccinated W/W^v mice and challenged them with *Helicobacter* 1 week later. In contrast to mast cell-reconstituted W/W^v mice administered with CT alone [66], mast cell-reconstituted vaccinated W/W^v mice recovered the ability to clear *Helicobacter* after vaccination. Indeed, 8 out of 10 mast cell-reconstituted vaccinated W/W^v mice had negative urease tests (fig. 3), whereas 5 out of 5 mast cell-reconstituted W/W^v mice administered with CT alone had positive urease tests [66].

How Can Mast Cells Be Involved in Vaccine-Induced *Helicobacter* Clearance?

Mast cells have been shown to be critical in bacterial clearance in a murine model of bacterial peritonitis [70, 71]. In this model, mast cells contribute to bacterial clearance by triggering the recruitment of polymorphonuclear leukocytes, in part via secretion of tumor necrosis factor- α during the acute phase of the bacterial infection, as well as by direct phagocytosis. Thus, in order to test whether recruitment of polymorphonuclear leukocytes is also responsible for the clearance of *Helicobacter* observed in vaccinated mice, we injected a depleting antineutrophil monoclonal antibody to vaccinated mice and challenged the animals with *H. felis*. Neutrophil depletion proved to have no impact on the efficacy of vaccination (data not shown) [66].

As mast cells have been reported to phagocyte and kill bacteria such as *E. coli* [72], we sought to test whether mast cells could directly phagocyte and kill *Helicobacter*. When BMMC were incubated with *H. pylori* for 6 h, no viable *H. pylori* were recovered at the end of the incubation period [66]. This observation indicates that mast cells have the potential to be directly involved in *Helicobacter* clearance without the need of prior vaccination or cognate help from specific CD4⁺ T cells. We then tested whether the role of mast cells in vaccination-induced bac-

terial clearance in vivo was merely dependent on their local recruitment and bactericidal activity. To challenge this hypothesis, we tested whether a nonvaccination-dependent increase in the gastric mast cell number would lead to the clearance of *Helicobacter* infection. We infected IL-9 transgenic mice [73] as well as IL-3-treated mice [74] with *H. felis*, as both transgenic overexpression of IL-9 and IL-3 treatment lead to gastric mastocytosis. Without vaccination, mice harboring a gastric mastocytosis failed to show *H. felis* clearance [66]. Therefore, although mast cells can kill *Helicobacter* in vitro, the presence of a high number of mast cells infiltrating the stomach mucosa is not sufficient to clear *Helicobacter* in the absence of vaccination.

Mast cells are responsive to aggregation of the Fc antibody receptors on their surface. Mast cells express Fc γ RIII, a low affinity activating receptor that preferentially binds complexed IgG and Fc ϵ RI, the high affinity IgE receptor [75]. The vaccination protocol did not induce serum anti-urease IgE antibodies in W/W^v or Balb/c mice [66], indicating that mast cells could not be activated via the high affinity IgE receptor in vaccination protocols. In addition, vaccinated W/W^v mice reconstituted with BMMC derived from Fc γ R-deficient mice still eradicated *Helicobacter* infection (unpubl. data). This demonstrated that aggregation of the Fc antibody receptors at the surface of mast cells is not involved in the mast cell-dependent vaccine-induced *Helicobacter* clearance.

Finally, mast cells and CD4⁺ T lymphocytes with a Th2 cytokine secretion phenotype have been described as collaborating to expel intestinal parasites [76, 77]. In our model, mast cell recruitment is reminiscent of the

recruitment that takes place during parasitic infection of the intestinal epithelium in mice. By analogy, it can be speculated that a similar recruitment and/or collaboration between mast cells and CD4⁺ T cells leads to *Helicobacter* clearance in the stomach after vaccination. Recently, it has been demonstrated by Cliffe et al. [78] that clearance of *Trichuris muris* from the intestine of infected mice is the consequence of an immune-dependent modulation of intestinal epithelial cell turnover. This increased epithelial cell turnover generates environmental conditions which are incompatible with the survival of the parasite in the intestine. We can envisage that crosstalks between CD4⁺ T cells, mast cells and possibly other unidentified actors such as the stomach epithelium would modify the environmental conditions of the stomach that might be incompatible with the survival of *Helicobacter*. Indeed, it is known that the survival of *Helicobacter* in the stomach depends both on pH and on adhesion of the bacteria to mucins [46]. Recently, Kawakubo et al. [79] showed that gastric mucin with O-glycans and terminal α 1,4-linked N-acetylglucosamine (which are present in the deeper portions of the stomach mucosa) appeared to function as a natural antibiotic against *Helicobacter*. One can postulate that the immune response generated by vaccination leads to an increase in the local expression of this natural antibiotic and to bacterial eradication.

The elucidation of the mechanisms leading to *Helicobacter* clearance following vaccination will thus increase our knowledge of the defense systems operating at the gastric mucosal surface and should be helpful in designing human vaccines and identifying other biotherapeutic tools able to clear gastric *Helicobacter* infection.

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